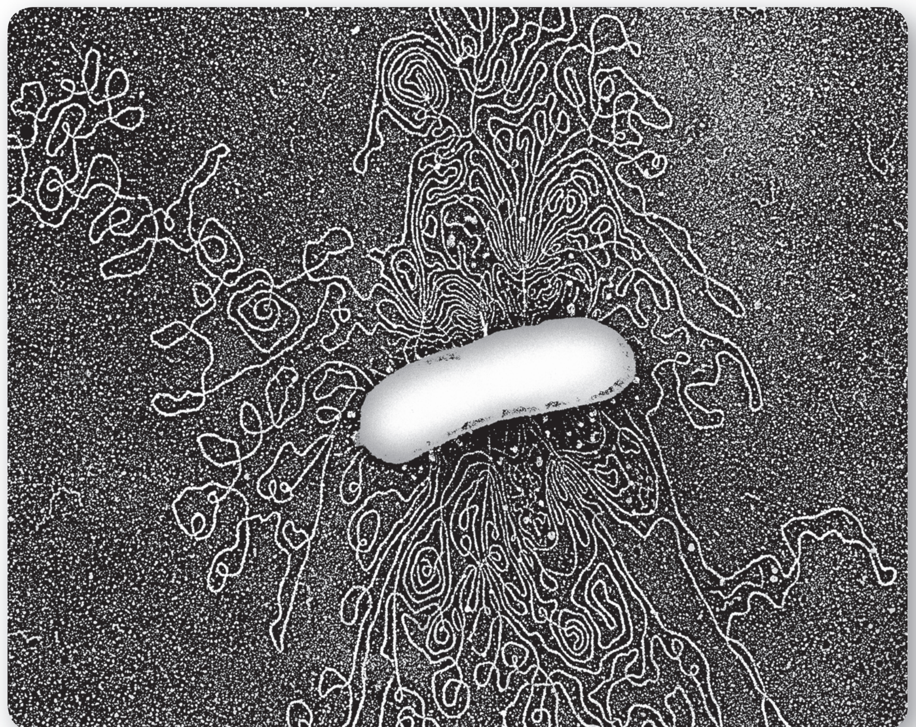
Microbial Biotechnology

After completing this chapter you should be able to:

- Provide examples of how prokaryotic and eukaryotic cell structures differ.
- Describe features of bacteria that make them useful for applications in biotechnology.
- Provide examples of how yeast are used in biotechnology.
- List examples of medically important proteins that are produced in bacteria using recombinant DNA technology.
- Explain how alcohol and lactic acid fermentation processes can be used to produce common foods and beverages.
- Describe the role microorganisms play in the development and production of vaccines; provide examples of different vaccines.
- Discuss why studying microbial genomes is valuable.
- Understand why scientists are interested in using synthetic genomes and synthetic biology for biotechnology applications.
- Explain the value of metagenomics; describe the goals of the Human Microbiome Project.
- Define bioterrorism, identify microorganisms that may be used as bioweapons, and discuss strategies to combat them.



Microbes, such as the *Escherichia coli* (*E. coli*) cell shown in the center of this photo, have a long history of applications in biotechnology. The cell wall and plasma membrane of this cell have been burst open to reveal the bacterial chromosome.

Dr. Gopal Murti/Photo Researchers, Inc.

Just when Italian art restorers were at a loss to save one of the world's largest collections of fourteenth- and fifteenth-century medieval frescoes (paintings done on plaster) decorating a cemetery in Pisa near the Leaning Tower, soil bacteria called *Pseudomonas stutzeri* came to the rescue. Restorers were trying to clean the frescoes of paint-clouding grime contained within glue that had been used to repair them after damage in World War II. Traditional chemical reagents only further damaged the paintings. Knowing that *P. stutzeri* could degrade nitrogens, plastic resins, and pollutants such as tetrachloroethylene, scientists cultured a strain of these microbes in the lab and applied them to the frescoes. Within 6 to 12 hours, the bacteria had cleaned approximately 80% of the glue residue, and the remaining residue was removed with a light washing. The next proposed project for these **microorganisms** is removing black crust from limestone and marble monuments in Greece.

Microorganisms, or **microbes,** are tiny organisms too small to be seen individually by the naked eye; they must be viewed with the help of a microscope. Although the most abundant microorganisms are bacteria, microbes also include viruses; fungi such as yeast and mold; algae; and single-celled organisms called protozoa. Bacteria have existed on the earth for over 3.5 billion years, and they greatly outnumber humans. It has been estimated that microbes comprise over 50% of the earth's living matter. Yet less than 1% of all bacteria have been identified, cultured, and studied in the laboratory. We are literally surrounded by bacteria. They live on our skin, in our mouths, and in our intestines; they are in the air and on virtually every surface we touch. Bacteria have also adapted to live in some of the harshest environments on the planet: polar ice caps, deserts, boiling hot springs, and under extraordinarily high pressure in deep-sea vents miles under the ocean's surface.

The rich abundance of bacteria and other microbes provides a wealth of potential biotechnology applications. Well before the development of gene-cloning techniques, humans used microbes in biotechnology. In this chapter, we primarily discuss the important roles bacteria have played in both old and new practices of biotechnology. We conclude by discussing the dangers of microbes as agents of bioterrorism.

FORECASTING THE FUTURE

Speculating on the future directions of microbial biotechnology is not particularly easy because there are so many active areas of research with great potential. Two areas of great intrigue are synthetic genomes and biofuels. As you will learn later in the chapter, a synthetic genome has been assembled for a bacterial strain and transplanted into another strain. What will be some of the future applications of this technology and of the field of synthetic biology? Will it eventually be possible for a synthetic eukaryotic genome to be produced? For biofuels, there is a great need to reduce our global dependence on fossil fuels and generate alternative energy sources. Can microbes be used in metabolic processes to create fuels or to break down materials to release components that can become fuels in a way that is effective and cost-efficient for widespread use? Microbial biotechnology is a dynamic field that is producing many new applications. We could forecast several other topics as hot areas of microbial biotechnology in the future, but we think it will be particularly interesting to keep an eye on developments in biofuels and synthetic biology.

1 The Structure of Microbes

Before you can consider the many applications of microbial biotechnology, you must be able to distinguish microorganisms from plant and animal cells. Recall that cells can be broadly classified based on the presence (eukaryotes) or absence (prokaryotes) of the nucleus that contains a cell's DNA. *Eukaryotic* cells include plant and animal cells; fungi such as yeast; algae; and single-celled organisms called protozoans, among which are amoebas like those you may have studied in high school biology. Unlike eukaryotic cells, *prokaryotic cells* lack most membrane-bound organelles, such as a nucleus. Prokaryotes include the **domains** (taxonomic categories above the kingdom level) **Bacteria** and **Archaea**—organisms that share properties of both eukaryotes and prokaryotes. The cellular structure of microorganisms is important in determining both where they will thrive and how they can be used in biotechnology. For example, Archaea that live in extreme environments such as very salty conditions are called halophiles or hot environments are called thermophiles; as a result, they have very unique metabolic properties. Moreover, structural features of bacteria, in particular, make them excellent microorganisms to use for biotechnology research.

Bacterial cells are much smaller (1–5 micrometers, or μm; 1 μm = 0.001 millimeter) than eukaryotic cells (10–100 μm) and have a much simpler structure. Bacterial cells also exhibit the following structural features:

- DNA is not contained within a nucleus and typically consists of a single circular chromosome that lacks histone proteins.

- Bacteria may contain plasmid DNA.
- Bacteria lack membrane-bound organelles.
- The cell wall that surrounds the cell (plasma) membrane is structurally different from the plant cell wall. Composed of a complex polysaccharide and protein substance called **peptidoglycan,** the cell wall forms a rigid outer barrier that protects the cells and determines their shape. In Archaea, this structure does not contain peptidoglycans.
- Some bacteria contain an outer layer of carbohydrates, which form a structure called the *capsule.*

Most bacteria are classified by the **Gram stain,** a technique in which dyes are used to stain the cell wall of bacteria. Gram-positive bacteria stain purple and have simple cell wall structures rich in peptidoglycans, whereas gram-negative bacteria stain pink and have complex cell wall structures with less peptidoglycan. Bacteria do not form multicellular tissues like animal and plant cells, although some bacteria can associate with each other to form chains or filaments of many connected cells.

Bacteria vary in their size and shape. The most common shapes include spherical cells called **cocci** (singular, coccus), rod-shaped cells called **bacilli** (singular, bacillus), and corkscrew-shaped or spirillar bacteria (Figure 1). As you study microbes, you will learn that the names of bacteria frequently give you with a tip about the shapes of those cells. For instance, *Staphylococci* are spherical bacteria that live on the surface of our skin. These grow in bunches likes grapes, which reflects their naming from a Greek term referring to bunches of grapes.

The single circular chromosome that comprises the genome of most bacteria is relatively small. Bacterial chromosomes average in the range of 2 million to 4 million base pairs in size, compared with 200 million base pairs for a typical human chromosome. Some bacteria also contain plasmids in addition to their chromosomal DNA. Plasmid DNA often contains genes that are not essential for life—for example, genes for antibiotic resistance and genes encoding proteins that form connecting tubes called *pili,* which allow bacteria to exchange DNA between cells. But plasmid DNA is an essential tool for molecular biologists because it can be used to clone pieces of DNA in recombinant DNA experiments.

Bacteria grow and divide rapidly. Under ideal growth conditions, many bacterial cells divide every 20 minutes or so, whereas eukaryotic cells often grow for 24 hours or much longer before they divide. Therefore, under favorable growth conditions in the laboratory, a small population of bacteria can divide rapidly to produce millions of identical cells. Because bacteria are so small, millions of cells can easily be grown on small dishes of agar or in liquid culture media. When grown on culture plates, each bacterial cell typically divides to form circular colonies that contain thousands or millions of cells (Figure 2a). For many applications in biotechnology, bacteria are often grown in fermenters that can hold several hundred or thousand liters of liquid culture medium (Figure 2b).

It is also relatively easy to make mutant strains of bacteria that can be used for molecular and genetic studies. Mutants can be created by exposing bacteria to x-rays, ultraviolet light, and a variety of chemicals that mutate DNA (mutagens). Literally thousands of mutant strains are available. For these reasons and many more, bacteria are not only the favorite organisms of many microbiologists but also ideal model organisms for studies in molecular biology, genetics, biochemistry, and biotechnology.

Yeasts Are Important Microbes Too

Although the primary focus of this chapter is applications of bacteria in biotechnology, **yeasts** have served many important roles in biotechnology. In fact, archeologists have uncovered recipes on ancient Babylonian tablets from 4300 B.C. for brewing beer using yeast, which is one of the oldest documented applications of

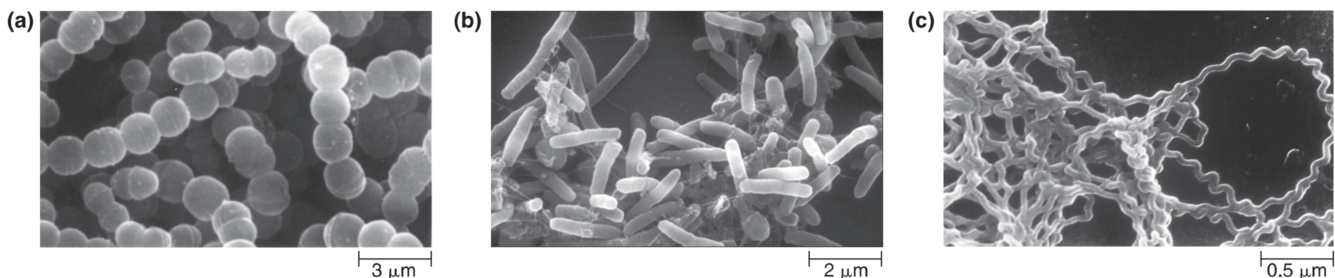


FIGURE 1 Shapes of Bacteria The most common shapes of bacteria are (a) spherical, (b) rod-shaped, and (c) corkscrew-shaped.

(a): S. H. Pincus and S. F. Hayer., **(b):** Manfred Kage/Photolibrary/Peter Arnold, Inc., **(c):** CNRI/Photo Researchers, Inc.

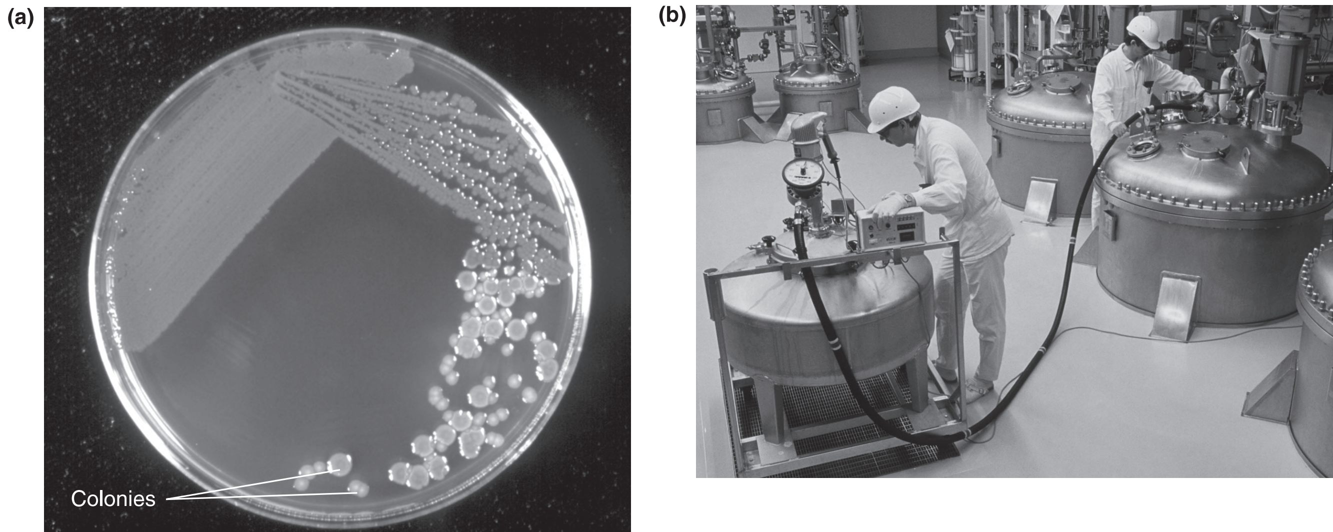


FIGURE 2 Bacteria in Culture (a) Most bacteria can be grown under a variety of conditions in liquid culture media or on a solid medium such as in the petri dish shown here. On solid media, many bacteria grow in circular clusters called colonies, which typically begin with a single cell that divides rapidly to produce a spot visible to the naked eye. A single colony may contain millions of individual bacterial cells. (b) Bacteria can also be grown in large quantities. The fermenters shown here contain several hundred liters of bacteria growing in liquid culture broth. These fermenters function as bioreactors that serve many purposes, such as growing bacteria for isolating recombinant proteins and culturing yeast to produce wine.
(a): John Durham/Photo Researchers, Inc., **(b):** Maximilian Stock Ltd/Photo Researchers, Inc.

biotechnology. Much more recently, yeast-producing recombinant human antibodies have been generated. Yeasts are single-celled eukaryotic microbes that belong to the **Kingdom Fungi.**

There are well over 1.5 million species of fungi, yet only around 10% of these have been identified and classified, so there is significant potential for identifying more valuable products from fungi. For instance, fungi are important sources of antibiotics and drugs that lower blood cholesterol. In addition to having many structures that are similar those of to other eukaryotic cells, such as plant and animal cells, yeasts also contain a number of membrane-enclosed organelles in the cytoplasm, a cytoskeleton, and chromosome structures similar to human chromosomes. Yeast cells also have larger genomes than most bacteria. These features make yeasts very valuable model organisms for studying chromosome structure, gene regulation, cell division, and cell-cycle control. Recall that yeast artificial chromosomes (YACs) played important roles in the Human Genome Project.

Different types of yeast vary greatly in size, but a majority are larger than bacteria and spherical, elliptical, or cylindrical in shape. Many can grow in the presence of oxygen **(aerobic conditions)** or in the absence of oxygen **(anaerobic conditions)** and under a variety of nutritional growth conditions. A wide number of different types of yeast mutants are also available. *Saccharomyces cerevisiae,* a commonly studied strain of yeast, was the first eukaryotic organism to have its complete genome sequenced. It has 16 linear chromosomes that contain over 12 million base pairs of DNA and approximately 6,300 genes. Mechanisms of gene expression in yeast resemble those in human cells. Several human disease genes have also been discovered in yeast and, by studying them, scientists can learn a great deal about how these genes function in human and our evolutionary relatedness to yeast.

A strain of yeast called *Pichia pastoris* has proven to be a particularly useful organism. *Pichia* grows to a higher density (biomass) in liquid culture than most laboratory strains of yeast, has a number of strong promoters that can be used for high production of proteins, and can be used in **batch processes** to produce large numbers of cells. In the next section we consider how microorganisms can be used by scientists as valuable tools for biotechnology research.

2 Microorganisms as Tools

Microorganisms in either their natural state or genetically modified forms have served as useful tools in a variety of fascinating ways.

Microbial Enzymes

Microbial enzymes have been used in applications from food production to molecular biology research. Because microbes are an excellent and convenient source of enzymes, some of the first commercially available enzymes isolated for use in molecular biology were DNA polymerases and restriction enzymes from bacteria. Initially isolated primarily from *E. coli,* DNA polymerases became available for a range of recombinant DNA techniques such as labeling DNA sequences to make probes and using the polymerase chain reaction (PCR) to amplify DNA.

Taq is a heat-stable enzyme essential for PCR that was isolated from the hot-spring Archaean *Thermus aquaticus*. Because of their ability to grow and thrive under extreme heat, these microbes are called **thermophiles** (from the Greek words meaning "heat-loving"). Many similar thermostable enzymes have been identified in other thermophiles. For example, Pfu DNA polymerase, a popular thermostable enzyme widely used for PCR, is derived from the thermophile *Pyrococcus furiosus,* a species of Archaea originally present in geothermally heated marine sediments. Several companies have permission from the U.S. government to prospect geysers in Yellowstone National Park to identify other potentially valuable microorganisms that might contain novel and valuable enzymes. Such so-called **bioprospecting** projects are occurring all around the world. Recently a strain of yet-to-be named bacteria was isolated from a hydrothermal vent on the floor of the northeast Pacific Ocean. This strain has been shown to survive at 121°C, which is believed to be the record so far for the upper temperature limit at which life can exist.

The enzyme **cellulase,** produced by *E. coli,* degrades cellulose, a polysaccharide that forms the plant cell wall. Cellulase is widely used to make animal food more easily digestible. Have you ever owned a pair of stone-washed denim jeans? These soft and faded jeans are not produced by a literal washing with stones. Instead, the denim is treated with a mixture of cellulases from fungi such as *Trichoderma reesei* and *Aspergillus niger*. These cellulases mildly digest cellulose fibers in the cotton used to make the pants, resulting in a softer fabric. The protease **subtilisin,** derived from *Bacillus subtilis,* is a valuable component of many laundry detergents, where it functions to degrade and remove protein stains from clothing. Several bacterial enzymes are also used to manufacture foods, such as carbohydrate-digesting enzymes called amylases that are used to degrade starches for making corn syrup.

Bacterial Transformation

Recall that *transformation*—the ability of bacteria to take in DNA from their surrounding environment—is an essential step in the recombinant DNA cloning process. In DNA cloning, recombinant plasmids can be introduced into bacterial cells through transformation so that the bacteria can replicate the recombinant plasmids. Most bacteria do not take up DNA easily unless they are treated to make them more receptive, so-called **competent cells.** One technique for preparing competent cells involves treating cells with an ice-cold solution of calcium chloride. Positively charged atoms (cations) in the calcium chloride disrupt the bacterial cell wall and membrane to create small holes through which DNA can enter. These cells can then be frozen at ultralow temperatures (–80°C to –60°C) to maintain their competent state and then be used in the laboratory as needed.

Once competent cells are prepared, they can be transformed with DNA relatively easily, as shown in **Figure 3a**. Typically, the DNA to be introduced into bacteria is inserted in a plasmid containing one or more antibiotic-resistance genes. The recombinant plasmid vector is mixed in a tube with the competent cells and the mixture placed on ice for a few minutes. The exact mechanism of transformation is not fully understood, but we do know that the cells must be kept cold, during which time DNA sticks to the outer surface of the bacteria, and the cold conditions probably also serve to create gaps in the lipid structure of the cell membrane that allow for the entry of DNA. Cations in the calcium chloride are thought to play a significant role in neutralizing the negative charges of phosphates in the cell membrane and the DNA that would otherwise cause them to repel each other. The cells are then heated briefly (for about a minute) at temperatures between 37°C and 42°C. During this brief heat "shock," DNA enters the bacterial cells.

After these cells have been allowed to grow in culture broth, they can be plated onto an agar medium containing antibiotics. Only those cells that were transformed with plasmid DNA containing the appropriate antibiotic-resistant genes will grow to produce colonies. This technique is called *antibiotic selection*. Plasmid DNA is replicated (cloned) by transformed

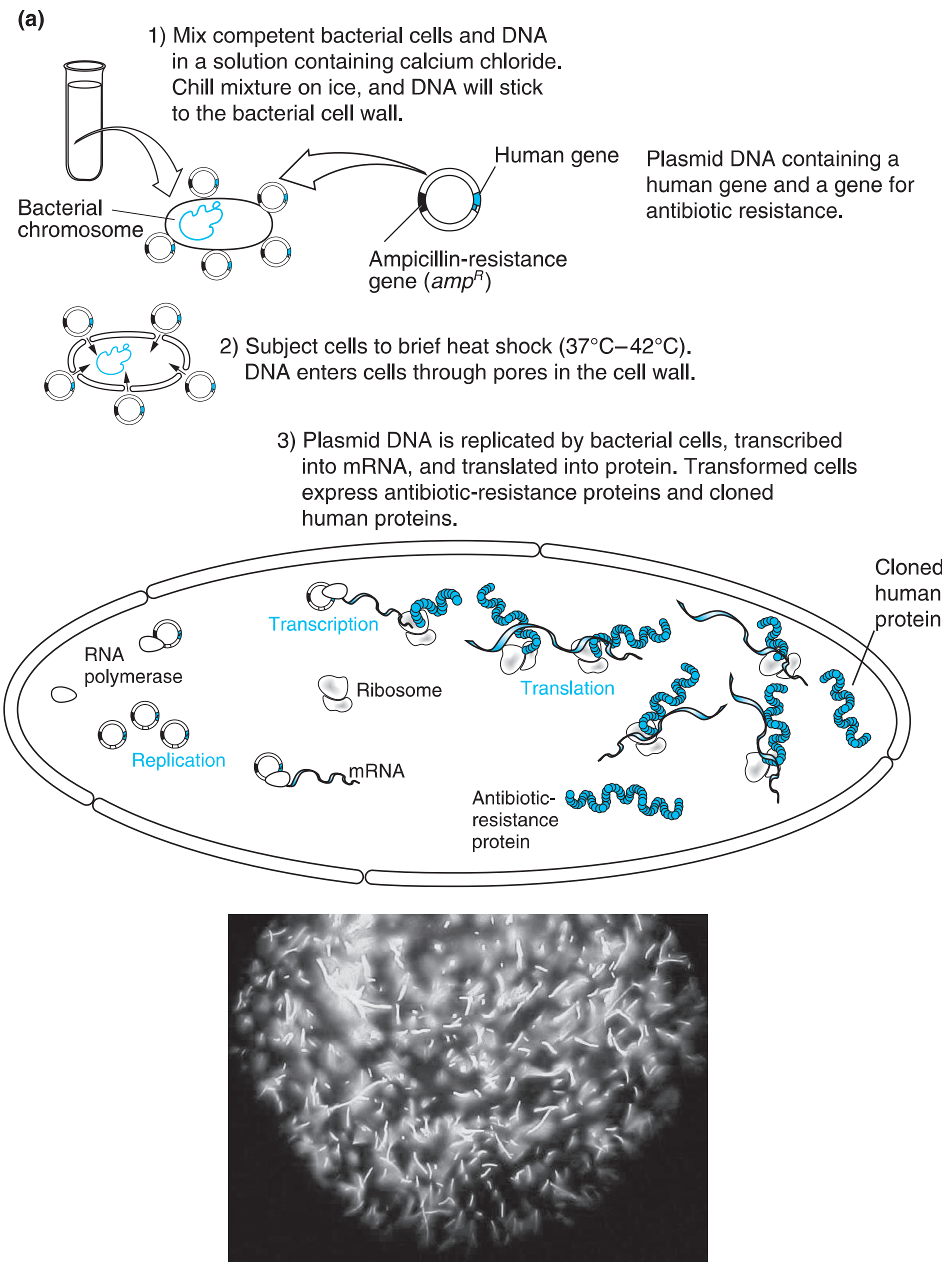


FIGURE 3 Transformation of Bacterial Cells (a) In a laboratory environment, most bacterial cells can be induced to take up foreign DNA by calcium chloride treatment. (b) *E. coli* transformed with a plasmid containing the jellyfish gene for green fluorescent protein (GFP). These bacterial cells glow bright green—a dramatic example of transformation—indicating that they have taken up plasmids containing the GFP gene and expressed GFP mRNA and protein.

Charlotte K. Mulvihill and Catherine Pongratz.

bacteria, and genes in the plasmid are transcribed and translated into protein. Thus, the transformed bacterial cells now express recombinant proteins.

This process is called transformation because one can “transform” the properties of bacterial cells by introducing foreign genes. Transformed cells have been genetically altered with new properties encoded by the DNA, enabling them to produce substances they would not normally produce. For example, *E. coli* can be transformed with a gene called green fluorescent protein (GFP), which comes from jellyfish (Figure 3b). As we will discuss in Section 3, bacterial cells have been transformed to express a large number of valuable genes including many human genes.

Electroporation

Another common technique for transforming cells is called **electroporation** (Figure 4). In this approach, an instrument called an electroporator produces a brief electrical shock that introduces DNA into bacterial cells without killing most of them. Electropora-

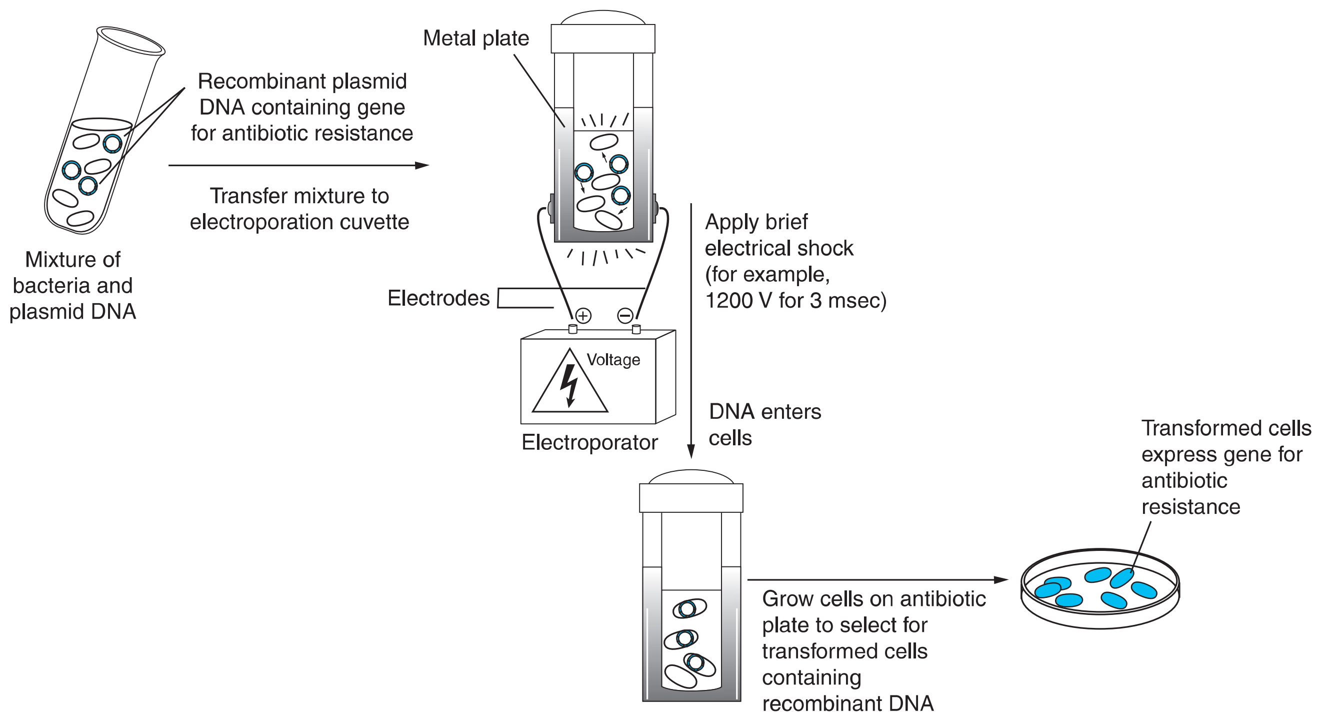


FIGURE 4 Electroporation Is a Rapid and Effective Technique for Transforming Bacteria In electroporation, a mixture of bacteria and plasmid DNA is placed into a cuvette. Upon the application of a brief electrical shock to the cuvette, DNA quickly enters cells. Cells containing recombinant DNA can then be selected for by growth on agar containing an antibiotic or another selection component.

tion offers several advantages over the calcium chloride treatment although competent cells are still required for electroporation. Electroporation is rapid, requires fewer cells, and can also be used to introduce DNA into many other cell types including yeast, fungi, animal, and plant cells. In addition, electroporation is a much more efficient process than calcium chloride transformation. A greater majority of cells will receive foreign DNA through electroporation than by calcium chloride treatment. Because of this, much less DNA can be used to transform cells (picogram amounts of DNA are sufficient). One drawback to this technique is that it is more costly than calcium chloride transformation because of the cost of an electroporator and competent cells that can tolerate electrical shock. Regardless of how bacterial cells are transformed, once the DNA of interest is introduced into bacteria, a variety of useful techniques can be carried out.

Cloning and Expression Techniques

In addition to replicating recombinant DNA, transformed bacteria are valuable because they can frequently be used to mass-produce proteins for a variety of purposes.

Creating bacterial fusion proteins to synthesize and isolate recombinant proteins

One popular technique for using bacteria for the synthesis and isolation of recombinant proteins involves making a **fusion protein.** There are a variety of ways to produce a fusion protein, but the basic concept of this technique is to use recombinant DNA methods to insert the gene for a protein of interest into a plasmid containing a gene for a well-known protein that serves as a “tag” (**Figure 5**). The tag protein then allows for the isolation and purification of the recombinant protein as a fusion protein. Plasmid vectors for making fusion proteins are often called **expression vectors** because they enable bacterial cells to produce or express large amounts of protein. Commonly used expression vectors include those that synthesize proteins such as maltose-binding protein (shown in Figure 5), glutathione S-transferase, luciferase, green fluorescent protein, and β-galactosidase.

Expression vectors incorporate prokaryotic promoter sequences so that, once the recombinant expression vector containing the gene of interest is introduced into bacteria by transformation, bacteria synthesize

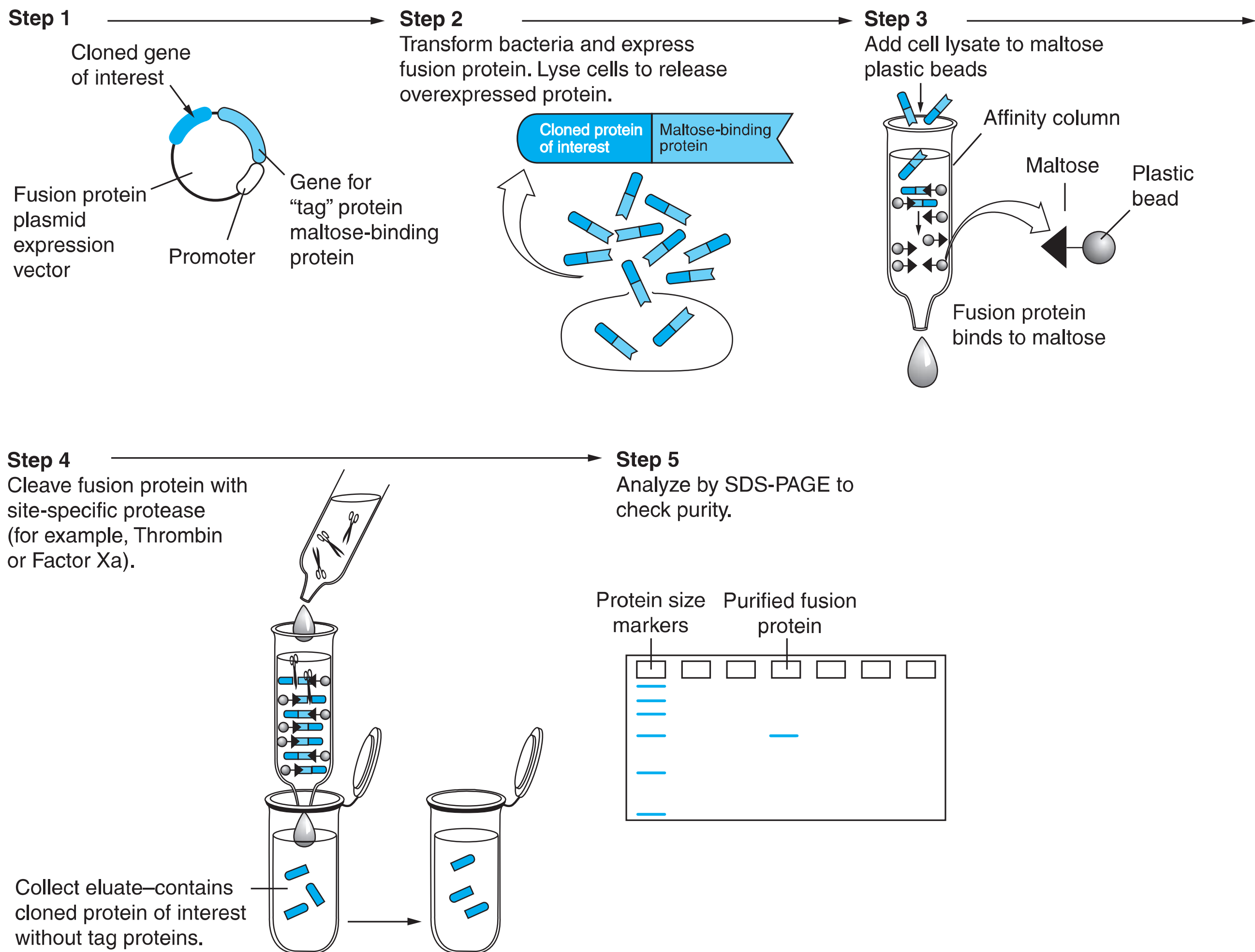


FIGURE 5 Fusion Proteins To make a fusion protein, a gene of interest is ligated into an expression vector. Bacterial cells are transformed with recombinant DNA. Transformed cells express the fusion protein, which is isolated by binding it to an affinity column.

mRNA and protein from this plasmid. The mRNA strands transcribed are hybrid molecules that contain sequence coding for the gene of interest and the tag protein. As a result, a fusion protein is synthesized from this mRNA consisting of the protein of interest joined to (fused) to the tag protein, in this case maltose-binding protein (Figure 5, step 2).

To isolate the fusion protein and separate it from other proteins normally made by the bacteria, cells are broken open (lysed) and homogenized to create a bacterial milkshake of sorts known as an *extract*. The extract is then passed through a tube called a *column*. One common approach is to fill a column with plastic beads coated with molecules that will bind to the tag protein portion of the fusion protein. This technique is called **affinity chromatography** because the beads in the column have an attraction, or "affinity," for binding to the tag protein. For example, in Figure 5, plastic beads are attached to the sugar maltose, which will be bound by maltose-binding protein. Next, an enzyme treatment that uses protein-cutting enzymes called *proteases* cuts off and releases the protein of interest from the tag protein. Some techniques for making fusion proteins incorporate short peptide tags of just a few amino acids. For example, poly-His tags are a short string of the amino acid histidine. One benefit of this approach is that, unlike maltose-binding protein and other tags that are large proteins, the small tags typically don't affect the structure and function of the protein they are fused to, so they usually don't need to be removed. Fusion protein techniques are used to provide purified proteins to study protein structure and function and used to isolate insulin and other medically important recombinant proteins (see Figure 8 later in the chapter).

E. coli and the Gram-negative rod-shaped bacterium *Bacillus subtilis* are commonly used microbes for producing fusion proteins. In particular, *B. subtilis* is a favored microbe for many applications when producing fusion proteins for human proteins because it will secrete them into growth media where they can easily be harvested and purified. And unlike some bacteria, *B. subtilis* often processes proteins in such a way as to maintain their three-dimensional folding and function.

Microbial proteins as reporters

According to recent estimates, close to three-fourths of all marine organisms can release light through a process known as **bioluminescence.** For marine fish, bioluminescence in lines of cells along the side of a fish and in its fins can be used to attract mates in dark ocean environments. Bioluminescence in many marine species is created by bacteria such as *Vibrio fisheri* that use the marine organism as a host (**Figure 6**). Bacteria such as *Vibrio* have been used as biosensors to detect cancer-causing chemicals called carcinogens, environmental pollutants, and chemical and bacterial contaminants in foods. *Vibrio fisheri* and another marine bioluminescent strain called *Vibrio harveyi* create light through the action of genes called *lux* genes. Several *lux* genes encode protein subunits that form an enzyme called **luciferase** (derived from the Latin *lux ferre*, meaning “light bearer”).

Luciferase is the same enzyme that allows fireflies to produce light. The *lux* genes have been cloned and used to study gene expression in a number of unique ways. For instance, by cloning *lux* genes into a plasmid, the *lux* plasmid can be used to produce fusion proteins. Also, *lux* genes can serve as valuable **reporter genes.** If inserted into animal or plant cells, the luciferase encoded by the *lux* plasmid cause these cells to fluoresce (Figure 6). In this manner, the *lux* plasmid is acting as a “reporter” to provide a visual indicator of gene expression.

Lux genes have been used to develop a fluorescent bioassay to test for tuberculosis (TB). TB is caused by the bacterium *Mycobacterium tuberculosis,* which grows slowly and can exist in a human for several years before TB develops (TB symptoms are discussed in Section 4). For the TB bioassay, scientists introduced *lux* genes into a virus that infects *M. tuberculosis.* Saliva from a patient who may be infected with *M. tuberculosis* is mixed together with the *lux*-containing virus. If *M. tuberculosis* is in the saliva sample, the virus infects these bacterial cells, which can be detected by their glowing. Similarly engineered strains of *E. coli* have been used to detect arsenic contamination in water. Reporter genes have many valuable roles in research and medicine. In the next section we explore a range of everyday applications that involve microbes.

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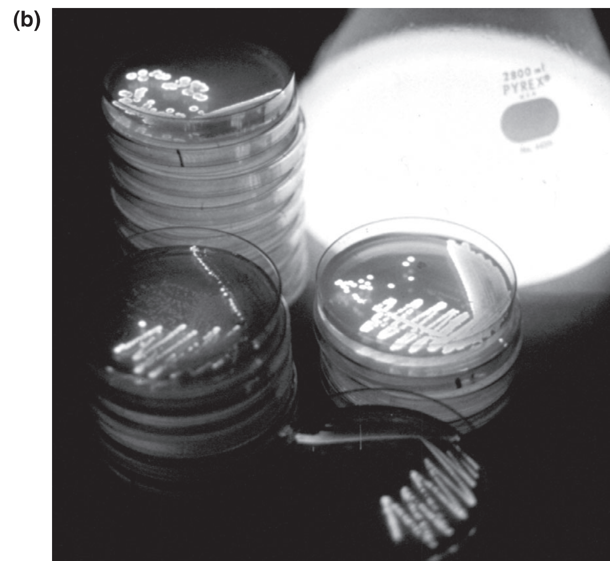


(c) The light-releasing chemical reaction catalyzed by luciferase

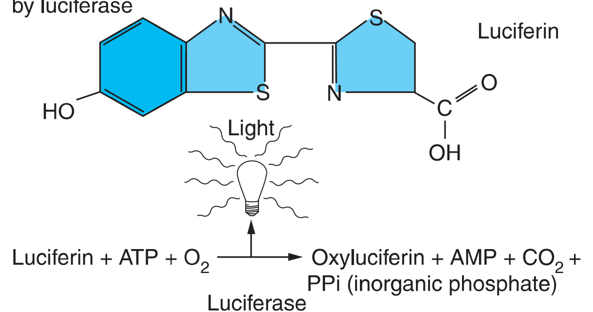


FIGURE 6 Bioluminescent Marine Bacteria Bioluminescent marine bacteria, such as *Vibrio fisheri* shown (a) glowing in the light-releasing organs of a deep-sea fish and (b) growing in the laboratory, generate light. (c) *Lux* genes encode the enzyme luciferase that uses oxygen and stored energy (ATP) to convert luciferin into oxyluciferin. This reaction releases light. *Lux* genes serve as reporter genes, allowing biologists to study gene expression. Expression is indicated by glowing cells.

(a): Jurgen Freund/bluegreenpictures, **(b):** Kenneth Lucus/Biological Photo Services.

3 Using Microbes for a Variety of Everyday Applications

Harnessing the great potential of microbes for making foods and for developing and producing new drugs are among the most common everyday applications of microbial biotechnology.

Food Products

Microbes are used to make many foods, including breads, yogurt, cheeses, and sauerkraut as well as beverages such as beer, wine, champagne, and liquors. As a child you probably learned the classic nursery rhyme "Little Miss Muffet." The tale of Miss Muffet, sitting on her tuffet, eating "curds and whey" might seem like an

TOOLS OF THE TRADE

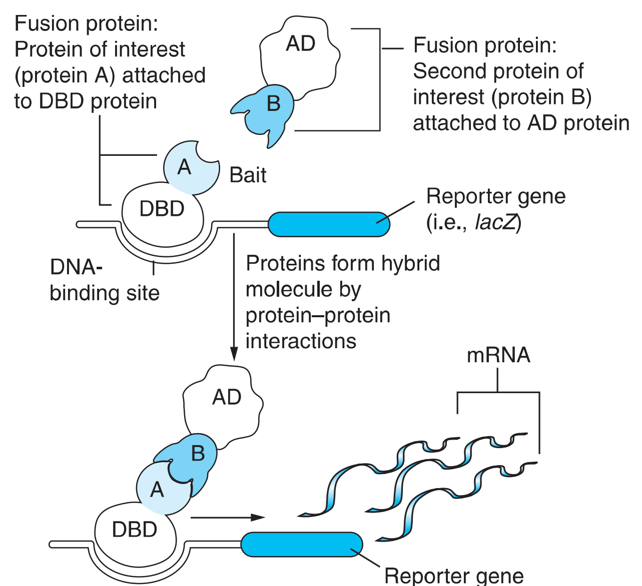
The Yeast Two-Hybrid System

The **yeast two-hybrid system** is an innovative technique for studying proteins that interact with each other; it provides a way of understanding protein function. Suppose you identified several proteins that you believed might interact and work together as part of a metabolic pathway for the synthesis of an important hormone in the body. The yeast two-hybrid system is an excellent technique to use to determine if these proteins do, in fact, bind to and interact with each other.

As shown in this figure, the gene for one protein of interest is cloned and expressed as a fusion protein attached to the DNA binding domain (DBD) of another gene. These DNA-binding sites are commonly found on proteins, such as transcription factors that interact with DNA. This protein is often called the "bait" protein because it is used to find other proteins called "prey" that may bind to it. The gene for the second protein of interest is fused to another gene that contains transcriptional activator domain (AD) sequences. AD proteins, also required for the attachment of DNA-binding proteins to DNA, stimulate the binding domain of a bait protein to interact with DNA-binding sites such as a promoter sequence for a reporter gene.

This modified DNA is introduced into yeast cells such as *Saccharomyces cerevisiae*. Neither the DBD fusion protein nor the AD fusion protein alone can stimulate transcription from this reporter gene. In the figure, the *lacZ* gene, which encodes the enzyme β-galactosidase, is a common reporter gene whose activity can easily be measured using simple colorimetric tests. Only a combination of both proteins, a "hybrid" or complex of both proteins, will stimulate expression of the reporter gene. Because scientists can create mutations of both proteins of interest and see how these mutations affect the ability of the two proteins to interact, we can learn a lot about the structure and function of those proteins.

The yeast two-hybrid system is a powerful example of how microbial biotechnology can be used for a research application.



The Yeast Two-Hybrid System for Studying Protein Interactions If a researcher wanted to know if two proteins (A and B) interacted with one another, one protein could be expressed as a fusion protein attached to the DNA binding domain (DBD) of another protein (such as a transcription factor) and the second protein attached to an activator domain (AD) protein. Neither fusion protein alone would be capable of binding to and stimulating transcription (mRNA production) of the reporter gene. But the resulting hybrid protein created by protein–protein interactions of the two fusion proteins would stimulate transcription of the reporter gene, which could easily be measured as an indicator of protein interactions between the A and B proteins.

improbable way to discuss biotechnology, but the treat in Miss Muffet's bowl was the result of biotechnology! Curds and whey are formed from coagulated milk, and milk coagulation is an important step during cheese production. To make cheese, milk from cows, goats, or sheep is treated to help it coagulate (*curd*). The watery liquid that remains after curd forms is called *whey.*

One way to make cheese from coagulated milk is to treat the milk with an acidic solution, but the best-tasting cheeses are typically made using the enzyme **rennin.** In the early days of cheese production, rennin was traditionally obtained by extracting it from the stomachs of calves and other milk-producing species such as goats, sheep, horses, and even zebras and camels. Rennin coagulates milk to produce curd by digesting a family of proteins called *casein,* which is a major component of milk. Digested casein forms an insoluble mixture of proteins that clumps (coagulates) in a process similar to what happens when milk spoils.

In the 1980s, using recombinant DNA techniques, scientists cloned rennin and expressed it in bacterial cells and fungi such as *Aspergillus niger*. Recombinant rennin (now called **chymosin**) from microbes is widely used by cheese makers as an inexpensive substitute for extracting rennin from calves. In 1990, rennin was the first recombinant DNA food ingredient approved by the Food and Drug Administration (FDA). For some types of cheese, certain strains of bacteria called lactic acid bacteria (*Lactococcus lactis, L. acidophilus*) are used for coagulation. These bacteria degrade casein and use an enzyme called *lactase* to break down sugars in the milk that are eventually used by bacteria for **fermentation.**

Fermenting microbes

Fermentation is an important microbial process that produces many food products and beverages including a variety of breads, beers, wines, champagnes, yogurts, and cheeses. One of the earliest applications of microorganisms—the brewing of beer and wine—involves fermentation by yeast (**Figure 7a**). To appreciate how making beer, wine, and bread requires microbes, you need to know more about the process of fermentation.

Animal and plant cells and many microbes obtain energy from carbohydrates such as glucose by using electrons from these sugars to create a molecule called **adenosine triphosphate (ATP).** ATP production occurs as a series of reactions. The first major reaction, **glycolysis,** converts glucose into two molecules of *pyruvate.* During this conversion, electrons are transferred from glucose to electron carrier molecules called *NAD*$^+$ (nicotinamide adenine dinucleotide), which capture electrons to produce NADH (Figure 7b). This molecule transports electrons to subsequent reactions in the process that results in ATP production. For certain

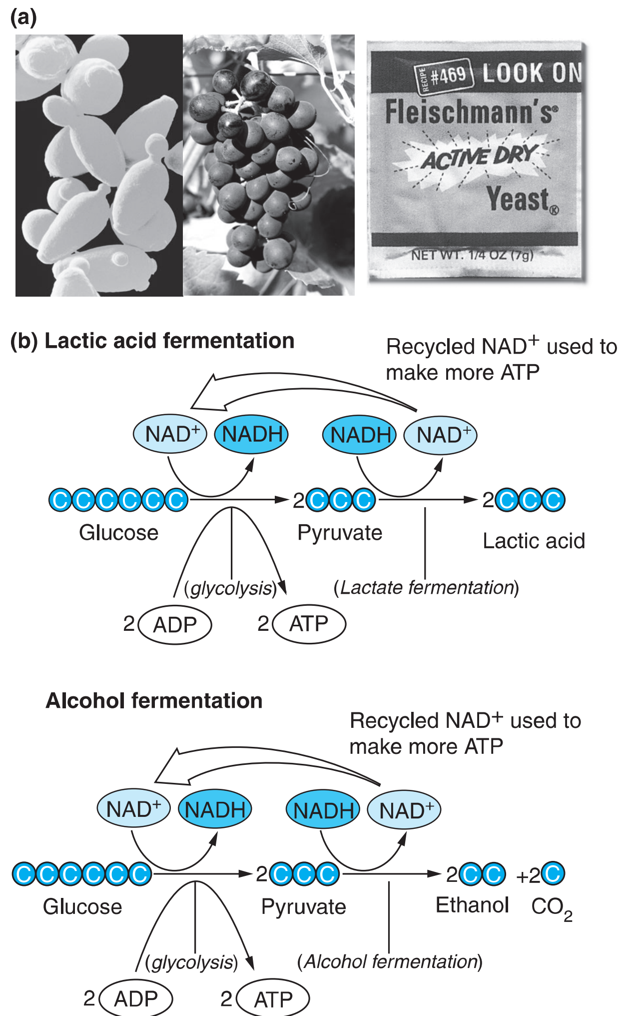


FIGURE 7 Fermentation Certain yeasts and bacteria are capable of producing energy from sugars (glucose) through fermentation. (a) Yeast such as *S. cerevisiae* (left), shown here, causes bread dough to rise; other strains of *Saccharomyces* grow on grapevines and are important for making wine (center photo). (b) Anaerobic bacteria that undergo lactic acid fermentation make lactic acid (lactate) as a waste product; alcohol-fermenting bacteria create ethanol and carbon dioxide (CO_2) as waste products.

(a): SciMAT/Photo Researchers, Inc., **(b):** Ian O'Leary/Dorling Kindersley.

bacteria and yeasts, oxygen is an important part of these electron transport reactions. Microbes that use oxygen for ATP production are called **aerobes** because they undergo oxygen-dependent (aerobic) metabolism.

Many microbes live in areas where oxygen is rare or absent, such as the intestines of animals, deep water, or soil. Because these organisms must survive without oxygen, they have evolved the ability to derive energy from sugars in the absence of oxygen (anaerobic conditions). This is fermentation, and microbes that use fermentation are called **anaerobes.** Fermentation is similar to glycolysis in that NAD$^+$ is used to capture electrons to make NADH and pyruvate; however,

neither the NADH nor the pyruvate has anywhere to go. In aerobic metabolism, oxygen is required to use electrons from NADH and pyruvate to make ATP, but under anaerobic conditions there is little or no oxygen, so the NADH and pyruvate cannot be used to make ATP. All organisms must recycle NADH into NAD^+. Fermentation enables many anaerobes to do this in the absence of oxygen, and some anaerobes are capable of either fermentation or aerobic respiration depending on the presence or absence of oxygen. In the absence of oxygen, anaerobes have evolved to acquire fermentation reactions as a way to solve the problem of recycling NADH into NAD^+. Fermenting microbes use pyruvate as an electron acceptor molecule to take electrons from NADH and thus to regenerate NAD^+ (Figure 7b). Two of the most common types of fermentation are **lactic acid fermentation** and **alcohol (ethanol) fermentation.**

In lactic acid fermentation, electrons from NADH are used to convert pyruvate into lactic acid, also called lactate; during alcohol fermentation, electrons from NADH convert pyruvate into ethanol. NAD^+ is regenerated when electrons are removed from NADH and transferred to pyruvate to make lactate or ethanol in the final step of fermentation. During alcohol fermentation, carbon dioxide gas is also produced as a waste product.

There are many strains of fermenting bacteria and yeast. Other types of fermentation create sauerkraut from cabbage and produce such useful products as acetic acid in vinegar, citric acid in fruit juices, and acetone and methanol, two chemicals often used in laboratories for cleaning glassware. Furthermore, these microbial products have the advantage of being produced more efficiently and cheaply than by other means. Lactic acid fermentation also occurs in human muscle cells during strenuous exercise. The burning you feel in your legs when you run because you are late to class is created by fermentation in muscle cells, creating lactic acid.

So how are fermenting microbes used to make foods and beverages? To make beer and wine, many processes rely on wild strains of yeast that live on grapevines and on such domestic strains of yeast as *Saccharomyces cerevisiae,* which are very good at alcohol fermentation. Large barrels, or fermenters, containing crushed grapes and yeast are mixed together under carefully controlled conditions. Fermenting yeast converts sugars from the grapes into alcohol. Fermentation rates are monitored and carefully controlled by changing both the amount of oxygen in the fermenter and the temperature.

By manipulating fermentation rates, wine makers can control the alcohol content of the brewing wine until the desired alcohol content and flavor are achieved. Bottles of champagne and other sparkling wines are capped while the yeast is still in the liquid and actively fermenting, thereby trapping carbon dioxide gas in the bottle and releasing it when the bottle is opened, producing the characteristic champagne bottle “pop.” Production of some wines relies also on lactic acid bacteria such as *Oenococcus oeni*, which will convert bitter-tasting malic acid that is formed during fermentation into softer-tasting lactic acid and thus give the wine a milder flavor and aroma.

Lactic acid–fermenting bacteria are used to produce cheeses, sour cream, and yogurts; the sharp or sour flavors of these products is primarily due to lactic acid. Yogurt was first created in Bulgaria and has been around for centuries. Yogurt production typically involves a blend of bacteria that often includes strains of anaerobic lactic acid–fermenting microbes such as *Streptococcus thermophilus,* a strain called *Lactobacillus* (*Lactobacillus delbrueckii* and *Lactobacillus bulgaricus*), and another strain called *Lactococcus* (*Lactococcus lactis*). Active cultures of these lactic acid–fermenting microbes are added to mixtures of milk and sugar in a fermenter, which is maintained at carefully controlled temperatures. Microbes in the mixture use sugars to produce lactic acid. Fruit and other flavorings may then be added to the yogurt before it is cooled to refrigeration temperature (4°C–5°C) to prevent changes in its composition. When you enjoy a spoonful of yogurt, you are also eating large numbers of fermenting microbes that are still in the yogurt. Lactic acid and other products of fermentation contribute to the sweet and sour taste of yogurt and assist in the coagulation of the yogurt.

Another lactic acid bacterium, *Lactobacillus sakei,* is found naturally on fresh meat and fish. *L. sakei* serves as a natural biopreservative in meat products such as sausage, where it wards off growth of other undesirable microbes that will spoil the food or cause illness. In addition to lactic acid, this microbe produces molecules called *bacteriocins,* which act as naturally occurring antimicrobial agents to kill other microbes.

Therapeutic Proteins

The development of recombinant DNA technology quickly led to using bacteria to produce such important medical products as therapeutic proteins. Insulin was the first recombinant molecule expressed in bacteria for use in humans. Here we use insulin production as an example of how microbes can be used to make therapeutic proteins.

Producing recombinant insulin in bacteria

Insulin is a hormone produced by cells in the pancreas called *beta cells.* When insulin is secreted into the

bloodstream by the pancreas, it plays an essential role in carbohydrate metabolism. One of its primary functions is to stimulate the uptake of glucose into body cells such as muscle cells, where the glucose can be broken down to produce ATP as an energy source. **Type I,** or **insulin-dependent, diabetes mellitus** occurs when beta cells do not produce insulin. The lack of insulin results in an elevated blood glucose concentration, which can cause a number of health problems such as high blood pressure, poor circulation, cataracts, and nerve damage. People with type I diabetes require regular injections of insulin to control their blood sugar levels.

Prior to recombinant DNA technology, insulin used to treat diabetes was purified from the pancreases of pigs and cows before being injected into diabetics. The purification process was cumbersome, expensive, and often produced impure batches of insulin. Also, purified insulin was ineffective in some individuals, and many others developed allergies to insulin from cows. In 1978, insulin was cloned into an expression vector plasmid, expressed in bacterial cells, and isolated by scientists at Genentech, a biotechnology company near San Francisco, California. In 1982, this recombinant human insulin, called Humulin, was the first biotechnology product to be approved for human applications by the U.S. Food and Drug Administration (FDA).

Bacteria do not normally make insulin, and producing human insulin in recombinant bacteria was a significant advance in biotechnology. It remains an outstanding example of microbial biotechnology in action. Human insulin consists of two polypeptides called the A (21 amino acids) and B (30 amino acids) chains or subunits; they bind to each other by disulfide bonds to create the active hormone (**Figure 8**). In the pancreas, beta cells synthesize both insulin chains as one polypeptide that is secreted and then enzymatically cut (cleaved) and folded to join the two subunits. When the human genes for insulin were cloned and expressed in bacteria, genes for each of the subunits were cloned into separate expression vector plasmids containing the *lacZ* gene encoding the enzyme β-galactosidase (β-gal) and then used to transform bacteria (Figure 8).

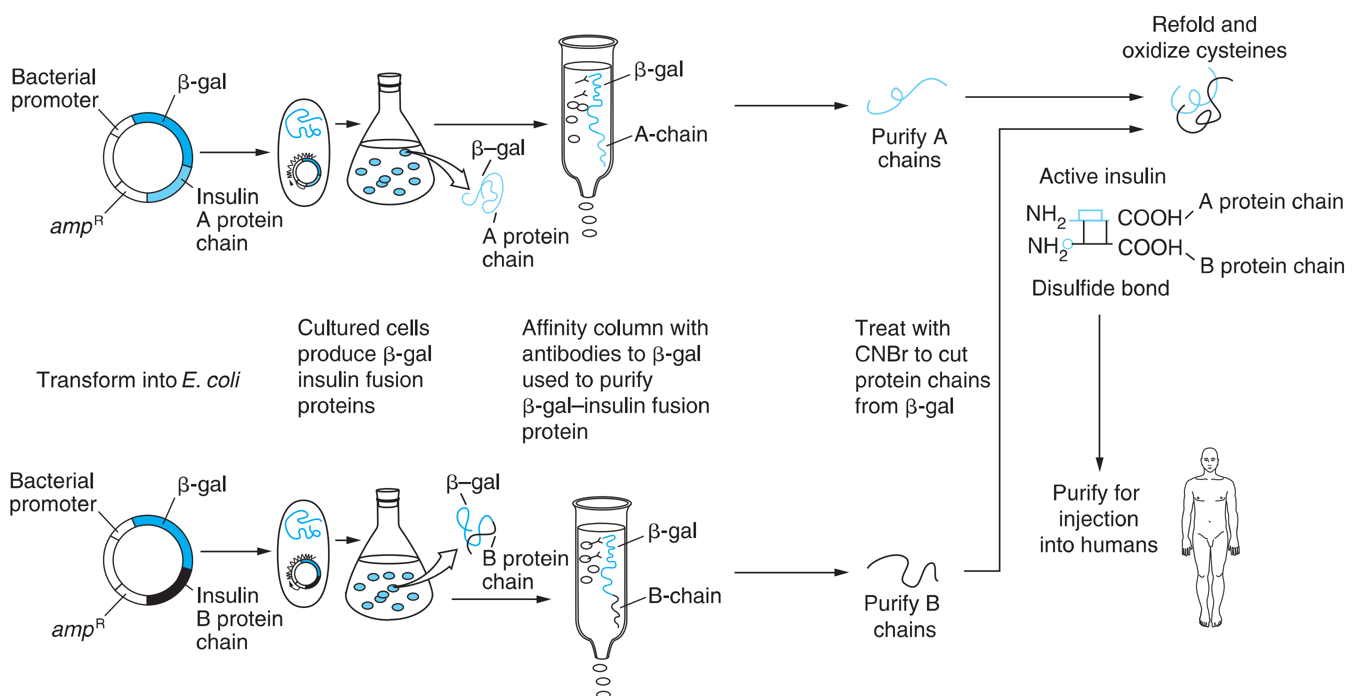


FIGURE 8 Using Bacteria for the Production of Human Insulin Insulin was the first protein expressed in recombinant bacteria to be approved for use in humans. Insulin consists of two protein chains (A and B) produced from separate genes. To make recombinant insulin, scientists cloned the insulin genes into plasmids containing the *lacZ* gene, which encodes the enzyme β-galactosidase. Recombinant plasmids were used to transform bacteria, enabling them to produce β-gal–insulin fusion proteins. Affinity chromatography was used to isolate fusion proteins, which were then chemically treated to separate the cloned insulin from β-gal proteins. Purified forms of the A and B protein chains of insulin could then combine to form active insulin, which is given to people with diabetes to control blood sugar levels.

Because the insulin genes are connected to the *lacZ* gene, when bacteria synthesize proteins from these plasmids, they produce a protein that contains β-gal attached to the human insulin protein to create a β-gal–insulin fusion protein. As we saw in Section 2, making a fusion protein enables scientists to isolate and purify a protein of interest such as insulin. Bacterial extracts are passed over an affinity column to isolate the β-gal–insulin fusion proteins (Figure 8). The fusion protein is chemically treated to cleave off the β-gal, releasing the insulin protein; then, purified A and B chains of insulin are mixed together under conditions that allow the two subunits to bind and form the active hormone. After further purification to conform to FDA guidelines, the recombinant hormone is ready for patient use as an injectable drug.

Shortly after insulin became available, growth hormone—used to treat children who suffer from a form of dwarfism—was cloned in bacteria and became available for human use. A short time later, a wide variety of other medically important proteins that were once difficult to obtain became readily available as a result of recombinant DNA technology and expressing proteins in bacteria. As shown in Table 1, many other therapeutic proteins with valuable applications for treating medical illness in humans have been expressed in and isolated from bacteria. A major category of medical products from recombinant bacteria are vaccines. We cover vaccines in Section 4.

Using Microbes against Other Microbes

Antibiotics are substances produced by microbes that inhibit the growth of other microbes. Antibiotics are a type of **antimicrobial drug,** a general category defined as any drug (whether produced by microbes or not) that inhibits microorganisms. Penicillin was the first antibiotic to be used widely in humans, and its discovery is an excellent example of how some microbes protect themselves from others by making

TABLE 1 THERAPEUTIC PROTEINS FROM RECOMBINANT BACTERIA

Protein	Function	Medical Application(s)
DNase	DNA-digesting enzyme	Treatment of patients with cystic fibrosis.
Erythropoietin	Stimulates production of red blood cells	Used to treat patients with anemia (low number of red blood cells).
Factor VIII	Blood clotting factor	Used to treat certain types of hemophilia (bleeding diseases due to deficiencies in blood clotting factors).
Granulocyte colony-stimulating factor	Stimulates growth of white blood cells	Used to increase production of certain types of white blood cells; stimulate blood cell production following bone marrow transplants.
Growth hormone (human, bovine, porcine	Hormone stimulates bone and muscle tissue growth	In humans, used to treat individuals with dwarfism. Improves weight gain in pigs and cows; stimulates milk production in cows.
Insulin	Hormone required for glucose uptake by body cells	Used to control blood sugar levels in patients with diabetes.
Interferons and interleukins	Growth factors that stimulate blood cell growth and production	Used to treat blood cell cancers such as leukemia; improve platelet counts; some used to treat different cancers.
Superoxide dismutase	An antioxidant that binds and destroys harmful free radicals	Minimizes tissue damage during and after a heart attack.
Tissue plasminogen activator (tPA)	Dissolves blood clots	Used to treat patients after heart attack and stroke.
Vaccines (e.g., hepatitis B vaccine)	Stimulate the immune system to prevent bacterial and viral infections	Used to immunize humans and animals against a variety of pathogens; also used in some cancer tumor treatments.

TABLE 2 COMMON ANTIBIOTICS

Antibiotic	Source Microbe	Common Uses of Antibiotic
Bacitracin	*Bacillus subtilis* (bacterium)	First-aid ointment and skin creams
Erythromycin	*Streptomyces erythraeus* (bacterium)	Broad uses to treat bacterial infections, especially in children
Neomycin	*Streptomyces fradiae* (bacterium)	Skin ointments and other topical creams
Penicillin	*Penicillium notatum* (fungus)	Injected or oral antibiotic used in humans and farm animals (cattle and poultry)
Streptomycin	*Streptomyces griseus* (bacterium)	Oral antibiotic used to treat many bacterial infections in children
Tetracycline	*Streptomyces aureofaciens* (bacterium)	Used to treat infections of the urinary tract in humans; commonly used in animal feed to reduce infections and stimulate weight gain

antimicrobial substances. Alexander Fleming was the microbiologist who, in 1928, discovered that colonies of the mold *Penicillium notatum* inhibited growth of the bacterium *Staphylococcus aureus*. When cultured together on a petri dish, *S. aureus* would not grow in a small zone of agar surrounding mold colonies. A dozen years later, scientists used *P. notatum* to isolate the drug they called penicillin, which was subsequently mass-produced and used to treat bacterial infections in humans.

A majority of antibiotics are isolated from bacteria, and most of these substances inhibit the growth of other bacteria. In the over 60 years since penicillin was discovered, thousands of other antibiotic-producing microbes have been discovered, and hundreds of different antibiotics have been isolated. Table 2 shows examples of common antibiotics and their source microbes.

How do antibiotics and other antimicrobial drugs affect bacterial cells? Most of these substances act in a few key ways. Typically they either prevent bacteria from replicating or kill microbes directly, which of course also prevents affected cells from replicating. Antibiotics can damage the cell wall or prevent its synthesis (which is how penicillin acts), block protein synthesis, inhibit DNA replication, or inhibit the synthesis or activity of an important enzyme required for bacterial cell metabolism (**Figure 9**).

You sneeze, your body aches, your nose is running, your throat is sore, and you can't sleep, but you still have an exam to take tomorrow afternoon. How will you do it? By going to your doctor and asking for antibiotics of course! But are antibiotics what you really need? Because antibiotics are effective only against

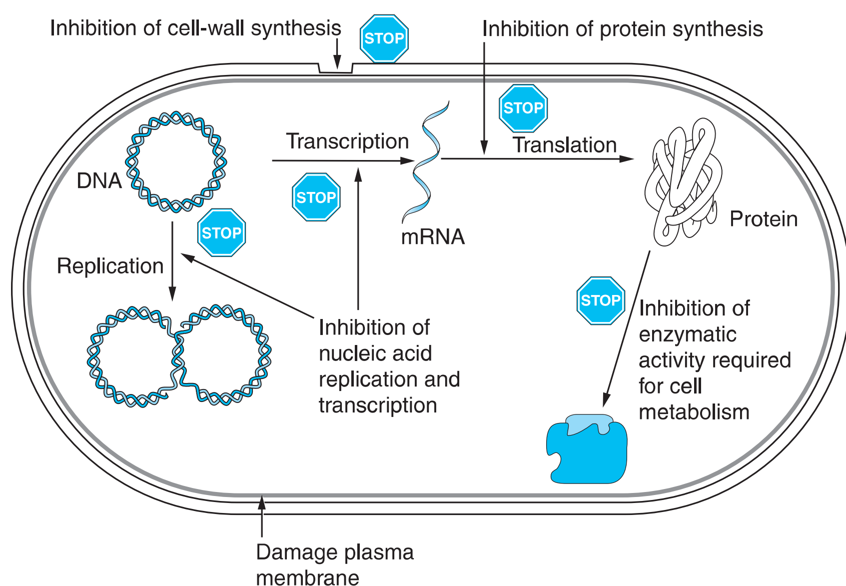


FIGURE 9 Antibiotics and Other Antimicrobial Drugs Work against Microbes in a Variety of Ways

bacteria; they do not work against viruses such as those that cause flu. Also, bacterial resistance to antibiotics has become a major problem. Improper use and overuse of antibiotics in humans and farm animals has led to dramatic increases in antibiotic-resistant bacteria, including some strains that do not respond at all to many antibiotics that were effective in the past.

Antibiotic-resistant strains of *S. aureus, Pseudomonas aeruginosa, Streptococcus pneumoniae, M. tuberculosis,* and many other deadly strains of human pathogens have already been detected in hospitals. Because most antibiotics attack a bacterial cell in a limited number of ways (Figure 9), resistance to one antibiotic often leads to resistance to many other drugs. Consequently, new antimicrobial drugs that are harmful to bacteria in different ways need to be developed for medical use as well as for treating food animals such as cows, pigs, and chickens. Marine microbiologists are discovering new strains of ocean microbes with novel antibiotics and anticancer compounds. From treetops to polar ice caps, deserts, and the ocean's depths, scientists are bioprospecting many microorganisms as potential sources of new antimicrobial substances.

Another way to create new antimicrobial drugs is to study bacterial pathogens and identify toxins and properties that disease-causing bacteria use to create illness. By understanding the factors involved in causing illness, scientists can develop new strategies to block bacterial replication. For instance, for certain bacteria, their ability to cause disease requires that they attach (adhere) to human tissues. Once attached, bacterial cells can multiply and then produce sufficient toxins to cause illness.

A Florida company called Oragenics received FDA approval to begin clinical trials of a recombinant form of *Streptococcus mutans* to evaluate its safety and efficacy for reducing tooth decay. Unlike naturally occurring strains of *S. mutans* found in the oral cavity, the recombinant strain cannot metabolize sugars to produce lactic acid. Because lactic acid dissolves enamel and dentin in teeth, it leads to cavities and tooth decay. Scientists will attempt to use recombinant *S. mutans* to colonize the oral cavity and replace natural strains of *S. mutans* and then determine if tooth decay is reduced. Recently, scientists at the University of California–Los Angeles (UCLA) created a sugar-free lollipop containing an ingredient in licorice that kills *S. mutans,* and these bacteria-killing lollipops are now available for purchase.

4 Vaccines

Antibiotics and vaccines have proven to be very effective for treating infectious disease conditions in humans and animals caused by **pathogens—**disease-causing microorganisms (**Figure 10**). However, pathogens with resistance to widely used antibiotics and vaccines have emerged and challenge the effectiveness of vaccines and antibiotics. Infectious diseases caused by microbes affect everyone, and worldwide over 60% of the causes of death among children before age 4 are due to infectious diseases. Without question our ability to prevent, detect, and treat infectious diseases is an important aspect of microbial biotechnology, and vaccines play a key role in this process.

The world's first vaccine was developed in 1796 when Edward Jenner demonstrated that a live cowpox virus could be used to vaccinate humans against smallpox. Smallpox and cowpox are closely related viruses. Smallpox epidemics ravaged areas of Europe, and an estimated 80% or more of Native Americans on the East Coast of the United States died from smallpox infections carried by European settlers in North America. Cowpox produces blisters and lesions on the udders of cows and produces similar skin ulcers in humans. Based on a milkmaid's claim that cowpox infections protected her from smallpox, Jenner prepared his vaccine. He took fluid from cowpox blisters on the milkmaid and used needles containing this fluid to scratch the skin of healthy volunteers. His first "patient" was an 8-year-old boy. A majority of Jenner's volunteers did not develop cowpox or smallpox even when subsequently exposed to persons infected

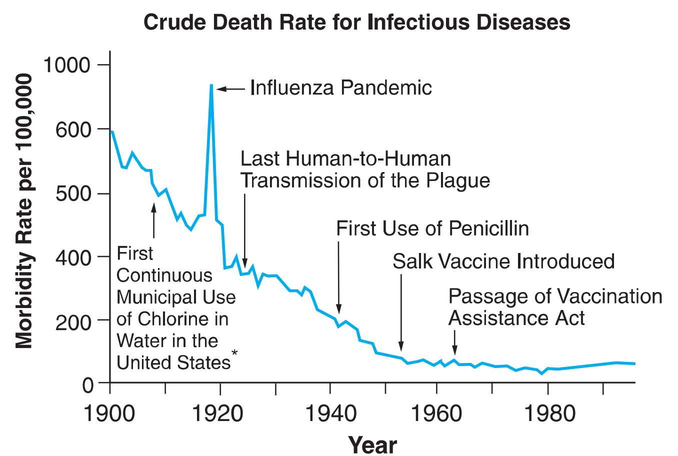


FIGURE 10 The Use of Antibiotics and Vaccines to Combat Infectious Diseases Caused by Microorganisms Even though the use of antibiotics and vaccines has decreased the incidence of human illness caused by microorganisms in the United States, strains of microbes that show resistance to many popular antibiotics and vaccines are emerging. New antibiotics and vaccines are required to fight these microbes.

*The American Society for Microbiology Report: Congressional Briefing. Infectious Disease Threats, 2001.

with smallpox. Exposure to cowpox fluid had stimulated the immune system of Jenner's volunteers to develop protection against smallpox.

These experiments demonstrated the potential of **vaccination** (named from the Latin word *vacca*, meaning "cow")—using infectious agents to provide immune protection against illness. Although the United States stopped routine vaccinations for smallpox in 1972, by 1980, subsequent widespread applications of the vaccine had eradicated this disease. In the United States many vaccines are routinely given to newborns, children, and adults. Although you may not remember your first vaccination (which usually occurs sometime from 2 to 15 months of age), you were probably vaccinated with the **DPT vaccine,** which provides several years of protection against three bacterial toxins called *d*iphtheria toxin, *p*ertussis toxin, and *t*etanus toxin. Diphtheria can cause breathing problems, paralysis, and heart failure. Pertussis causes whooping cough, which involves episodes of coughing paralysis so severe that it becomes hard for infants to eat, drink or breathe. Tetanus can cause lockjaw, preventing opening of the mouth.

Another childhood vaccine is the **MMR** (*m*easles-*m*umps-*r*ubella) vaccine. As you may know, measles results in a rash, fever, and cough; it can also cause a variety of other complications such as ear infections, seizures, and even death. Mumps causes fever, swollen glands, and headaches, but it can also lead to deafness. Rubella, or German measles, causes a fever, rash, and arthritis.

You were probably also vaccinated with **OPV** (*o*ral *p*olio *v*accine) for the poliovirus, a strain that infects neurons in the spinal cord, causing a devastating paralysis called poliomyelitis (polio). The OPV has dramatically decreased the incidence of polio. It has virtually been eliminated in North America, South America, and most of Europe; however, it still exists in some areas of the world. Polio, once a much more common disease, ravaged millions of children worldwide prior to 1954, when Jonas Salk developed the first vaccine for polio. Salk's original vaccine required injection; Albert Sabin developed the current version, which can be taken by mouth, in 1961. To understand how vaccines work, you must be familiar with the basic aspects of the human immune system.

A Primer on Antibodies

The immune system in humans and other animals is extremely complex. Numerous cells throughout the body work together in intricate ways to recognize foreign materials that have entered our body and mount an attack to neutralize or destroy those materials. Foreign substances that stimulate an immune response are called **antigens.** They may be whole bacteria, fungi, and viruses or individual molecules such as proteins or lipids found on pollen. For instance, people with food allergies have immune responses to proteins, carbohydrates, and lipids in certain foods.

The immune system typically responds to antigens in part by producing antibodies. This response is called **antibody-mediated immunity** (**Figure 11**). When

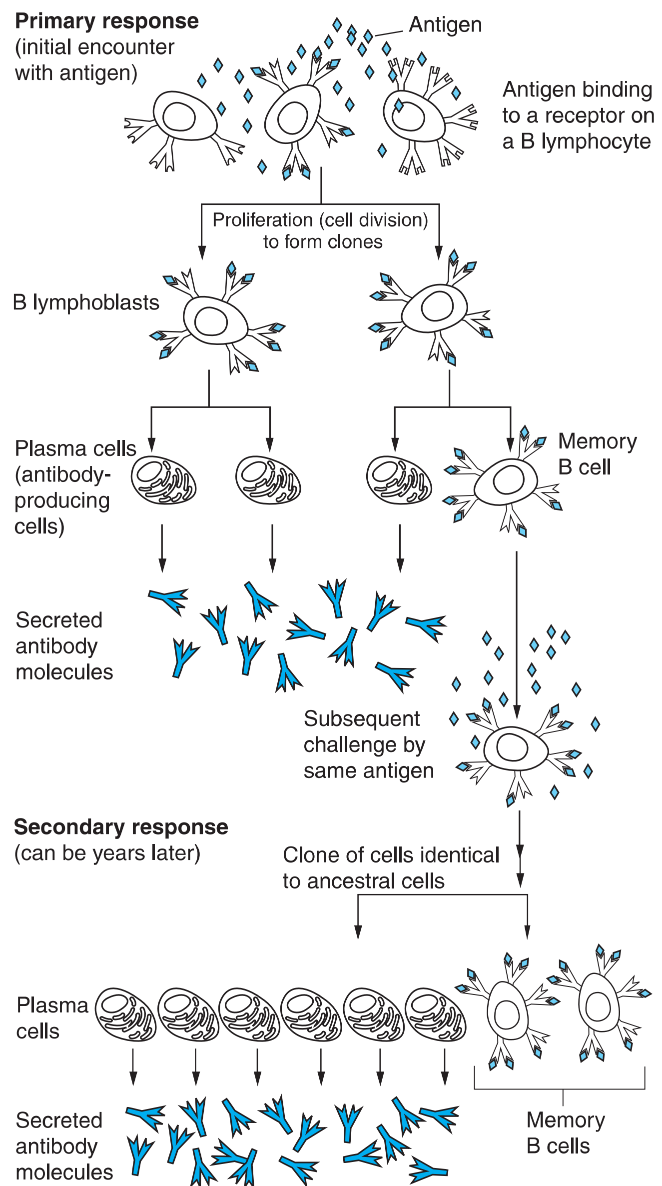


FIGURE 11 Antigens Stimulate Antibody Production by the Immune System In response to the initial antigen exposure, which comprise whole cells or individual molecules, B cells divide repeatedly to form many other B cells (clones). B cells differentiate into plasma cells, which produce antibodies specific to the antigen. During this process, memory B cells are formed. If a person is exposed to the same antigen again in the future, even many years later, memory B cells can recognize and produce a stronger and more rapid response to the antigen.

they are exposed to antigens, **B lymphocytes** (simply called **B cells**), which are a type of white blood cell or **leukocyte,** recognize and bind to antigen. **T lymphocytes (T cells)** play essential roles in helping B cells recognize and respond to antigen. After antigen exposure, B cells develop to form **plasma cells,** which produce and secrete antibodies. Most antibodies are released into the bloodstream, but there are also antibodies in saliva, tears, and the fluids lining the digestive system, among others.

Another purpose of antibody production is to provide lasting protection against antigens. During the process of B-cell development, some B cells become "memory" cells, which have the ability to recognize foreign materials years later and in response grow and produce more plasma cells and antibodies, which provide the body with long-term protection against antigens (Figure 11).

Antibodies are very specific for the antigen for which they were made, but how do these proteins protect the body against foreign materials? Many antibodies bind to and coat the antigen for which they were made (**Figure 12**). After antigens are covered with antibodies, a type of leukocyte called a **macrophage** can often recognize them. Macrophages are cells that are very effective at phagocytosis (which literally means "cell eating," derived from the Greek terms *phago,* "eating," and *cyto,* "cell"). In phagocytosis, macrophages engulf antigen covered with antibody; then, organelles in the macrophage called *lysosomes* unleash digestive enzymes that degrade the antigen (Figure 12). When the antigen is a foreign cell such as a bacterium, some antibodies are involved in mechanisms that rupture the cell through a process called *cell lysis.*

We are constantly being exposed to antigens, against which our immune system develops antibodies. But sometimes our natural production of antibodies is not sufficient to protect us from pathogens such as smallpox, viruses that cause hepatitis, and **human immunodeficiency virus (HIV),** the cause of **acquired immunodeficiency syndrome (AIDS).**

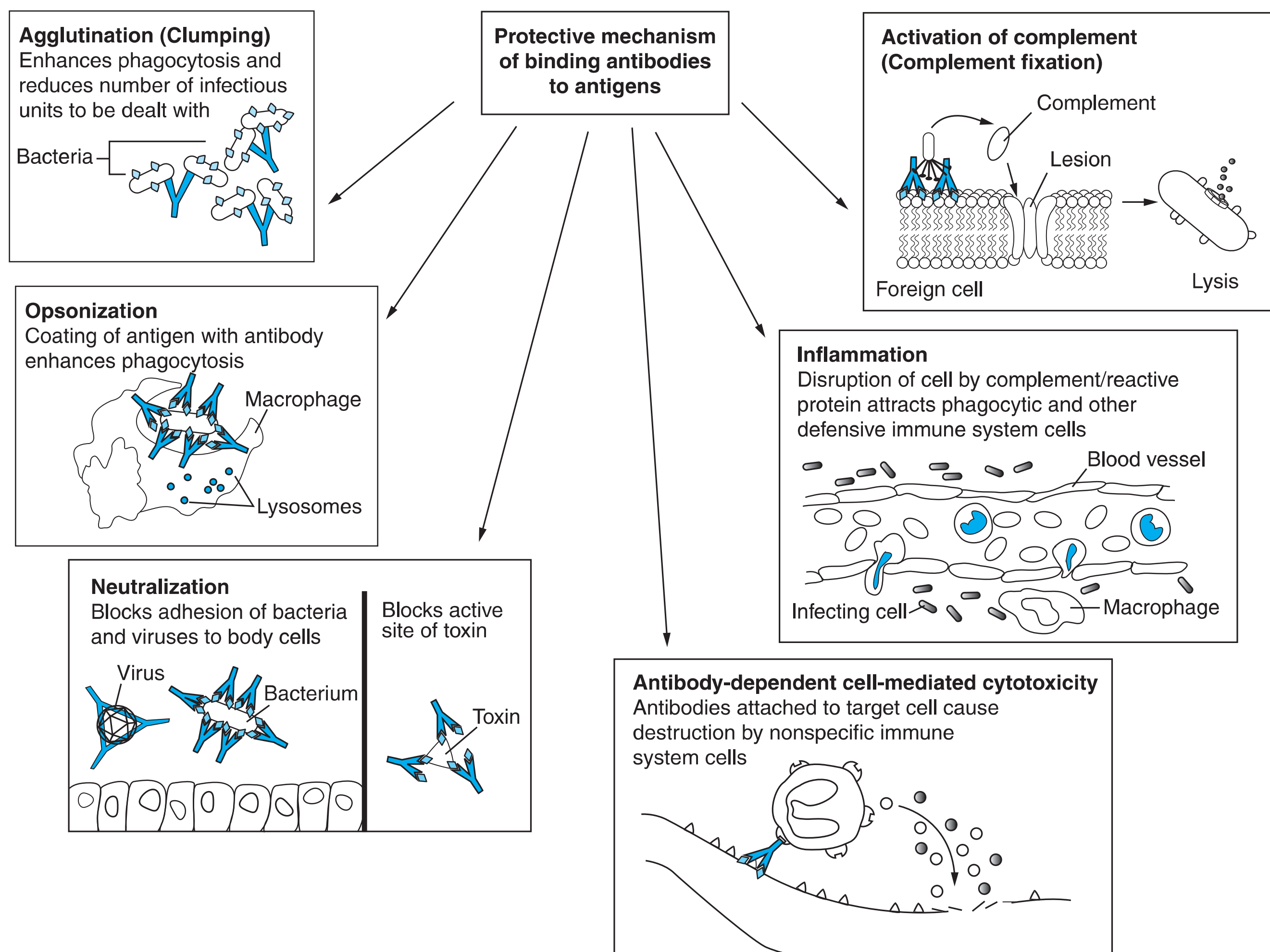


FIGURE 12 Mechanisms of Antibody Action Antibodies can inactivate and destroy antigens in a number of ways.

YOU DECIDE

Microbes on the Loose

One of the many controversies surrounding microbial biotechnology is the prospect that recombinant microbes can enter the environment, through accidental introduction or intentional release, as in the ice-minus studies described below. If recombinant microbes are loose in the environment, how can we know what will ultimately happen to these organisms? The first field application of genetically engineered bacteria was developed at the University of California by plant pathologist Steven Lindow and colleagues. They identified a common strain of bacteria called *Pseudomonas syringae,* which makes bacterial proteins that stimulate ice crystal formation. Lindow's group created ice-minus bacteria by removing the ice protein–producing genes from *P. syringae*. They proposed that releasing ice-minus bacteria onto plants would cause the ice-minus bacteria to crowd out normal, ice-forming *P. syringae* and provide frost-sensitive crop plants with protection from the cold, thus extending the growing season and increasing crop yields.

Surrounded by a great deal of controversy, Lindow received approval in 1987 to test *P. syringae* on a crop of potatoes. Around the same time, other scientists received permission to test ice-minus bacteria on strawberries in a small town in California. This was the first time that genetically altered microbes were ever intentionally released into the environment in the United States. In both experiments, a majority of plants were damaged by activists concerned about the release of genetically altered microbes. Ice-minus *P. syringae* have shown some promise for frost protection, but they have not been as effective at crowding out the growth of normal ice-forming *P. syringae* as Lindow and others had hoped.

Because gene transfer between bacteria is a natural process that occurs in the wild, scientists are concerned about horizontal gene transfer, the spread of genes to related microbes. As a result of genetic recombination and the creation of new genes, new strains of microbes with different characteristics based on the genes they inherit may be produced.

- What would happen if recombinant microbes could transfer genetically altered genes into other microorganisms for which they were not originally intended?
- For instance, what would happen if ice-minus bacterial genes were transferred to strains of bacteria that are accustomed to living under cold conditions?
- Once recombinant microbes escape or are released into the environment, we cannot simply call them back into the lab if we do not like what they are doing in the field.
- Can we prevent the escape of genetically altered microbes in field experiments? It is a difficult if not impossible task when wind, rain, and other weather elements are involved.

Should genetically engineered microbes be released even in "controlled" experiments that might result in beneficial applications of biotechnology? You decide.

Biotechnology can help our immune systems by boosting our immunity through the use of vaccines.

Types of Vaccines: How Are Vaccines Made?

Vaccines are parts of a pathogen or whole organisms that can be given to humans or animals by mouth or by injection to stimulate the immune system against infection by those pathogens. When people or animals are vaccinated, their immune systems recognize the vaccine as an antigen and respond by making antibodies and B memory cells. By stimulating the immune system, the vaccine has pressured it into stockpiling antibodies and immune memory cells that can go to work on exposure to the real pathogen in the future, should such exposure occur. Therefore many but not all vaccines are designed to be *preventative* or *prophylactic* (by providing protection against a pathogen should you be exposed) and not *therapeutic*—that is, a cure once you have become infected or developed a particular condition. Remember also that vaccination is used in pets, farm animals, zoo animals, and even wild animals.

So how are vaccines made? Four major strategies are generally used to create immune responses using vaccines.

1. **Subunit vaccines** are made by injecting portions of viral or bacterial structures, usually proteins or lipids from the microbe, to which the immune system responds. A fairly effective vaccine against hepatitis B virus was one of the first examples

of a subunit vaccine, and vaccines for tetanus, anthrax, and meningococcal disease (which you may have been vaccinated for, because this vaccine is required by many colleges and universities) are also subunit vaccines.

2. **Attenuated vaccines** involve using live bacteria or viruses that have been weakened through aging or by altering their growth conditions to prevent their replication after they are introduced into the recipient. The Sabin vaccine for polio is an attenuated vaccine. So are the MMR, tuberculosis, cholera, and chickenpox (varicella) vaccines as well as many others.
3. **Inactivated (killed) vaccines** are prepared by killing the pathogen and using the dead or inactive microorganism for the vaccine. A mixture of inactivated poliovirus is used in the Salk vaccine against polio. The rabies vaccines administered by injection to dogs, cats, and humans, the DPT vaccine, and the influenza (flu) vaccine, which has become common in recent years, are also examples of inactivated vaccines. Inactivated flu vaccine can also be delivered as a nasal spray.
4. **DNA-based vaccines** have been attempted but so far they have not proven to be widely effective. However, in 2005, the USDA approved the world's first licensed DNA vaccine, a vaccine against West Nile virus (WNV). Developed by Fort Dodge Laboratories of Fort Dodge, Iowa, this vaccine is designed to protect horses from WNV, a mosquito-borne virus. Equine infections of WNV are on the rise, and about a one-third of horses infected with WNV will die or will have to be euthanized. In 2007, the USDA approved the first therapeutic cancer vaccine in the United States for any species, human or animal, for canine melanoma. This vaccine consists of a plasmid containing a gene for a human enzyme (tyrosinase).

Immunity from vaccinations can fade with time, particularly for inactivated vaccines, which often do not produce a strong immune response. As a result, many vaccines require immunization *booster* shots every few years to restimulate the immune system so that it will continue to provide protective levels of antibodies and immune memory cells. For instance, the DPT vaccine is effective for about 10 years, as is the tetanus vaccine, and the flu vaccine that you may have received requires annual injections because new strains of influenza virus are developing each year.

Attenuated and inactivated vaccines were among the first vaccines developed. Some subunit vaccines against bacteria were made prior to recombinant DNA technology by growing bacterial pathogens in liquid culture. Many bacteria release proteins into the surrounding media, and these proteins can be purified and mixed with compounds that will help stimulate an immune response when injected into humans. But as scientists have learned more about the molecular structure of many pathogens, attempts at making recombinant subunit vaccines have become more popular.

For instance, **hepatitis B** is a blood-borne virus transmitted by exposure to body fluids, sexual intercourse, and contaminated blood transfusions. Hepatitis B causes deadly liver diseases. When vaccines for hepatitis B were first prepared, scientists isolated the virus from the blood of infected patients and then used biochemical techniques to purify viral proteins. These proteins were then injected into humans as a vaccine. The hepatitis B vaccine is recommended for international travelers, particularly people visiting Africa and Asia, and health care workers and others who may come in contact with hepatitis-infected persons or their body fluids. Currently a majority of subunit vaccines, including the vaccine for hepatitis B, are made using recombinant DNA approaches in which the vaccine is produced in microbes.

To produce the recombinant subunit vaccine for hepatitis B, scientists cloned genes for proteins on the outer surface of the virus into plasmids. Yeasts transformed with these plasmids are used to express large amounts of viral protein as fusion proteins, which are then purified and used to vaccinate people against hepatitis B infections. This approach is a common strategy for producing subunit vaccines, although sometimes fusion proteins are expressed in bacteria or in cultured mammalian cells. In, 2005, the pharmaceutical company Merck received FDA approval of a recombinant subunit vaccine (Gardasil) against cervical cancer and the first cancer vaccine to be approved by the FDA. Gardasil targets four specific strains of **human papillomavirus (HPV),** which cause about 70% of cervical cancers (HPV strains 16 and 18) and a large percentage of genital warts (caused by HPV strains 6 and 11). Cervical cancer affects 1 in 130 women, nearly half a million women worldwide, and approximately 70% of sexually active women will become infected with HPV during their lifetimes. In the United States, more than 10,000 women each year contract cervical cancer and around 4,000 die of the disease.

Given as a series of three booster shots, Gardasil is designed as a prophylactic vaccine, for use in girls and women aged 9 through 26, which means that it is taken to provide immune protection prior to exposure to HPV. Merck is also seeking FDA approval to expand Gardasil use to women aged 27 to 45 and potentially for use in teen boys. Merck recommends that Gardasil be given to female preteens prior to their becoming sexually active. Several states have pending legislation requiring that schoolchildren be vaccinated with Gardasil.

In Texas, for example, the governor signed an executive order making HPV vaccination compulsory for girls ages 11 to 12. Compulsory vaccination has become a very controversial issue (see problem 8 in the Questions & Activities at the end of this chapter) and Merck has since ceased its efforts to lobby for state laws requiring compulsory vaccination.

Ideally, the immune system can be most effective during the early stages of exposure to an infectious agent, when immune cells can attack the pathogens as soon as they enter the body. Disease-causing viruses use a number of complicated ways to infect cells, replicate, and cause disease. For instance, **human immunodeficiency virus-1 (HIV-1),** the causative agent of AIDS**,** infects human immune cells by binding to a cell and injecting its RNA genome (Figure 13). The enzyme reverse transcriptase copies the HIV genome into DNA. HIV and other viruses that transcribe their RNA genomes into DNA are called **retroviruses.** After the viral genome has been copied, it is transcribed to make RNA and translated to produce viral proteins that assemble to create more viral particles that are released from infected cells. We present this brief overview of viral replication because each stage essentially represents a potential target for antiviral drugs, including some vaccines.

Bacterial and Viral Targets for Vaccines

Pathogens are changing all the time, giving rise to both drug- and vaccine-resistant strains and new strains of disease-causing bacteria and viruses. More infectious microbes exist than there are vaccines. As a result, there are many research priorities for improving existing vaccines and producing new ones, and biotechnology companies have in excess of 50 targets for vaccine development. Several of the major vaccine targets include Dengue fever, Hepatitis C and E, sexually transmitted diseases such as herpes, gonorrhea and chlamydial infection, methicillin-resistant *Staphylococcus aureus* (MRSA), pneumococcal disease, rotavirus, and West Nile Virus. In addition, nonpathogenic human diseases such as Alzheimer's disease, multiple sclerosis, allergies, drug addiction, diabetes, and hypertension are targets for *therapeutic* vaccines. Here, we consider a few of the many targets for new vaccines.

The flu is caused by a large number of viruses that belong to the **influenza** family of viruses. Although most people experience flu symptoms that last for a few days and can be readily treated by over-the-counter medications, influenza kills some 500,000 to 1 million people worldwide each year, including about 34,000 deaths in the United States. Because flu viruses mutate so rapidly, no one-size-fits-all vaccine protects against all strains. New flu vaccines are generated each year based on the three main flu virus strains that are expected to be prevalent during the upcoming flu season. Viruses for this vaccine are grown in eggs.

The **World Health Organization (WHO)**—an international group that monitors infectious diseases and epidemics—has established centers in over 80 countries so that it can collect and screen samples of influenza for analysis and then develop vaccine treatment strategies. Infectious disease scientists are considering the development of a "global lab" to monitor strains of influenza viruses around the world, replicate these viruses, and then use recombinant DNA techniques to produce subunit vaccines in response to new viral strains detected. This strategy is a surveillance and rapid-response approach to keeping up with new pathogens and producing vaccines as needed. In the future, it will likely be implemented for many different disease-causing organisms.

Influenza A represents one of the greatest potential threats to human health through a *pandemic,* a global outbreak. Pandemic strains of influenza A have arisen in the past. In 1918, influenza virus killed at least 20 million people. Other pandemics occurred in 1957 and 1968, and epidemiologists predict that a future pandemic could be much worse than previous episodes. Of recent concern was the emerging strain of **avian flu (H5N1),** which received a lot of media attention because of its presence in chickens. Variations in influenza A are due to two glycoproteins called hemagglutinin (HA) and neuraminidase (NA), which project from the surface of the virus. Avian flu is an influenza A subtype called H5N1 because of the variation of these two proteins contained in this strain (abbreviated as H and N when used in strain names). In 2003, strain H5N1 caused a pandemic in chickens in Asia that resulted in the killing of over 200 million birds in an effort to halt the spread. Scientists were concerned that this strain, like other viruses, may mutate and make the jump into humans. Although a few isolated cases of human infection were seen, widespread infection did not occur; however, bird-to-pig transmission of the virus has occurred and mutation of the virus in pigs could produce a strain that would infect humans. Because of the devastation such a virus could cause, the WHO declared development of a vaccine to protect against H5N1 a high priority and vaccines were subsequently produced. In 2009, **swine flu (H1N1)** led to significant public health concerns, but vaccination against this virus has proven effective at controlling its spread in humans.

Tuberculosis (TB), caused by the bacterium *Mycobacterium tuberculosis,* is responsible for between 2 and 3 million deaths each year. Inhaled particles of *M. tuberculosis* can infect lung tissue, creating lumpy lesions called *tubercles.* The spread of TB has been effectively

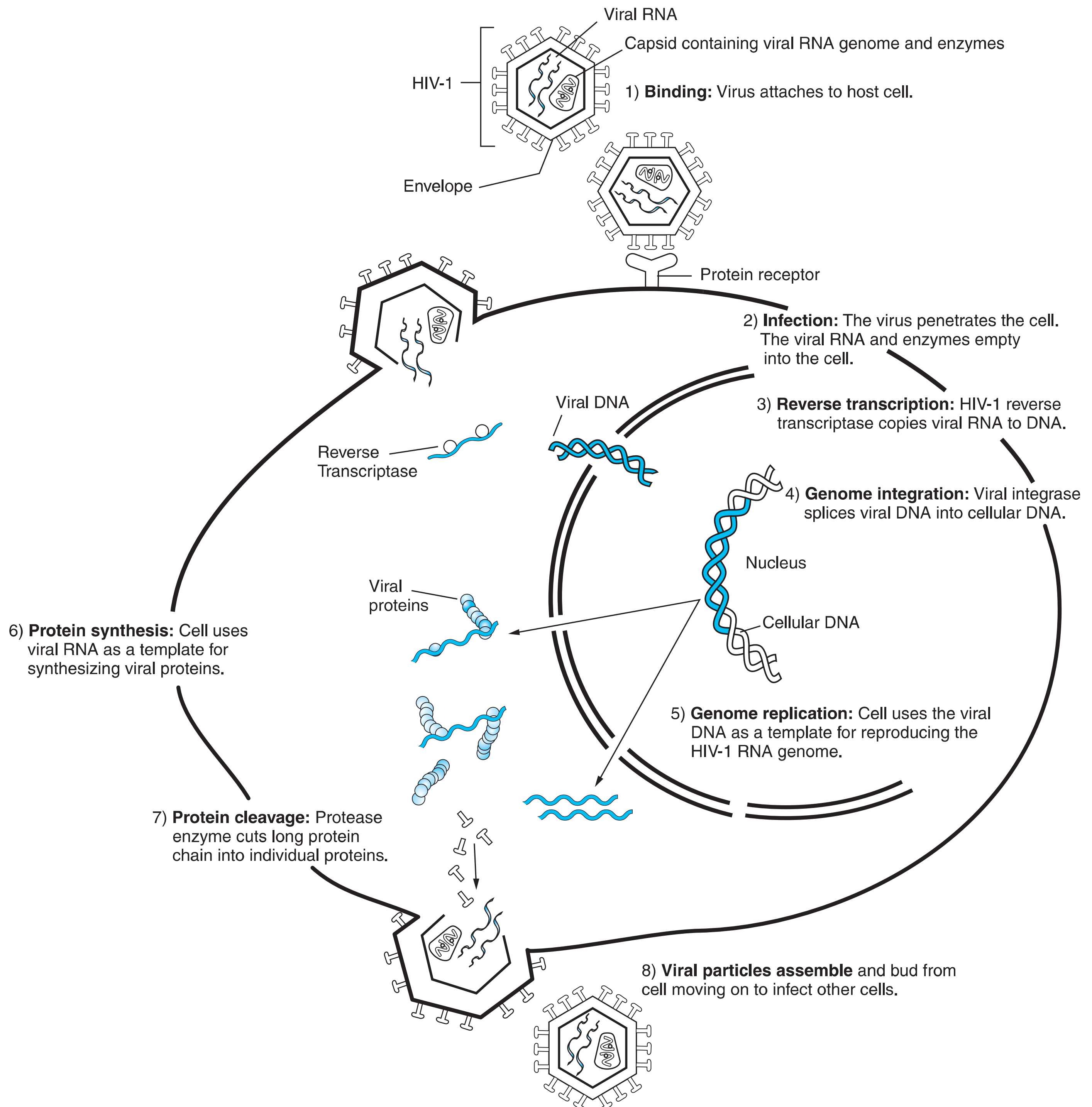


FIGURE 13 HIV-1 Life Cycle Each stage of HIV-1 replication is a potential target for antiviral drugs and some vaccines.

controlled in many areas of the world by the use of antibiotics and vaccines. But there has been a resurgence of TB because *M. tuberculosis* has proved to be very adept at evolving new strains that are resistant to treatment. Concern over such new strains is so great that in 1993, the WHO declared TB a global health emergency, and a number of research initiatives were launched. The Bill and Melinda Gates Foundation, together with other organizations, have provided over $30 million for these efforts. The genome for *M. tuberculosis* has been sequenced and, as a result, new proteins have been discovered, leading to the development

of new TB vaccines, many of which are currently in clinical trials.

Malaria is caused by the protozoan parasite *Plasmodium falciparum* and transmitted by insects. Worldwide, *Plasmodium* strains are developing resistance to the most commonly used antimalarial drugs. Although these drugs have been effective in parts of the world, the death rate from malaria is still unacceptable. Each year approximately half a billion cases of *Plasmodium* infections occur in children and cause nearly 3 million deaths. Whole-genome microarrays have been made for *Plasmodium* to help scientists identify new gene targets for inhibiting this parasite.

On another front, more than 33 million people are infected by **HIV** worldwide. The need for a vaccine to treat and stop the spread of HIV is critical if we are to curb this devastating disease and eventually eliminate the AIDS epidemic. Several vaccines for the prevention of HIV infections or the treatment of HIV-infected individuals have been tried in humans; most of these vaccines target viral surface proteins, but to date none of these have lived up to their promise. In 2007, a promising vaccine from Merck failed in phase II clinical trials.

One obstacle facing HIV vaccine scientists is the high mutation rate of HIV. Consequently, the multisubunit vaccines called *cocktails*—those that contain mixtures of many viral proteins, together with antiviral drugs that block viral replication—may be a more effective strategy to combat HIV than using either vaccines or antiviral drugs alone. Similar strategies are being developed for treating other viruses, such as hepatitis B and C.

In the next section, we will consider the many reasons for sequencing microbial genomes and the tools used to carry out this work.

5 Microbial Genomes

In 1995, the Institute for Genomic Research, which played a major role in the Human Genome Project, reported the first completed sequence of a microbial genome when they published the sequence for *Haemophilus influenzae.* Since then, over 1,000 microbial genomes have been published, and work is being carried out on the genomes for several hundred other microbes. In 1994, as an extension of the Human Genome Project, the U.S. Department of Energy initiated the **Microbial Genome Program (MGP).** A goal of the MGP is to sequence the entire genomes of microorganisms that have potential applications in environmental biology, research, industry, and health, such as bacteria that cause tuberculosis, gonorrhea, and cholera, as well as genomes of protozoan pathogens such as the organism (*Plasmodium*) that causes malaria.

Why Sequence Microbial Genomes?

Streptococcus pneumoniae, the bacterium that causes ear and lung infections, including pneumonia, kills approximately 3 million children worldwide each year. Infections of *S. pneumoniae,* which can also cause bacterial meningitis, have been effectively treated since 1946. But many of these vaccines are ineffective in young children, who are particularly susceptible to infection and serious health consequences. In 2001, the *S. pneumoniae* genome was completely sequenced, and many genes encoding previously undiscovered proteins on the surface of the bacterium were identified. Researchers are optimistic that this new understanding of the *S. pneumoniae* genome will lead to new treatments for pneumonia, including gene therapy approaches to rid children of infections that may persist for years.

This is just one example of the potential power of genomics at work. By sequencing microbial genomes, scientists will be able to identify many secrets of bacteria, from genes involved in bacterial cell metabolism and cell division to genes that cause human and animal illnesses. In addition, researchers will find bacterial genes that may enable scientists to develop new strains of microbes that can be used in bioremediation and to reduce atmospheric carbon dioxide and other greenhouse gases, to find disease-causing organisms in food and water, to detect biological weapons, to synthesize plastics, to make better food products, and to produce genetically altered bacteria as biosensors for detecting harmful substances, among many other examples.

Our ability to sequence microbial genomes is also expected to lead to new and rapid diagnostic methods and ways to treat infectious conditions. For instance, if scientists sequence genes encoding cell-surface proteins that coat a particular bacterial pathogen, they may be able to use these proteins to generate new diagnostic tools, vaccines, and antimicrobial agents.

Bacteria perform a wealth of biochemical activities, which is reflected in their genomes. Of the microbial genomes sequenced to date, approximately 45% of the genes identified produce proteins of unknown function, and approximately 25% of genes discovered produce proteins that are unique to the bacterial genome sequenced. Therefore the potential for identifying new genes and proteins with unique properties that may have important applications in biotechnology is very high.

Selected Genomes Sequenced to Date

Of the millions of different bacteria that have been identified, which ones are of greatest interest to microbial

TABLE 3 SELECTED MICROBIAL GENOMES

Bacterium	Human Disease Condition (megabases, mB)	Approximate Genome Size	Approximate Number of Genes
Bacillus anthracis	Anthrax	5.23	5,000
Borrelia burgdorferi	Lyme disease	1.44	853
Chlamydia trachomatis	Eye infections, genitourinary tract infections (e.g., pelvic inflammatory disease)	1.04	896
Escherichia coli O157:H7	Severe food-borne illness (diarrhea)	4.10	5,283
Haemophilus influenzae	Serious infections in children (eye, throat, and ear infections, meningitis)	1.83	1,746
Helicobacter pylori	Stomach (gastric) ulcers	1.66	1,590
Listeria monocytogenes	Listeriosis (serious food-borne illness)	2.94	2,853
Mycobacterium tuberculosis	Tuberculosis	4.41	3,974
Neisseria meningitidis (*MC58*) infections	Meningitis and blood	2.27	2,158
Pseudomonas aeruginosa	Pneumonia, chronic lung infections	6.30	5,570
Rickettsia prowazekii	Typhus	1.11	834
Rickettsia conorii	Mediterranean spotted fever	1.30	1,374
Streptococcus pneumoniae	Acute (short-term) respiratory infection	2.16	2,236
Yersinia pestis	Plague	4.65	4,012
Vibrio cholerae	Cholera (diarrheal disease)	4.00	3,885

Sources: Sawyer, T. K. (2001). Genes to Drugs. *Biotechniques* 30(1): 164–168. TIGR Microbial Database (www.tigr.org/tdb/mdb/mdbcomplete) and Gold: Genomes OnLine Database (wit.integratedgenomics.com/GOLD).

genome researchers? As shown in Table 3, the bacterial genomes that have received the most attention are those from microbes responsible for serious illnesses and diseases in humans. For example, recently the genome for *Pseudomonas aeruginosa* was completed. It is a major human pathogen causing urinary tract infections, a number of skin infections, and persistent lung infections that are a significant cause of death in cystic fibrosis patients. *P. aeruginosa* is a particularly problematic bacterium because it is resistant to many antibiotics and disinfectants commonly used to treat other microbes. Learning more about the genes involved in the metabolism, replication, and the breakdown of compounds (such as antibiotics) in *P. aeruginosa* will be greatly helped by an understanding of its genome.

Another one of the first microbes targeted for genome studies was *Vibrio cholerae,* which is typically found in polluted waters in areas of the world with poor sanitary practices. This bacterium causes the disease **cholera,** which is characterized by severe diarrhea and vomiting, leading to massive fluid loss, which can cause shock and even death. Strains of antibiotic-resistant *V. cholerae* are causing recurring problems in Asia, India, Latin America, and even areas of the Gulf Coast in the United States. Understanding the genome of *V. cholerae* will help scientists identify toxin genes, genes for antibiotic resistance, and other genes that will augment our current methods for combating this microbe. Genome biologists are also focusing on microorganisms that may be used as biological weapons in a terrorist attack. In Section 7, we discuss why and how microbes can be used as bioweapons and what can be learned by studying their genomes.

Scientists have been studying the genetics of lactic acid bacteria for about 35 years helping an effort to understand how these bacteria contribute to the flavor and texture of cheeses, milk, and other products we discussed earlier. Genome projects have been completed for several dozen dairy-related lactic acid bacteria. For example, scientists recently sequenced the genome for

Lactococcus lactis, a strain that is important for making cheese. Such projects have already helped food scientists better utilize different strains to make specific cheeses with enhanced flavor characteristics and to refine culture conditions to maximize the growth abilities of different microbes.

Metagenomic Studies Sequence Genomes from Microbial Communities

Metagenomics involves the sequencing of genomes for entire communities of microbes. Metagenomics projects are sequencing microbial genomes from environmental samples of water, air, and soils as well as from oceans throughout the world, glaciers, mines—virtually every corner of the globe. Estimates also suggest that over 99% of currently known microbial diversity exists in organisms that cannot be cultured. Currently a number of metagenomics projects have been launched around the world, involving international teams of investigators sequencing marine microbes and soil microbes (there is a "terragenome" project under way).

Human genome pioneer J. Craig Venter left Celera in 2003 to form the J. Craig Venter Institute (JCVI), and he has played a central role in establishing the field of metagenomics. One of the institute's initiatives is a global expedition to sample marine and terrestrial microorganisms from around the world and to sequence their genomes. On what is called the Sorcerer II Expedition, Venter and his researchers are traveling the globe by yacht on a sailing voyage that has been described as a modern-day version of Charles Darwin's famous treks on the HMS *Beagle.*

A pilot study the institute conducted on the Sargasso Sea off Bermuda yielded around 1,800 new species of microorganisms and over 1.2 million novel DNA sequences. Samples of water from different layers in the water column are passed through high-density filters of various sizes to filter out microbes. DNA is then isolated from the microbes and used for shotgun cloning and then sequenced with computer-automated sequencers that are kept running on board nearly around the clock. This expedition has great potential for identifying new microbes and genes with novel functions, including commercially valuable genes. For example, the Sargasso Sea project identified hundreds of photoreceptor genes. Some microorganisms rely on photoreceptors for capturing light energy to power photosynthesis. Scientists are interested in learning more about photoreceptors to help develop ways in which photosynthesis may be used to produce hydrogen as a fuel source. Medical researchers are also very interested in photoreceptors because, in humans and many other species, photoreceptors in the eye are responsible for vision. The Sorcerer II expedition has sequenced well over 6 billion base pairs (bp) of DNA from more than 400 uncharacterized microbial species. These sequences contain 7.7 million previously uncharacterized sequences encoding more than 6 million different potential proteins.

The Human Microbiome Project

In 2008 the National Institutes of Health initiated the **Human Microbiome Project,** a $115-million, 5-year metagenomic project to complete the genomes of an estimated 600 to 1,000 microorganisms, bacteria, viruses, and yeasts that live on and inside humans. Microorganisms comprise some 1% to 2% of the human body, outnumbering human cells by about 10 to 1. Many microbes, such as *E. coli* in the digestive tract, have important roles in human health, and of course other microbes make us ill. In addition, 1,200 different bacteriophages (recall that phages are viruses that infect bacteria) are found in the gut, and we know virtually nothing about more than half of them.

The Human Microbiome Project has several major goals, including:

- Determining if individuals share a core human microbiome.
- Understanding how we acquire and maintain microbial communities.
- Understanding how changes in the microbiome can be correlated with changes in human health and the conditions (such as stress and diet) that affect the microbiome.
- Developing new methods, including bioinformatics tools, to support analysis of the microbiome.
- Addressing ethical, legal and social implications raised by human microbiome research. Does this sound familiar? Recall that addressing ethical, legal and social issues was a goal of the Human Genome Project (HGP).

It is estimated that the microbiome consists of 100 to 1,000 times the number of human genes. The Human Microbiome Project is still very much in its infancy as a project but it has already sequenced 500 microbial genomes. So far about 3.3 million human gut microbe genes characterized to date appear to be very similar among over 100 individuals. The saliva microbiome is also highly similar from individual to individual, and a Human Oral Microbiome database has been established from these studies; it seeks to develop linkages between the oral microbiome and oral health. More than 700 types of microbes grow in the mouth alone, contributing to oral health issues such as bad breath, plaque formation, and tooth decay. Scientists have characterized the genomes for dandruff-causing bacteria and fungi of the

scalp. We have also learned that gut microbes play a role in obesity and that the gut microbial environment changes from childhood to adulthood. Similar projects are under way to study the microbiomes of dogs and other animals. Keep an eye on microbiome and other metagenomics projects, because they will provide very interesting data about the microbes among us.

Viral Genomics

The study of viral genomes is another hot area of research (Table 4). This is true in part because many deadly viruses mutate quickly in response to vaccine and antiviral treatments. Antiviral drugs are designed to work in several ways. Some antiviral drugs block viruses from binding to the surface of cells and infecting cells; others block viral replication after the virus has infected body cells. Research on viral genomes helps scientists learn how viruses cause disease and leads to the development of new and effective antiviral drugs.

Creating Synthetic Genomes: A Functional Synthetic Genome Is Produced for a Bacterial Strain

In 2010 JCVI scientists published the first report of a functional **synthetic genome.** In this project they designed and had chemically synthesized more than a thousand 1,080-bp segments covering the entire 1.08-Mb genome of the bacterium *Mycoplasma mycoides* (**Figure 14**). To assemble these segments correctly, the segments had 80-bp sequences at each end, which overlapped with their neighbor sequences. These sequences were cloned in *E. coli*. Then, using the yeast *Saccharomyces cerevisiae,* they assembled the sequences into eleven separate 10-kb assemblies, which were eventually combined to completely span the entire *M. mycoides* genome. The assembled genome, called JCVI-syn1.0, was then transplanted into a close relative *M. capricolum* as recipient cells, resulting in a new cell

YOU DECIDE

Should We Create Life Synthetically?

The JCVI synthetic genome experiments are of limited applicability thus far and will likely be very challenging to apply to other cells types. But if applications of synthetic biology eventually become routine, will we have simplified or demystified life, making it less sacred? Should humans be such “creators” of life? Of course we have been creating life in the lab for a long time; **in vitro** fertilization is one such example. But synthetic biology is a very different approach. Not surprisingly, one fear raised was that bioterrorists could use synthetic genome strategies to recreate deadly bacteria or viruses (read about bioweapons in the next section on bioterrorism) from benign bacteria and viruses. To what extent should we use this technology to remake naturally occurring cells with features we deem better or more desirable? In the future, could synthetic genomes be used to create life from inanimate components? Should this be done if it is technically possible? You decide.

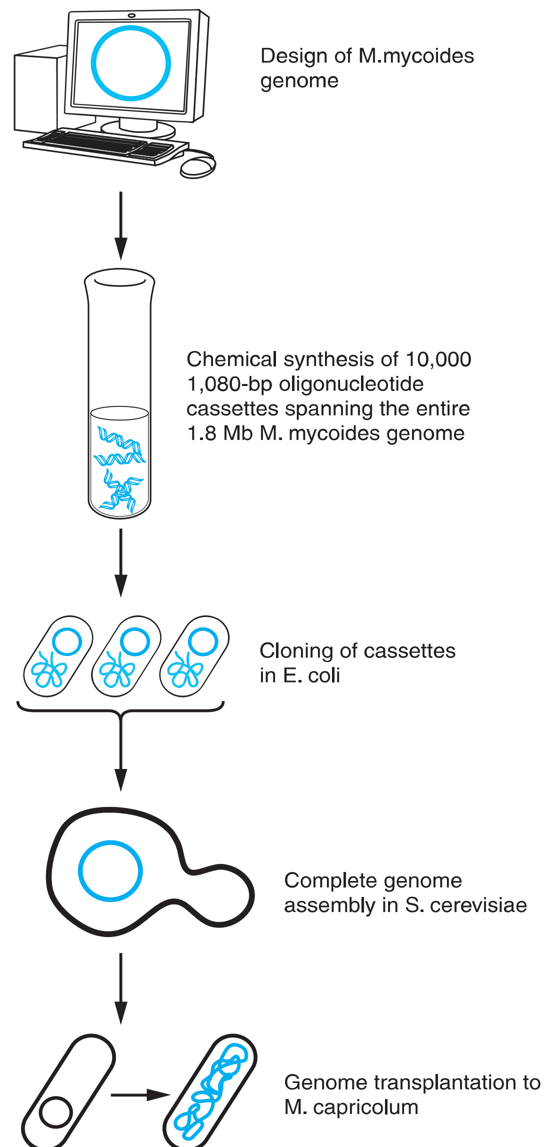


FIGURE 14 Building a synthetic version of the 1.08-Mb *Mycoplasma mycoides* genome JCVI-syn1.0. Shown here is an overview of the approach used to produce *M. mycoides* JCVI-syn1.0.

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with the JCVI-syn1.0 genotype and the phenotype of a new strain of *M. mycoides*. As shown in **Figure 15**, JCVI determined that the recipient cells were taken over to become JCVI-syn.10 *M. mycoides,* in part because they were shown to express the *lac*Z gene, which was incorporated into the synthetic genome. Selection for tetracycline resistance and a determination that recipient cells also made proteins characteristic of *M. mycoides* and not *M. capricolum* were also used to verify strain conversion.

One particularly impressive accomplishment of these experiments was that the synthetic DNA was "naked" DNA, because it did not contain any proteins from *M. mycoides;* therefore it was capable of transcrib-

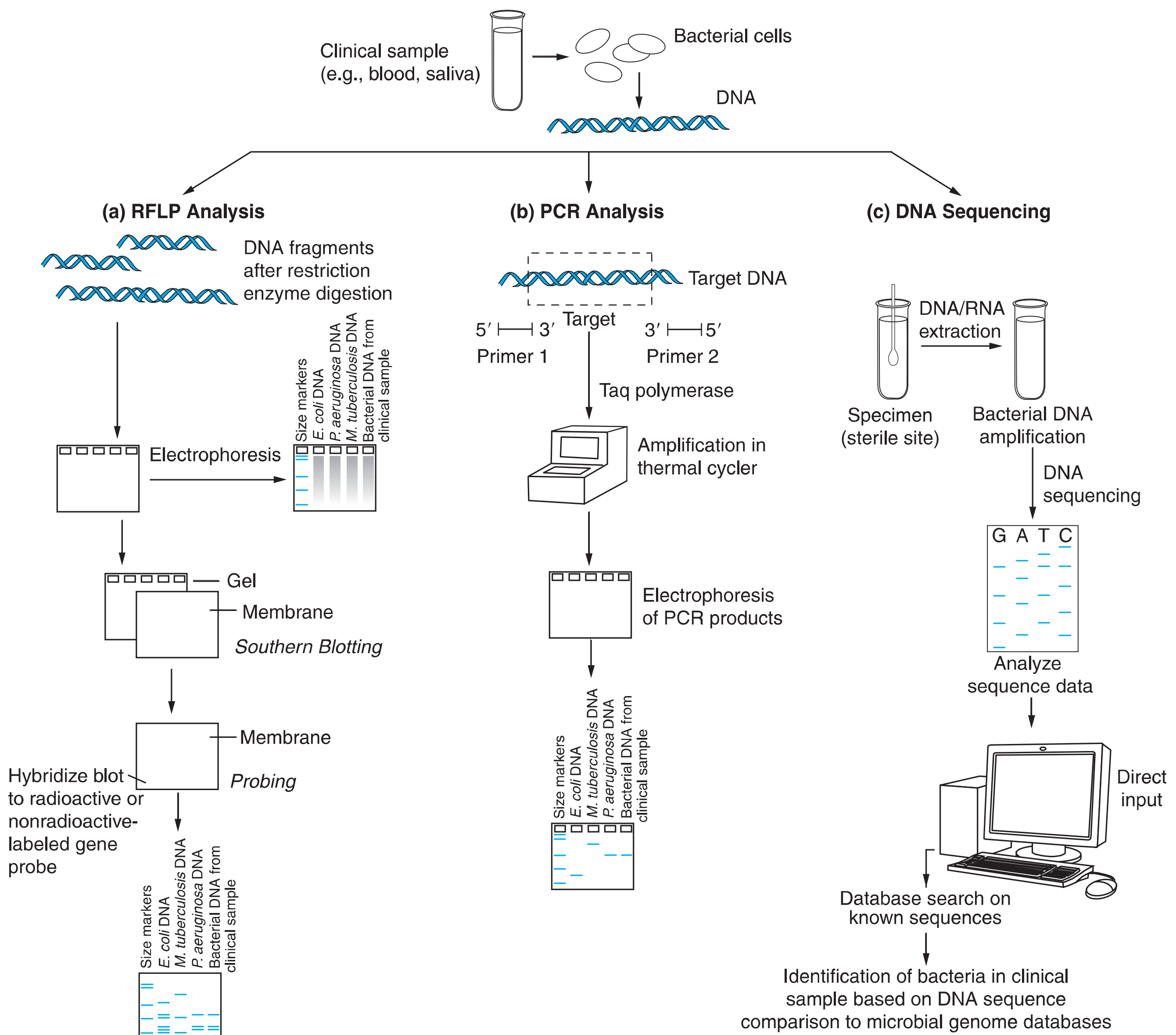

FIGURE 15 **Using Molecular Techniques to Identify Bacteria** Many molecular techniques are available for identifying bacteria. (a) For some pathogens, isolated DNA (which may come from a clinical sample such as blood or saliva) can be digested by restriction enzymes, separated by agarose gel electrophoresis followed by Southern blotting (RFLP analysis). Banding patterns of DNA fragments can be compared to reference strains of known bacteria to allow for a positive identification. In this example, DNA from the clinical sample matches *P. aeruginosa*. (b) PCR has the advantage of being much more sensitive than RFLP analysis; therefore only small clinical samples and small amounts of DNA are required, allowing for early treatment of an infection. (c) DNA-sequencing strategies are also commonly used for microbial identification.

TABLE 4 EXAMPLES OF MEDICALLY IMPORTANT VIRAL GENOMES THAT HAVE BEEN SEQUENCED

Virus	Human Disease or Illness	Year Sequenced
Ebola virus	Ebola hemorrhagic fever	1993
Hepatitis A virus	Hepatitis A	1987
Hepatitis B virus	Hepatitis B	1984
Hepatitis C virus	Hepatitis C	1990
Herpes simplex virus, type I	Cold sores	1988
Human immunodeficiency virus (HIV-1)	Acquired immunodeficiency syndrome (AIDS)	1985
Human papillomavirus	Cervical cancer	1985
Human poliovirus	Poliomyelitis	1981
Human rhinovirus	Common cold	1984
Influenza A virus		
• Subtype H5N1 (Avian flu)	Severe flu	2007
• Subtype H5N1 (Swine flu)	Severe flu	2009
Severe acute respiratory coronavirus (SARS-CoV)	Severe acute respiratory syndrome (SARS)	2003
Variola virus	Smallpox	1992

ing all of the appropriate genes and translating all of the protein products necessary for life as *M. mycoides*. This is not a trivial accomplishment. The synthetic genome effectively rebooted the *M. capricolum* recipient cells to change then from one form to another. When this work was announced, J. Craig Venter claimed "This is equivalent to changing a Macintosh computer into a PC by inserting a new piece of PC software."

Venter and others have used recombinant DNA technology to construct synthetic copies of viral genomes. Researchers at Stony Brook University in New York made headlines when they assembled approximately 7,500 bp of synthetically produced DNA sequences to synthesize proteins and lipids; these were assembled into a recreated polio virus: the first synthetically made virus. The genome for the 1918 influenza strain responsible for the pandemic was also assembled in this way.

But Venter's recent work with *M. mycoides* JCVI-syn1.0 9 is hailed as a defining moment in the emerging field of **synthetic biology.** This work did not create life from an inanimate object, since it was based on converting one living strain into another. There are many fundamental questions about synthetic genomes and genome transplantation that need to be answered. But clearly these studies provided key "proof of concept" that synthetic genomes could be produced, assembled, and successfully transplanted to create a microbial strain encoded by a synthetic genome and bring scientists closer to producing novel synthetic genomes incorporating genes for specific traits of interest.

What are potential applications of synthetic genomes and synthetic biology? JCVI claims that their ultimate goal is to create microorganisms that can be used to synthesize biofuels. Other possibilities exist, such as creating synthetic microbes with genomes engineered for bioremediation, producing alternative fuels, synthesizing new biopharmaceutical products, developing genetically programmed bacteria to help us heal, and making "prosthetic genomes." Work on synthetic genomes and synthetic biology has led to speculation of a future world in which there will be new bacteria and perhaps new animal and plant cells designed and even programmed to be controlled as we want them to be.

6 Microbes for Making Biofuels

The United States alone requires about 140 billion gallons of fuel per year to satisfy current needs, and we are producing only about 5 billion gallons per

year of ethanol as a **biofuel** produced from grain. The production of biofuels has the potential to provide an alternative energy source and reduce global warming resulting from the burning of fossil fuels. To produce ethanol, alcohol-fermenting microbes convert glucose and other sugars in grain to ethanol, but this process is not cost-effective or efficient. It takes a lot of corn kernels to produce relatively small amounts of ethanol. Although, as you will see, cellulosic biomass such as corn stalks is a readily available and abundant source of sugars for making ethanol, but breaking down glucose from cellulose is no simple matter. The trick is to break the cellulose down into individual glucose molecules, which can then be used to create ethanol by fermentation processes.

Cellulose in the plant cell wall is somewhat resistant to breakdown naturally in the environment. You also know that you and I cannot digest cellulose; therefore it contributes fiber to out diet. Some techniques use chemical treatments to help loosen the cellulose structure, but these chemicals inhibit many microbes that could be used to make ethanol.

Many companies working on alternative energy sources consider cellulosic sugars for ethanol production as a very sustainable source of biofuels for reducing the world's reliance on fossil fuels. One concept is to create biorefineries in which leftover biomass from conventional crops—such as corn husks and stalks, wood waste such as chips, saw dusts, and yard clippings—would incorporate microbial enzymes to process sugars with great efficiency from biomass into fuels. Researchers are improving strains of bacteria, such as *Zymomonas mobilis,* to help with this process. Key genes encoding enzymes that will convert sugars into ethanol at higher rates than yeasts can do have been engineered into these bacteria, but their effectiveness in scale-up processes for making ethanol is unproven so far.

Recombinant DNA technology is being used to produce *E. coli* with an increased ability to produce ethanol, as well as bacteria with an increased ability to ferment sugars into ethanol by alcohol fermentation. Others have genetically engineered *E. coli* to secrete cellulose-degrading enzymes. In addition, major bioprospecting efforts are ongoing around the world to identify bacteria and algae that produce useful enzymes—enzymes that could help to process biomass into fuel. Later, we will briefly discuss how bioremediation approaches are investigating the use of microbes to degrade components in sediments as a way of generating energy. Although the future potential of biofuels is unclear, it is expected that research over the next few years will result in significant improvements in biofuel production.

7 Microbial Diagnostics

We have repeatedly seen that microorganisms cause a number of diseases in humans, pets, and agriculturally important crops. Scientists can use a variety of molecular techniques to detect and track microbes—an approach called **microbial diagnostics.**

Bacterial Detection Strategies

Before the development of molecular biology techniques, microbiologists relied on biochemical tests and bacteria cultured on different growth media to identify strains of disease-causing bacteria. For example, when doctors take a throat culture, they use a swab of bacteria from your throat to check for the presence of *Streptococcus pyogenes,* a bacterium that causes strep throat. Even though these and other similar techniques still have an important place in microbial diagnosis, techniques in molecular biology allow for the rapid detection of bacteria and viruses with great sensitivity.

Molecular techniques such as restriction fragment length polymorphism (RFLP) analysis, PCR, and DNA sequencing, can be used for bacterial identification (Figure 15). If the genome of the pathogen is large and produces too many restriction enzyme fragments, which prevent visualizing individual DNA bands on an agarose gel, DNA may be subjected to Southern blot analysis (Figure 15).

Many databases of RFLPs, PCR patterns, and bacterial DNA sequences are available for comparison of clinical samples. For example, if a doctor suspects a bacterial or viral infection, samples including blood, saliva, feces, and cerebrospinal fluid from the patient can be used to isolate bacterial and viral pathogens. DNA from the suspected pathogen is then isolated and subjected to molecular techniques such as PCR (Figure 15). PCR is an important tool for diagnostic testing in clinical microbiology laboratories and widely used to diagnose infection caused by microbes such as the hepatitis viruses (A, B, and C), *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (both of which cause sexually transmitted diseases), HIV-1, and many other bacteria and viruses.

Tracking Disease-Causing Microorganisms

Scientists also use molecular biology techniques to track patterns of disease-causing microbes and the illnesses and outbreaks of illnesses they may cause. As you know, microbes play important roles in the production of dairy products such as yogurts and cheeses. But these dairy products are also susceptible to contamination with pathogenic microorganisms. Information about the microbes in milk can be used to determine the quality of milk and milk spoilage. Bacterial contam-

ination of food is a significant problem worldwide. You have probably heard of the bacterium *Salmonella,* which can contaminate meats, poultry, and eggs. *Salmonella* can infect the human intestinal tract causing serious diarrhea and vomiting, symptoms commonly called food poisoning.

After successfully responding to a 1993 outbreak of meat contaminated with *E. coli,* the **Centers for Disease Control and Prevention (CDC)** and the U.S. Department of Agriculture created a network of DNA-detecting laboratories to expand its coverage and boost its response time. This network, called **PulseNet,** enables biologists, using DNA fingerprinting approaches, to rapidly identify microbes involved in a public health condition. Results can be compared with a database to identify outbreaks of microbes in contaminated foods and to decide how to respond so that a minimal number of people are affected.

Approximately 76 million cases of food-borne disease due to microbes occur in the United States each year, causing well over 300,000 hospitalizations and approximately 5,000 deaths. In the United States alone, the *E. coli* strain O157:H7 causes close to 20,000 cases of food poisoning each year. This infectious strain is lethal. PulseNet now monitors *E. coli* O157, *Salmonella, Shigella,* and *Listeria,* and many non-food-borne diseases such as tuberculosis.

Microarrays for Tracking Contagious Diseases

Microarrays have created new approaches for detecting and identifying pathogens and for examining host responses to infectious diseases. For example, microarray pioneer Affymetrics Inc. has developed the SARS-CoV GeneChip, which contains approximately 30,000 probes representing the entire viral genome for the coronavirus that causes **severe acute respiratory syndrome (SARS).** The SARS virus is a highly contagious respiratory virus that has infected approximately 9,000 people and killed nearly 900 since it was first detected in November 2002. Similar chips are being used to detect the flu strains H1N1 and H5N1, which we discussed previously.

Microarray approaches are also being used to study gene expression changes that occur when an organism is infected with a pathogen (**Figure 16**), providing a "signature" for infection by a particular organism. With these chips, patterns of genes that are stimulated or inhibited by a pathogen can be analyzed as a hallmark signature unique to that particular pathogen. Notice how the three pathogens used in Figure 16 stimulate different sets of genes in mice.

In the next section, we provide a brief glimpse of biological agents that can pose a threat as weapons and discuss how biotechnology can be used to detect and combat bioterrorism.

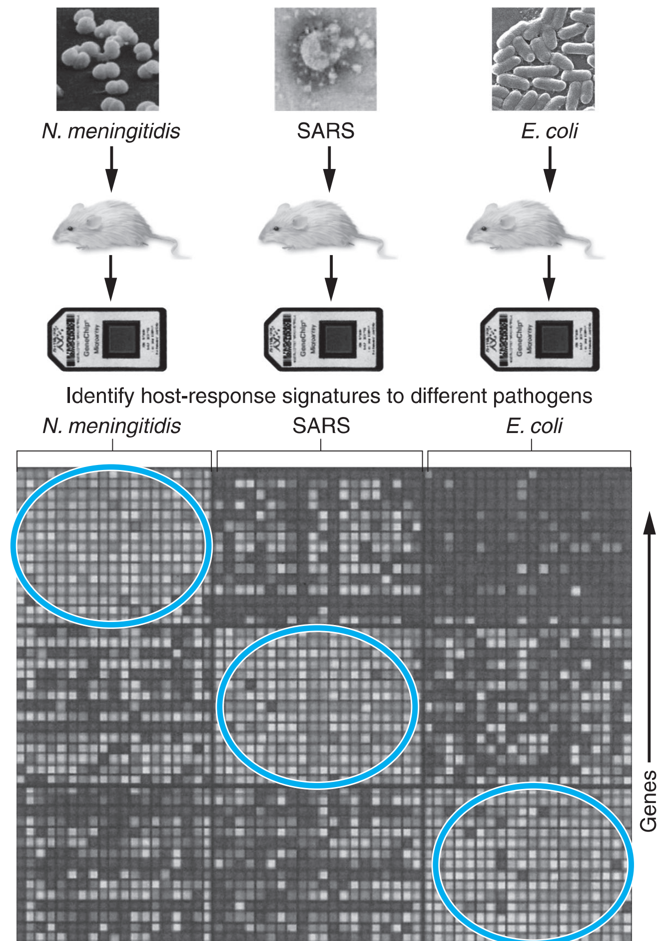

FIGURE 16 Host-Response Gene Expression Signatures for Pathogen Identification In this animal model, mice were infected with *Neisseria meningitidis* (the bacterium that causes meningitis), SARS, or *E. coli,* and microarray analysis was carried out to examine changes in gene expression following infection by each pathogen. In this example, actively expressed genes are shown as light spots. Notice how each pathogen activates a specific subset of genes (circled), creating a gene expression "signature" that researchers can use to identify the infecting pathogen.

(a): Eye of Science/Photo Researchers, Inc., **(b):** C. D. Humphrey and T. G. Ksiaze/CDC., **(c):** Janice Carr/CDC/ National Escherichia, Shigella, Vibrio Reference Unit at CDC.

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8 Combating Bioterrorism

The tragic events of September 11, 2001, were the most catastrophic attacks of terrorism on American soil. In the weeks that followed these horrific tragedies, America and the world were also served notice of a bioterrorism threat when letters contaminated with dried powder spores of the bacterium *Bacillus anthracis* were

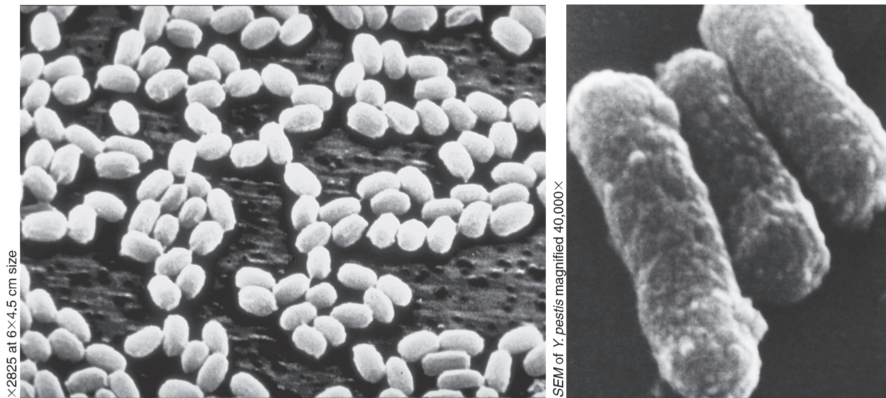

FIGURE 17 Deadly Microbes as Bioweapons Potential bioweapons might include the organisms that cause anthrax (*Bacillus anthracis*, left) and plague (*Yersinia pestis*, right).

(a): A Dowsett/Photo Researchers, Inc., **(b):** T. H. Chen and S. S. Elberg, Inf. Imm 15:972-977.

mailed to two senators, other legislators, and members of the press. Toxic proteins from *B. anthracis* cause significant damage to cells of the skin, respiratory tract, and gastrointestinal tract, depending on how a person is exposed to the microbe. As a result of this anthrax exposure, 5 people died and another 22 people became ill. These events raised our awareness that bioterrorism activities could cause devastating harm should biological agents be released in large quantities. **Bioterrorism** is broadly defined as the use of biological materials as weapons to harm humans or the animals and plants we depend on for food. Biotechnology by its very definition is designed to improve the quality of life for humans and other organisms. Unfortunately, bioterrorism represents a most extreme abuse of living organisms.

Bioterrorism has been a legitimate concern for centuries. In the fourteenth century, bodies of bubonic plague victims were used to spread the bacterium *Yersinia pestis,* which caused bubonic plague during wars in Russia and other countries. This subsequently played a part in causing the Black Death pandemic that ravaged Europe. Early European settlers of the New World spread measles, smallpox, and influenza to Native Americans. Although the introduction of these diseases may not have been intentional, it led to the deaths of hundreds of thousands of Native Americans because they had virtually no immunity to these pathogens. Between 1990 and 1995, aerosols of toxins from the bacterium *Clostridium botulinum* were released at crowded sites in downtown Tokyo, Japan, although no infections resulted from these attempts. In the last few decades, many other lesser known and, fortunately, unsuccessful incidents have occurred around the world.

Microbes as Bioweapons

New strains of infectious and potentially deadly pathogens are evolving every day all around the world. The threat posed by these disease-causing microorganisms, which could be used as **bioweapons,** may conjure images of science fiction novels; however, the potential for a bioterrorism attack is real and of significant concern.

Even though thousands of different organisms that infect humans are potential choices as bioweapons, most experts believe that only a dozen or so organisms could feasibly be cultured, refined, and used in bioterrorism (Table 5). These agents include bacteria such as *Bacillus anthracis,* the gram-positive bacillus that causes anthrax, and deadly viruses such as smallpox and Ebola (**Figure 17**). The possibility that

YOU DECIDE

Should Pathogen Genome Sequences Remain in Public Databases?

Science as a process relies on scientists sharing data and information through presentations at conferences, in publications, and via Web resources such as databases. Now that genomes have been completed for many human pathogens—such as those that cause cholera, anthrax, meningitis, and smallpox—there has been considerable debate about whether genome sequences for potential bioweapon microbes should be publicly available in DNA databases. Media reports on this subject raise concern that terrorists could use such sequence data to develop recombinant proteins for pathogen toxins as a way to produce bioweapons. Others have suggested that terrorists could use genome data to make more effective “superweapon” pathogens resistant to existing vaccines and drugs. However, many genome scientists have spoken in favor of keeping pathogen sequences in public databases, citing the importance of open access to information and claiming that there is so much that we don’t understand about the genomes of these pathogens that no human health threat is posed by making their sequences available. What do you think? Should pathogen genome sequences remain in public databases? You decide.

TABLE 5 POTENTIAL BIOLOGICAL WEAPONS

Agent	Disease Threat and Common Symptoms
Brucella (bacteria)	Different strains of *Brucella* infect livestock such as cattle and goats. They can cause brucellosis in animals and humans. Prolonged fever and lethargy are common symptoms. The disease can be mild or life-threatening.
Bacillus anthracis (bacterium)	Anthrax. Skin form (cutaneous) produces skin-surface lesions that are generally treatable. Inhalation anthrax initially produces flu-like symptoms leading to pulmonary pneumonia, which is usually fatal.
Clostridium botulinum (bacterium)	Botulism. Caused by ingestion of food contaminated with *C. botulinum* or its toxins. Varying degrees of paralysis of the muscular system created by botulinum toxins are typical. Respiratory paralysis and cardiac arrest often cause death.
Ebola virus or Marburg virus	Both are highly virulent viruses that cause hemorrhagic fever. Symptoms include severe fever, muscle/joint pain, and bleeding disorders.
Francisella tularensis (bacterium)	Tularemia. Lung inflammation can cause respiratory failure, shock, and death.
Influenza viruses (a large, highly contagious group)	Influenza (flu). Severity and outcome depend largely on the strain of the virus.
Rickettsia (several bacteria strains)	Different strains cause diseases such as Rocky Mountain spotted fever and typhus.
Variola virus	Smallpox. Chills, high fever, backache, headache, and skin lesions.
Yersinia pestis (bacterium)	Bubonic plague. High fever, headache, painful swelling of lymph nodes, shock, circulatory collapse, organ failure, and death within days after infection in a majority of cases.

unknown organisms could be used as bioweapons is disquieting because they would probably be very difficult to detect and neutralize. However, a little-known microbe would probably be difficult to deliver as a bioweapon.

As a bioweapon, smallpox, which is a disease caused by the variola virus, is of concern for several reasons. Virtually all humans are susceptible to smallpox infection because widespread vaccination stopped over 20 years ago, when the last case of confirmed smallpox was reported. At that time the WHO declared the disease to be eradicated throughout the world, in large part as a result of vaccines. Recent outbreaks of a monkey smallpox strain, however, may revive smallpox as a concern. Following the anthrax events of 2001, based on concern about the use of smallpox as a bioweapon, the U.S. government started to work with biotechnology companies to mass-produce and stockpile supplies of smallpox vaccines.

Targets of Bioterrorism

Antibioterrorism experts expect that bioterrorists will target cities or events where large numbers of humans gather at the same time. Experts, however, have had a poor track record of predicting where and how such acts might occur. At best, we can speculate that there may be many different potential ways to deliver a bioweapon to injure or kill humans. Bioterrorists are most likely to use a limited number of approaches to achieve quick and effective results. Widespread application of most agents might occur via some type of aerosol release in which small particles of the bioweapon are released into the air and inhaled. The aerosol could be created by grinding the bioweapon into a fine powder and producing a "silent bomb" that would release a cloud of the bioweapon into the air. This aerosol cloud would be gas-like, colorless, odorless, and tasteless. Such a silent attack could go undetected for several days. If exposed to a biological agent, in the days following an attack, a few people might develop early symptoms, which physicians might misdiagnose, or their symptoms might closely resemble common illnesses. Meanwhile, if the biological agent could be spread from human to human, larger numbers of people in other states and even other countries would become infected as infected individuals traveled from place to place and spread their illness. Experts

also suspect that biological agents might be delivered by crop-duster planes or disseminated into water supplies.

In addition to the direct threat to humans, experts are concerned with preventing bioweapon attacks that could cause severe damage to crops, food animals, and other food supplies (see Table 6). Not only could such an attack affect human health, but this approach could also cripple the agricultural economy of a country if food animals such as cows were infected. If there were general concerns about the safety of beef and milk, many consumers would likely shy away from buying these products for fear of contamination.

Using Biotechnology Against Bioweapons

As was evident during the anthrax incidents of 2001, the United States is generally unprepared for an attack with biological weapons. Numerous agencies including the American Society for Microbiology, the USDA, the CDC, the Department of Health and Human Services, and Congress have worked to develop legislation for minimizing the dangers of bioterrorism and responding to possible strikes. For instance, the U.S. Postal Service has implemented technologies for sanitizing mail by using x-rays or ultraviolet light.

In 2004, the U.S. federal government appropriated approximately $6 billion to be spent over 10 years to combat biological and chemical terrorism through an initiative called Project BioShield. A main goal of BioShield is to develop and purchase substantial quantities of vaccines and drugs to treat or protect Americans from bioweapons. Even with increased budgets, new laws, and international treaties, no measures can guarantee that bioterrorism will never occur or that we would be able to detect and protect people against illness from such an attack if it were to occur. Worldwide efforts to prevent bioterrorism are essential, but how could biotechnology help to detect bioweapons and respond to an attack if it did occur?

Field tests are an essential step in the detection of an attack. Some field tests involve antibody-based tests such as ELISAs to determine whether pathogens, or specific molecules from a pathogen, are present in an air or water sample. Such units were put in place around the Pentagon during the anthrax scare of 2001, the Gulf War, and the wars in Afghanistan and Iraq. These units draw in air and use antibodies to detect pathogens in the air. This technique is flawed because many of these instruments are not very sensitive and cannot detect small quantities of a pathogen. In fact, these sensors have been known to detect harmless microbes that live naturally in the environment. Newer, more sensitive biosensors must be developed. Similar detection tests using PCR have been developed.

Variations of protein-based assays include rapid handheld immunoassays developed by U.S. Navy scientists and protein microarrays used by the military to detect airborne pathogens such as anthrax spores and the smallpox virus (**Figure 18**). Designed for use in lab settings or even quick diag-

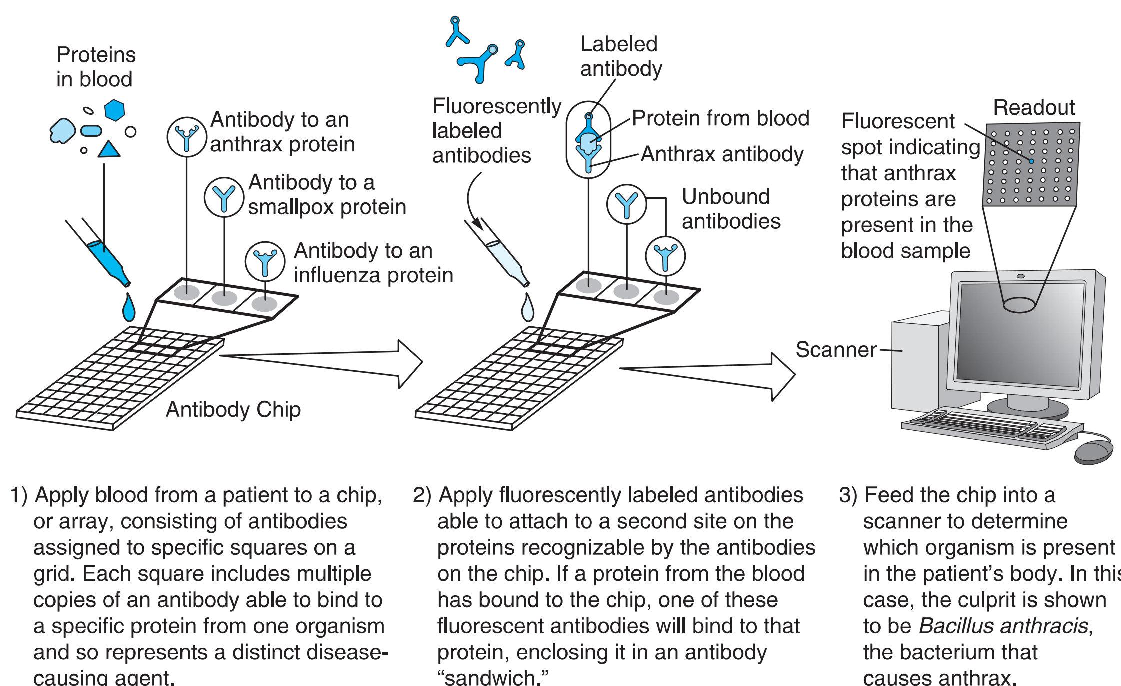

FIGURE 18 Protein Microarrays for Detecting Bioweapon Pathogens

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nostic tests in field settings (some field-based microarrays use diamond-coated surfaces that make them particularly durable), these arrays detect whole pathogens or pathogen components such as proteins or spores.

Emergency response teams and cleanup crews will also be called to action to evaluate the extent of contamination and determine appropriate cleanup procedures based on the bioweapon released (**Figure 19**). Should an attack occur, treatment drugs such as antibiotics will be needed. Countries must build a stockpile of drugs and vaccines that can be widely distributed to large numbers of people if necessary. The basic problem with vaccines is that they must be administered *before* exposure to bioweapons in order to be effective—giving them to infected individuals after an attack is useless. Since 2001, the CDC has increased the U.S. stockpile of smallpox vaccine. Mandatory vaccination of certain military personnel and a voluntary campaign to vaccinate health care workers and those first responders most likely to come in contact with the virus was also instituted. However, even drugs and vaccines may be ineffective if a bioterrorist attack involves organisms that have been engineered against most conventional treatments or if an unknown organism is used as the bioweapon. For instance, you may recall that the antibiotic ciprofloxacin (Cipro) was in high demand during the anthrax threats of 2001.

FIGURE 19 **Battling Bioterrorism** Hazardous material workers from the U.S. Coast Guard decontaminate a coworker after working inside the Hart Senate Office Building to clean the building of anthrax spores during the anthrax attacks in 2001. Battling bioterrorism will require the coordinated efforts of many individuals, from scientists, physicians, and politicians to emergency response teams and cleanup personnel.

AP Images.

An unfortunate reality is that somewhere in this world, someone may be working to plan an attack with biological weapons. Will we be prepared?

TABLE 6 POTENTIAL BIOLOGICAL PATHOGENS FOR A BIOWEAPONS ATTACK ON FOOD SOURCES

Disease	Target/Vector	Agent
Animal diseases		
Foot-and-mouth disease	Livestock	Foot-and-mouth virus
African swine fever virus	Pigs	African swine fever
Plant diseases		
Stem rust for cereals (fungus)	Oat, barley, wheat	*Puccinia* spp.
Southern corn leaf blight (fungus)	Corn	*Bipolaris maydis*
Rice blast (fungus)	Rice	*Pyricularia grisea*
Potato blight (fungus)	Potato	*Phytophthora infestans*
Citrus canker (bacterium)	Citrus	*Xanthomonas axonopodis* pv. *citri*
Zoonoses		
Brucellosis (bacterium)	Livestock	*Brucella melitensis*
Japanese encephalitis (flavivirus)	Mosquitoes	Japanese encephalitis virus
Cutaneous anthrax (bacterium)	Livestock	*Bacillus anthracis*

Source: Gilmore, R. (2004): U.S. Food Safety Under Siege? *Nature Biotechnology*, 22: 1503–1504.

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