Chapter 61: Adaptive Immunity: B Cells & Antibodies

INTRODUCTION

B cells perform two important functions: (1) they differentiate into plasma cells that produce antibodies and (2) they differentiate into long-lasting memory cells that respond robustly and rapidly to reinfection.

Antibodies are the principal defense used by the immune system to *prevent* infection because, by binding to the microbes' surfaces, they can **inhibit them from attaching to target cells** and/or **help innate killing mechanisms**. Antibodies can also **inhibit toxins** such as those made by tetanus and diphtheria. Vaccines work by raising protective, or **neutralizing**, antibodies.

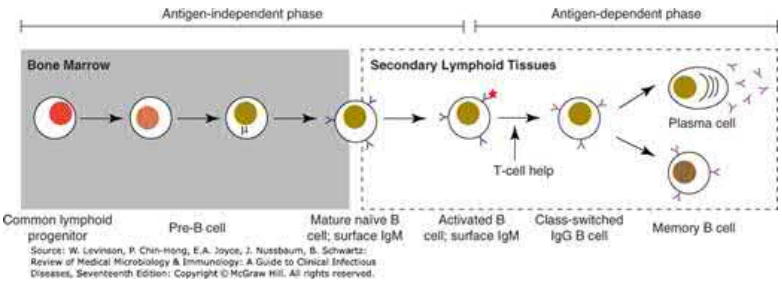
Advances in cell biology have allowed the generation of large quantities of engineered **monoclonal antibodies**. The ability of these antibodies to strongly bind a specific antigen with very limited "cross-reactive" binding of other antigens is the basis for many common diagnostic tests and an increasing array of therapies for various diseases (see Monoclonal Antibodies section later in this chapter).

B-CELL MATURATION

As described in Chapter 59, B cells come from stem cells called **common lymphoid progenitors**, which give rise to all lymphocytes. Each mature B cell represents a **clone**, a group of cells arising from a precursor, all of which have the same heavy chain and light chain rearrangements to form the same **B-cell receptor (BCR)**. Because the BCR—and secreted antibodies—from the clone and its progeny all have the same antigen specificity, these antibodies are called **monoclonal**. Figure 61–1 depicts an overview of the phases of B-cell maturation.

FIGURE 61–1

Maturation of B cells. B cells arise from lymphoid progenitor stem cells and differentiate into pre-B cells expressing μ heavy chains in the cytoplasm and then into mature B cells expressing monomer IgM on the surface. This occurs independent of antigen. Activation of B cells, class switching, and differentiation into memory B cells and plasma cells occurs after exposure to antigen (red star) and is enhanced by T-cell help. μ = mu heavy chains in cytoplasm; Y = IgM (blue) or IgG (purple). (Adapted with permission from Stites DP, Terr A: *Basic & Clinical Immunology*, 7th ed. New York, NY: McGraw Hill; 1991.)



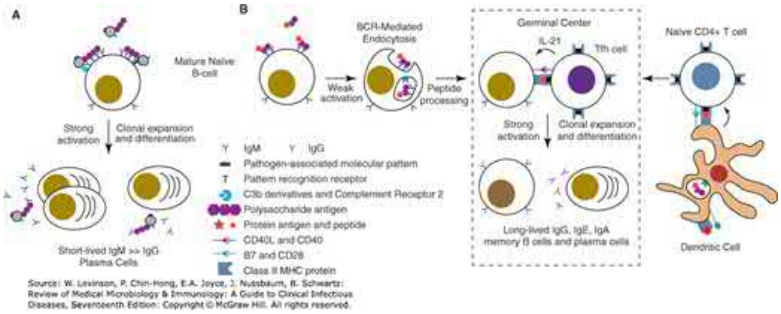
B-CELL ACTIVATION

B cells constitute about 30% of circulating lymphocytes. In lymph nodes, they are located in **follicles**; in the spleen, they are located in the **white pulp**. They are also found in gut-associated lymphoid tissue (GALT) such as Peyer's patches. They express the chemokine receptor **CXCR5**, which guides them toward chemokines produced in a region called the **B-cell follicle**. B cells reside in the follicles and survey the lymph and bloodstream for antigens.

After binding an antigen, B cells are stimulated to proliferate and "class switch." Like T cells, B cells generally require two signals to become activated. **Signal 1** is the binding of antigen to the BCR (Figure 61–2). Binding of multiple BCRs leads to *cross-linking* in which the BCRs are clustered together, increasing the signals sent into the cell. The more BCRs that are cross-linked by antigen, the stronger the signal will be. As we will discuss below, **signal 2** can come from a variety of sources. What the various types of signal 2 have in common is that they are *inflammatory*, meaning they only accompany foreign antigens that represent a real threat to the host.

FIGURE 61–2

Overview of B-cell activation. **A: T-cell–independent Response.** T-cell–independent antigens are large multivalent structures, often polysaccharides (purple) of a bacterial cell (gray circles), that cross-link many IgM receptors to achieve a strong activation signal 1. Signal 2 might include complement C3b derivatives (light blue) bound to the bacterial cell (shown on upper left of cell), or pathogen-associated molecular patterns (gray bars, shown binding to pattern recognition receptor on upper right of cell). Without T-cell help, these responses are short-lived and dominated by IgM plasma cells, although some IgG is also generated. Note that the IgM receptors initially recognize and bind the polysaccharide, so all resulting antibodies will be specific for that polysaccharide. **B: T-cell–dependent Response.** A T-cell–dependent antigen must contain at least some protein component. The B-cell receptor (BCR) binds a specific part of the antigen, it is endocytosed by the B cell, and the peptides are processed and complexed with class II major histocompatibility complex (MHC) proteins. During the germinal center reaction, B cells compete to present antigen to peptide-specific T follicular helper (Tfh) cells that had been previously activated by dendritic cells presenting the same peptide fragment of the antigen. In the germinal center, the B-cell clones that receive more CD40 ligand (CD40L) and cytokines are able to proliferate, class switch, and become long-lived memory B cells and plasma cells.



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Naïve B cells are short lived; without signal 2, they fail to achieve activation and are either **deleted** by apoptosis or become **anergic**, a state of nonresponsiveness. The requirement for inflammatory input from signal 2 at the time of activation is a safeguard so that B cells are not inadvertently activated by harmless antigens. Upon activation, B cells can either differentiate into long-lived **plasma cells**, which secrete more antibody, or into long-lived **memory B cells**, which wait in the follicles of the secondary lymphoid organs to respond to a reinfection.

T-Cell–Independent Activation

In some circumstances, B cells can be activated by a strong signal 1 and signal 2 and *do not need* T-cell help (see Figure 61–2A). Antigens that activate B cells without T-cell help are usually large multivalent molecules such as the chains of repeating sugars that make up bacterial capsular polysaccharide. The repeated subunits act as a **multivalent antigen** that cross-links many IgM antigen receptors on the B cell and sends a strong activating signal 1 into the B cell. Other macromolecules, such as lipids, DNA, and RNA, can also provide signal 1 to the B cell if these antigens are recognized by its surface BCR.

During **T-cell–independent activation**, the B cell's signal 2 can come from various **innate** (T-cell–independent) inflammatory sources:

1. B cells have **pattern recognition receptors**, which recognize pathogen-associated molecular patterns (PAMPs)
2. B cells have the **complement receptor CR2**, which recognize cleavage products of C3b released during complement activation (see Chapter 63)
3. Vaccine **adjuvants** can activate a B cell without requiring T-cell help (see Chapter 57).

These events typically occur *outside* the B-cell follicle, and the plasma cells generated by T-cell–independent activation are relatively short-lived.

The T-cell–independent response is significant because it is the main response to bacterial capsular polysaccharides, which are not proteins and

therefore not recognized by T cells. For example, the pneumococcal polysaccharide vaccine contains the surface polysaccharides of the most common serotypes of *Streptococcus pneumoniae* along with an adjuvant but no carrier protein. Together, the polysaccharide (signal 1) and adjuvant (signal 2) strongly activate B cells. However, because the vaccine does not contain peptides, activation of B cells by these polysaccharides is considered to be T-cell–independent.

T-Cell–Dependent Activation

The previous example illustrates an important concept for vaccine design, but, in general, antibodies generated independently of T-cell help are short-lived and are less specific for their antigens compared with antibodies generated with T-cell help. The strongest and most specific antibody response requires the participation of **dendritic cells** (DCs) and **T cells**. To describe T-cell–dependent activation of B cells, we first need the activation of naïve T cells (see Figure 61–2B, right side). As described in Chapter 60, CD4-positive T cells are activated by DCs presenting a foreign peptide complexed with class II major histocompatibility complex (MHC) proteins, along with co-stimulation.

Consider a T cell activated by a peptide. As it undergoes clonal proliferation, some of the offspring will differentiate into **T follicular helper (Tfh) cells** (see Chapter 60). Upon activation, a Tfh cell **turns off CCR7**, which held it in the T cell zone, and **turns on CXCR5**, pulling it into the B-cell follicle.

While this happens, antigen fragments of the same foreign entity circulate into the B-cell follicles of the secondary lymphoid tissue and interact directly with the antigen receptors (which are membrane-bound IgM molecules) of **naïve B cells**. The recognized epitopes of these circulating antigens might be lipid, polysaccharide, or nucleic acid components, but some component of the antigen may also contain the same peptide. The B cell then uses its BCR to take up the antigen into endosomes, and the antigen is processed. **This B cell can now function as an antigen-presenting cell**. The antigen is processed and its peptide components are complexed with class II MHC molecules and presented on the B cell's surface to interact with the Tfh cells at the border of the T-cell zone. Note that the Tfh cell recognizes the peptide, but the antibody from that B cell will recognize whatever lipid, polysaccharide, nucleic acid, or protein that initially bound to its BCR; the antibody is *not* determined by the peptide in the DC-T cell interaction.

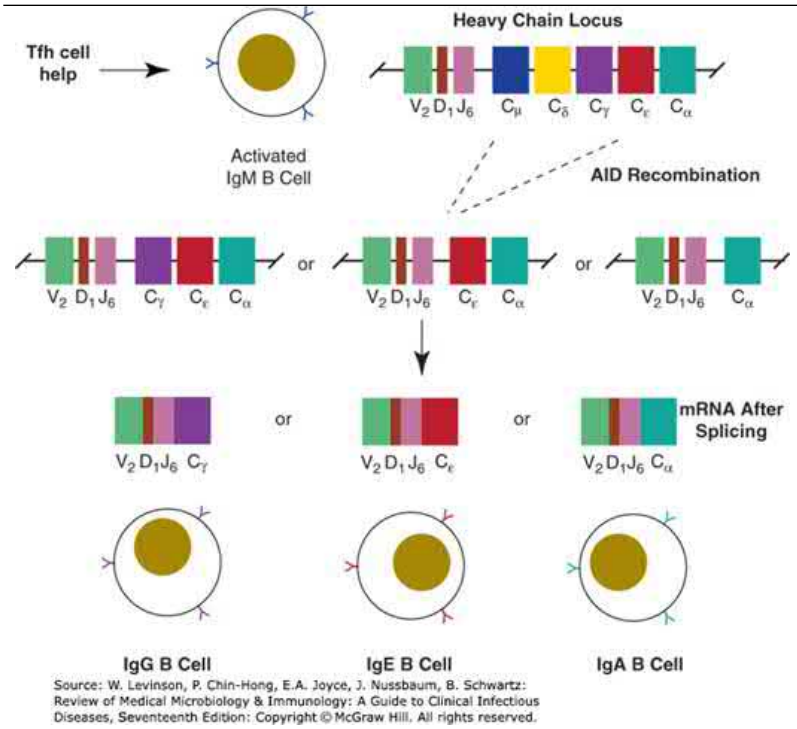
Class Switching & Affinity Maturation

If a Tfh cell recognizes the antigen peptide presented by the B cell's class II MHC molecules, the Tfh cell provides two key signals back to the B cell: first, **CD40 ligand** (**CD40L**) molecules on the Tfh cell bind to **CD40** on the B cell; and second, the Tfh cells produce the cytokine **interleukin (IL)-21**. Together, these signals have three important effects on the B cells:

1. **Rapid proliferation**
2. **Class switching,** changing from using the Cμ segment to using one of the other heavy chain C_H segments (Cγ, Cε, or Cα) (see Figure 61–3)
3. **Somatic hypermutation**

FIGURE 61–3

Class switching. T-cell help induces activation-induced cytidine deaminase (AID)-driven class switching. Activated IgM-positive B cells receive help from T follicular helper (Tfh) cells, including CD40 ligand (CD40L) and interleukin (IL)-21. This causes AID to create double-strand DNA breaks in the heavy chain locus that remove Cμ and Cδ and bring the VDJ region adjacent to one of the other C regions, either γ, ε, or α. After RNA splicing, the B cell begins to express IgG, IgE, or IgA instead of IgM.



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Genetic deficiency of the gene encoding CD40L causes an immunodeficiency called **hyper-IgM syndrome**. Patients with this disease have very high immunoglobulin (Ig) M levels and very little IgG, IgA, and IgE because their B cells are unable to receive T-cell help and therefore are unable to proliferate and "class switch." Hyper-IgM syndrome is characterized by severe bacterial infections (see Chapter 68). As a B-cell clone divides, switches its class, and hypermutates, the newly formed cluster of cells is called a **germinal center**.

Both B-cell class switching and somatic hypermutation are directed by the enzyme **activation-induced cytidine deaminase** (AID). For class switching, AID makes double-strand breaks in the DNA of the C_H locus of the heavy chain, removing the intervening DNA between the VDJ region and either Cγ, Cε, or Cα (see Figure 61–3). This causes *irreversible* switching of that IgM-positive B cell to instead express surface IgG, IgE, or IgA. The decision of whether to switch to IgG, IgE, or IgA is made based on the cytokine signals that the B cell receives:

1. **IL-21 plus gamma interferon (IFN-γ) → IgG.** This makes sense because IFN-γ is the cytokine associated with macrophage activation, and it is the same cytokine that generates the antibody most associated with opsonization and phagocytosis.

2. **IL-21 plus IL-4 → IgE.** This makes sense because IL-4 is one of the main cytokines associated with Th-2 immunity, and it is the same cytokine that generates the antibody most associated with mast cell, basophil, and eosinophil activity. Patients with allergic diseases caused by excess IgE often have excess IL-4.

3. **IL-21 plus various "mucosal" cytokines → IgA.** This makes sense because the cytokines in mucosal barriers induce antibodies that are secreted across mucosal surfaces. (A deficiency in the gene encoding the receptor for some these cytokines causes **IgA deficiency**, which can present with serious sinopulmonary and gastrointestinal infections.)

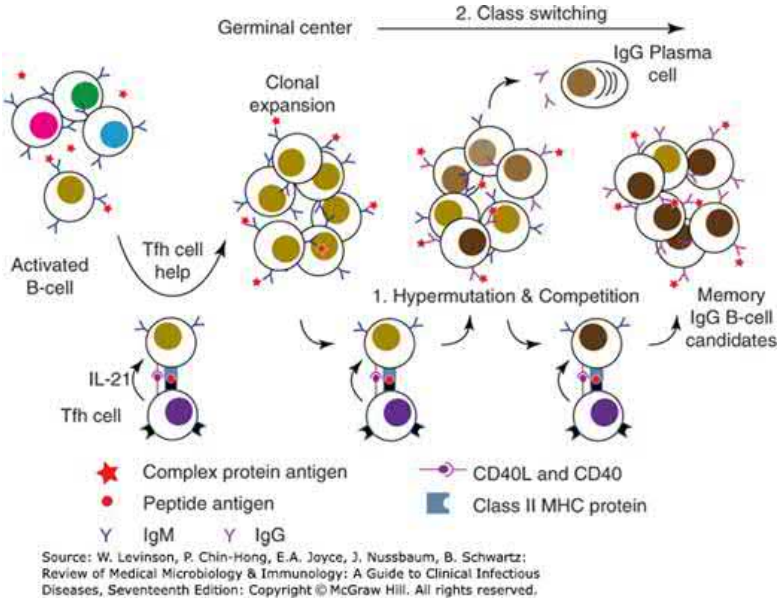
Recall that the variable region of an antibody is responsible for binding to antigen, and because the variable region is not affected by *class switching*, the resulting IgG, IgE, or IgA antibodies should have the same antigen specificities. However, AID does something else. It also makes **nucleotide substitutions** in the gene regions that encode the V_H and V_L chains. This results in the exchange of new amino acids into the antigen-binding *hypervariable* region, **massively increasing the potential diversity of the B-cell pool**.

With successive cell division and new mutations, the enlarging pool of B cells continue to **compete** for the circulating antigens that are present in the follicle; those B cells with higher affinity will be more likely to bind and take up the antigens and therefore more likely to present the correct peptides to CD40L-positive Tfh cells, whereas B cells with lower affinity immunoglobulins will be outcompeted, will not receive the Tfh cell's survival signals, and therefore will die. This process is called **affinity maturation**, and over multiple rounds of cell division, mutation, competition, and selection, a pool of

highly specific B-cell clones *evolves* from the initial germinal center (Figure 61–4). Many germinal centers in many secondary lymphoid organs are engaged with each infection, ensuring a broad **polyclonal** antibody response.

FIGURE 61–4

Germinal center reaction. B cells compete for antigen to receive T-cell help in the germinal center reaction. Naïve IgM-positive B cells survey for antigens in the B-cell follicle. Those that bind antigen (light brown) are selectively activated to engulf and process the antigen and present the peptides to T follicular helper (Tfh) cells. If a Tfh cell recognizes the peptide, it provides help (CD40L and interleukin [IL]-21), and B-cell clonal expansion initiates the germinal center reaction: (1) Repeated rounds of activation-induced cytidine deaminase (AID)-driven somatic hypermutation in the clones alter the specificity of the surface IgM for the antigen. Clones that out-compete their neighbors for antigens in the follicle (darker brown nuclei) enable them more interactions with Tfh cells, leading to progressive affinity maturation. (2) Tfh cytokines induce AID-driven class switching. (Note: In this case, gamma interferon [IFN-γ] signaled the B cells to class switch to IgG. If the Tfh cells provided IL-4, the B cells would class switch to IgE.) The successful clones either become long-lived plasma cells that leave the follicle or have the potential to become circulating memory B cells expressing IgG.



T-cell "help," in the form of IL-21 and CD40L, is also the main stimulus that drives B cells to differentiate into long-lived **plasma cells**. It might seem that this requirement of T-cell help to make plasma cells is unnecessarily cumbersome, but remember that the T cells have been carefully selected in the thymus *not* to see "self" peptides, and therefore, the involvement of T cells in B-cell activation is an additional safeguard against autoimmunity. Compared with T-cell–independent activation, the presence of Tfh cells generates higher titers of IgG, IgA, and IgE antibodies, longer-lived plasma cells, and a stronger response upon reinfection.

The concept of T-cell help for B cells was used in making an improved pneumococcal "conjugate" vaccine. The polysaccharides from common serotypes of *S. pneumoniae* were **conjugated to a highly immunogenic protein**. The vaccine is taken up by DCs, which process the *protein component* to be recognized by the T cells that become Tfh cells. In contrast, the B cells that are activated by the vaccine recognize the *polysaccharide component*, but once they bind the polysaccharide, they also take up and process the conjugated protein. Like the DCs, the B cells process this protein component and present the peptides to the newly activated Tfh cells. In this way, T-cell help is recruited to the follicle, generating high titers of antibody specific for the polysaccharide (see Figure 61–2).

THE PRIMARY RESPONSE

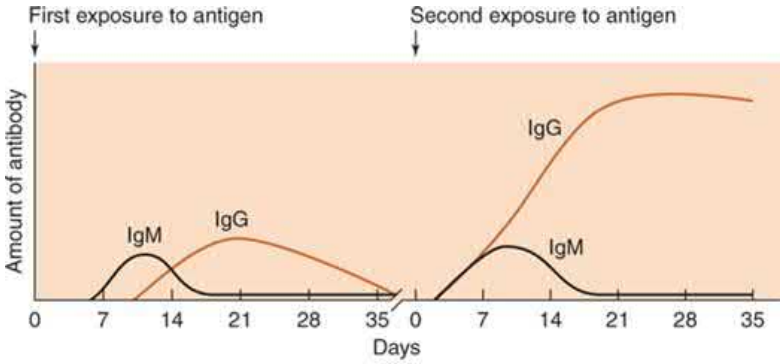
The **primary response** occurs the *first* time that an antigen is encountered. This usually involves T-cell–dependent activation of B cells, as described earlier (see Figure 61–2). Most of the B cells activated from an initial exposure undergo class switching and affinity maturation and differentiate into **plasma cells**. Plasma cells secrete thousands of antibody molecules per second for a life span that lasts from a few days to months.

The first antibodies are detectable in the serum after around **7 to 10 days** in a primary response but can be longer depending on the nature and dose

of the antigen and the route it takes to the secondary lymphoid organ (e.g., bloodstream or draining lymphatics). Antibody levels continue to rise for several weeks and then decline. As shown in Figure 61–5, the **first** antibodies to appear in the primary response are IgM, followed by IgG, IgE, or IgA, as more class-switched plasma cells are generated. IgM levels decline earlier than IgG levels because these plasma cells have shorter life spans.

FIGURE 61–5

Antibody synthesis in the primary and secondary responses. In the primary response, immunoglobulin (Ig) M is the first type of antibody to appear. In the secondary response, IgG and IgM appear but IgG shows a more rapid rise and a higher final concentration than in the primary response.



THE SECONDARY RESPONSE

A small fraction of activated B cells become **memory B cells**, which can remain quiescent in the B-cell follicles but are activated rapidly upon reexposure of their surface BCR to antigen.

When there is a second encounter with the same or closely related antigen, months or years after the primary response, the secondary response is both more **rapid** (the lag period is typically only **3 to 5 days**) and generates **higher** levels of antibody than did the primary response (see Figure 61–5). The memory B cells, which underwent some degree of affinity maturation during the primary response, now proliferate to form a new germinal center and repeat the process of affinity maturation before generating new plasma cells.

This means that, with each exposure, antibodies tend to bind antigen with **higher affinity** because the memory B cells are subjected to further rounds of affinity maturation (see Figure 61–4). During the secondary response, more long-lived IgG plasma cells are generated, meaning secondary IgG levels are *higher* and *tend to persist longer* (see Figure 61–5). This concept is medically important because the protection from the first dose of a vaccine can be "boosted" with repeat doses.

RESPONSE TO MULTIPLE ANTIGENS ADMINISTERED SIMULTANEOUSLY

When two or more antigens are encountered at the same time, the host reacts by producing antibodies to all of them. Combined immunization is widely used and shown to be just as effective as single immunization (e.g., the diphtheria, tetanus, pertussis [DTP] vaccine and the measles, mumps, rubella [MMR] vaccine).

EFFECTOR FUNCTIONS OF ANTIBODIES

The primary function of antibodies is to protect against infectious agents or their products (Table 61–1). Antibodies provide protection by:

1. **Activating complement** to lyse cell membranes and drive inflammation (see Chapter 63)
2. **Opsonize** bacteria, with or without complement
3. Stimulate immune cells' Fc receptors to kill a target cell, also called **antibody-dependent cellular cytotoxicity (ADCC)**

4. Bind and **neutralize** toxins and viruses.

TABLE 61–1

Important Functions of Immunoglobulins

Immunoglobulin	Major Functions
IgG	Main antibody in the secondary response. Opsonizes bacteria, making them easier to phagocytize. Fixes complement, which enhances bacterial killing. Neutralizes bacterial toxins and viruses. Crosses the placenta.
IgA	Secretory IgA prevents attachment of bacteria and viruses to mucous membranes. Does not fix complement.
IgM	Produced in the primary response to an antigen. Fixes complement. Does not cross the placenta. Antigen receptor on the surface of B cells.
IgD	Uncertain. Found on the surface of many B cells as well as in serum.
IgE	Mediates immediate hypersensitivity by causing release of contents from the granules of mast cells and basophils upon exposure to antigen (allergen). Defends against worm infections by causing release of enzymes from eosinophils. Does not fix complement. Important host defense against tissue-invasive helminth infections.

Note that this last mechanism—the binding of an antibody's Fab to its target—is independent of the Fc region. This mechanism is the basis for many *therapeutic blocking antibodies*. Some examples include antibodies that inhibit cytokines, chemokines, or other pathogenic proteins.

Opsonization is the process by which antibodies make microbes more easily ingested by phagocytic cells. This occurs by either of two reactions: (1) the Fc portion of IgG interacts with its receptors on the phagocyte or (2) IgG or IgM activates complement to yield C3b, which interacts with its receptors on the surface of the phagocyte.

Table 61–2 is a summary of the properties of the various classes of immunoglobulins.

TABLE 61–2

Properties of Human Immunoglobulins

Property	IgG	IgA	IgM	IgD	IgE
Percentage of total immunoglobulin in serum (approximate)	75	15	9	0.2	0.004
Serum concentration (mg/dL) (approximate)	1000	200	120	3	0.05
Sedimentation coefficient	7S	7S or 11S[1]	19S	7S	8S
Molecular weight (×1000)	150	170 or 400[1]	900	180	190
Structure	Monomer	Monomer or dimer	Monomer or pentamer	Monomer	Monomer
H chain symbol	γ	α	μ	δ	ε
Complement fixation	++	–	++	–	–
Transplacental passage	++	–	–	–	–
Mediation of allergic responses	–	–	–	–	++
Found in secretions	–	++	–	–	–
Opsonization	++	–	–[2]	–	–
Antigen receptor on B cell	–	–	++	?	–
Polymeric form contains J chain	–	++	++	–	–

[1]The 11S form is found in secretions (e.g., saliva, milk, and tears) and fluids of the respiratory, intestinal, and genital tracts.

[2]IgM opsonizes indirectly by activating complement. This produces C3b, which is an opsonin.

ISOTYPES & ALLOTYPES

Two antibodies with the same antigen specificity might nevertheless have important differences:

1. **Isotype** antibodies are defined by their Fc regions. For example, the different antibody **classes** (IgM, IgD, IgG, IgA, and IgE) are different isotypes; the constant regions of their H chains (μ, δ, γ, α, and ε) are different.

2. **Allotype** antibodies might be of the same isotype but have additional features that **vary among individuals**. They vary because the genes that encode the light and heavy chains are polymorphic, and individuals can have different alleles. For example, the γ heavy chain contains an allotype called Gm, which varies by one or two amino acids between individuals.

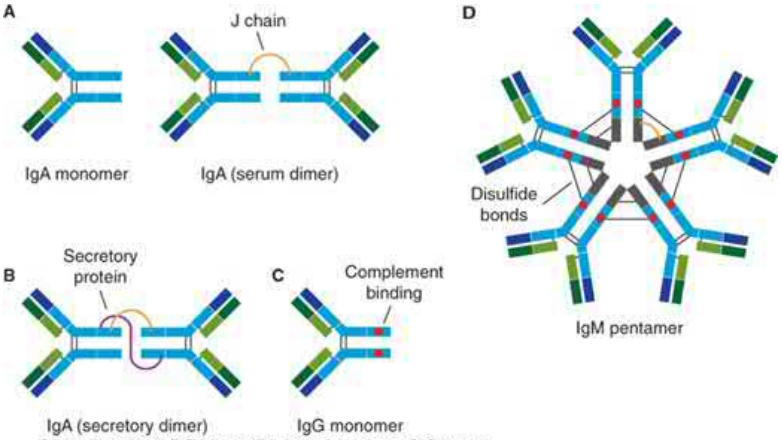
PROPERTIES OF ANTIBODY ISOTYPES (CLASSES)

IgG

Each IgG molecule consists of two L chains and two H chains linked by disulfide bonds (molecular formula H2L2) (see Figure 61–6C). Because it has two identical antigen-binding sites, it is said to be **divalent**. There are four **subclasses**, IgG1 to IgG4, based on differences in the H chains and on the number and location of disulfide bonds. IgG1 makes up most (65%) of the total IgG. IgG2 is directed against polysaccharide antigens and is an important host defense against encapsulated bacteria.

FIGURE 61–6

Structure of immunoglobulin (Ig) A and IgM, showing light chains in green and heavy chains in blue. **A:** Serum IgA monomer and dimer, linked by a J chain (orange). **B:** Secretory IgA dimer, linked by a J chain with an additional secretory protein (purple). **C:** IgG monomer, with complement-binding domains (red ovals). **D:** Pentamer of IgM molecules, which have a fourth C_H domain (gray), complement-binding domains, and a single J chain linking two adjacent molecules. Disulfide between the C_H domains maintains the pentamer structure. (Note that the IgM molecules have a fourth C_H domain.) (Adapted with permission from Stites D, Terr A, Parslow T: *Basic & Clinical Immunology*, 8th ed. New York, NY: McGraw Hill; 1994.)



IgG is the predominant antibody in the **secondary response** and constitutes an important defense against bacteria and viruses (see Table 60–1). IgG is the only antibody to **cross the placenta**; only its Fc portion binds to receptors on the surface of placental cells (see Table 61–2). This receptor, called **FcRn**, transports maternal IgG across the placenta into the fetal blood. IgG is therefore the **most abundant immunoglobulin in newborns**. This is an example of passive immunity because the IgG is made by the mother, not by the fetus (see Chapter 57). Another important attribute of IgG is that it is one of the two immunoglobulins that can activate complement; IgM is the other (see Chapter 63).

IgG is the immunoglobulin that **opsonizes**. It can opsonize (i.e., enhance phagocytosis) because there are receptors for the γH chain, called Fcγ receptors, on the surface of phagocytes. These Fcγ receptors are also found on natural killer (NK) cells, which are responsible for **ADCC**. If a target cell's membrane antigens are bound by the Fab portion of an IgG antibody (e.g., if the cell is infected by a virus), then the Fc portion of these antibodies can activate the surface Fcγ receptors of the NK cell. This triggers the NK cell to release its cytotoxic mediators, including **perforins** and **proteases**, killing the target cell.

IgG has various sugars attached to the heavy chains, especially in the C_H2 domain. The medical importance of these sugars is that they determine whether IgG will have a proinflammatory or antiinflammatory effect. For example, if the IgG molecule has a terminal *N*-acetyl glucosamine, it is proinflammatory because it will bind to mannose-binding ligand and activate complement (see Chapter 63 and Figure 63–1). In contrast, if the IgG has a sialic acid side chain, then it will not bind and becomes anti-inflammatory. Thus, IgG proteins specific for a single antigen that are of the same clone (i.e., made by the same plasma cell) can, at various times, possess different properties depending on these sugar modifications.

IgA

IgA is the main immunoglobulin in **secretions** such as colostrum, saliva, tears, and respiratory, intestinal, and genital tract secretions. It prevents attachment of microorganisms (e.g., bacteria and viruses) to mucous membranes. Secreted IgA consists of two H2L2 units plus one molecule each of J (joining) chain and secretory component (Figure 61–6A and B). (The J chain is only found in IgA and IgM, which are the only immunoglobulins that exist

as multimers. The J chain helps form the disulfide bonds that bind multiple heavy chains into a multimer.) The secretory component is a polypeptide synthesized by epithelial cells that provides for IgA passage to the mucosal surface. It also protects IgA from being degraded by proteases in the intestinal tract. In serum, some IgA exists as monomeric H2L2.

IgM

IgM is the main immunoglobulin produced early in the **primary response**. It is present as a monomer on the surface of virtually all B cells as the BCR. In serum, IgM is a **pentamer** composed of five H2L2 units plus one molecule of J (joining) chain (see Figure 61–6D). IgM cannot bind to Fcγ receptors to facilitate opsonization or ADCC. However, IgM does bind to complement, and the resulting C3b is an opsonin (see Chapter 63). Because the IgM pentamer has 10 antigen-binding sites, it has the **highest avidity** of the immunoglobins and is the **most efficient** in agglutination, complement activation, and other antibody reactions. It can be produced by the fetus in certain infections.

IgD

This immunoglobulin has no known antibody function but may function as an antigen receptor. It is present on the surface of many B lymphocytes and in small amounts in serum.

IgE

IgE is medically important for two reasons: (1) it mediates immediate (anaphylactic) hypersensitivity (see Chapter 65) and (2) it participates in host defenses against certain parasites (e.g., helminths [worms]) (see Chapter 56). The Fc region of IgE binds to **Fcε receptors** on the surface of mast cells and basophils. Bound IgE then becomes a receptor for antigen (allergen). When the antigen-binding sites of adjacent IgEs are cross-linked by allergens, several mediators are released by the cells, and immediate (anaphylactic) hypersensitivity reactions occur (see Figure 65–1). Although IgE is present in **trace** amounts in normal serum persons with allergic reactivity have greatly increased amounts, and IgE may appear in external secretions. IgE does not bind to complement and does not cross the placenta.

IgE may provide host defense against certain important helminth (worm) infections, such as *Strongyloides, Trichinella, Ascaris*, and the hookworms *Necator* and *Ancylostoma*. The larvae of these worms migrate through tissue causing the increased serum IgE level seen in these infections. Because worms are too large to be ingested by phagocytes, they are killed by eosinophils that release worm-destroying enzymes. IgE specific for worm proteins binds to **Fcε receptors** on eosinophils, triggering the ADCC response with release of major basic protein from the eosinophil's granules.

ANTIBODIES IN THE FETUS

In general, the fetus and the newborn have an underdeveloped immune system that responds weakly to infections and vaccines. Antibodies in the fetus are primarily IgG acquired by transfer of maternal IgG across the placenta. This is why it is important to confirm the mother's vaccine history to ensure the newborn will be protected.

After birth, it generally takes months until newborn infants can make IgG (and other isotypes, such as IgM and IgA), so most vaccines are delayed by several months after birth to ensure the newborn's immune system has developed enough to respond. During this time, maternal IgG gradually declines, and protection from maternal IgG is lost by 3 to 6 months. The risk of infections begins to increase over this time, which is why the first set of vaccines is usually recommended by 2 months.

ANTIBODIES IN THE DIAGNOSIS OF DISEASES

Immunoglobulins themselves can also be detected with **anti-immunoglobulin antibodies**. This is used to diagnose patients with an infection. In other words, rather than trying to detect a particular microbe, it is often easier to detect the antibody specific for that microbe using an anti-immunoglobulin antibody. This is the basis for many tests for infections, including human immunodeficiency virus (HIV) tests. It can also be used to detect self-reactive antibodies, which cause autoimmune diseases such as myasthenia gravis. In addition, antibodies can also be used to treat various diseases as discussed in the section entitled "Creating Therapeutic Antibodies" below.

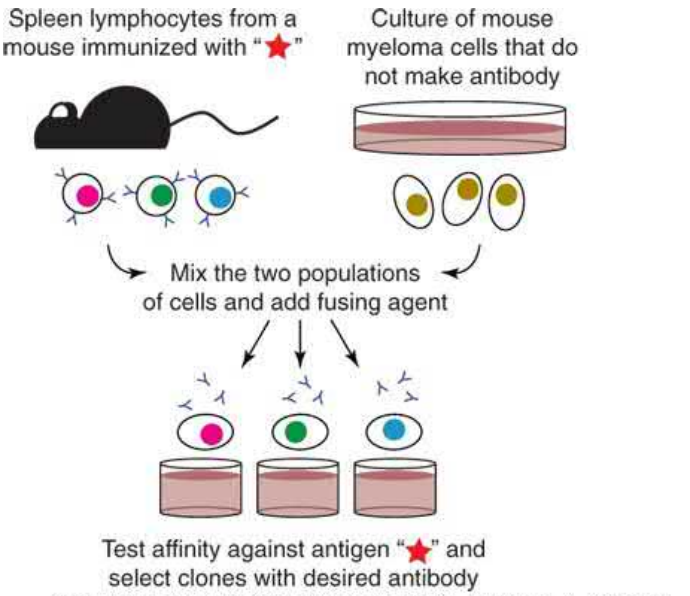
MONOCLONAL ANTIBODIES

The antibodies you make against a single vaccination or infection are usually made by **many different clones** of B cells (i.e., they heterogeneous, or **polyclonal**). Antibodies that arise from a **single clone of cells** are homogeneous, or **monoclonal**. Often, a plasma cell malignancy, such as **multiple myeloma**, can be diagnosed because a single proliferating clone might produce abnormally high levels of a monoclonal immunoglobulin, usually IgG.

The remarkable **specificity** of an antibody's hypervariable region for its target molecule has made monoclonal antibodies an invaluable resource in laboratory research and clinical applications. In the 1970s, the first method of generating virtually unlimited quantities of monoclonal antibodies in the laboratory was described (Figure 61–7). **Hybridoma cells**, formed by the fusion of two different cells, can be created by (1) isolating **spleen cells** from an animal (e.g., a mouse) previously immunized with an antigen of interest and (2) mixing these cells in a culture dish with **mouse myeloma cells** (which grow indefinitely in culture but do not make antibodies) so that the two cell types fuse. The newly formed *hybridoma* cells produce antibodies against the antigen of interest. More recent advances have eased production of therapeutic antibodies (see box "Creating Therapeutic Antibodies") that are now used in a variety of clinical situations, including prevention of infectious disease.

FIGURE 61–7

Overview of the approach to generating monoclonal hybridomas. A mouse is immunized with an antigen of interest (red star). Spleen cells are isolated and mixed with mouse myeloma cells, which can grow indefinitely in culture but do not make antibody. A "fusing agent" is added to encourage the two cell types to combine. The resulting antibody-producing *hybridoma clones* are sorted into culture wells, one cell to each well. Their antibodies are screened against the antigen of interest, and clones that make high-affinity antibodies are selected to be cultured to make antibody indefinitely.



CREATING THERAPEUTIC ANTIBODIES

Hybridoma antibodies were the first monoclonal antibodies to be generated but have significant limitations as therapeutics because different animal species have different heavy chains. This means that many mouse antibodies, for example, bind poorly to human Fc receptors and, therefore, cannot initiate ADCC. These antibodies are also immunogenic if given repeatedly, resulting in hypersensitivity reactions.

This problem was solved with new techniques to cut and fuse pieces of DNA, which allowed the creation of **chimeric** monoclonal antibodies in which the DNA encoding the mouse spleen cell *variable* regions is fused to DNA encoding the human *constant* regions (Figure 61–8). These antibodies are about 65% human. The advantage of the mouse variable region is that it is easy to obtain mouse spleen cells that make antibodies against, for example, a human or viral protein injected into the mouse. The names of chimeric antibodies end with the suffix *–ximab*, such as infliximab (antitumor necrosis factor [TNF]) and rituximab (anti-CD20).

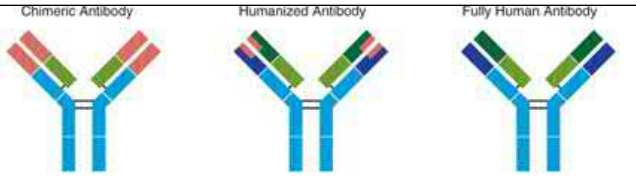
The variable portion of a chimeric antibody is nonhuman, meaning it can still be immunogenic in a human patient. The next generation of **humanized** monoclonal antibodies is generated by replacing *all* of the mouse DNA with the equivalent human DNA sequence *except* the small part encoding the hypervariable (antigen-binding) region (see Figure 61–8). The resulting antibodies are about 95% human, further reducing the chances of an immune reaction. The names of humanized antibodies end with the suffix *–zumab*, such as omalizumab (anti-IgE) and pembrolizumab (anti-PD-1).

Fully human monoclonal antibodies are the next generation of therapeutics that have even greater affinity and virtually eliminate the risk of hypersensitivity (see Figure 61–8). The entire human heavy and light chain gene loci can be fully expressed, either by cultured cells or by mutant strains of mice in which the mouse immunoglobulin genes have been replaced. This results in an enormous potential diversity of 100% human antibodies that can be tested against antigens of interest. (These antibodies might not reflect the range and effector function of human antibodies generated during a "real-world" infection, and efforts are under way to address this by isolating B cells from patients after, for example, a viral infection, and *immortalizing* these cells in order to generate large amounts of their antibodies.) The names of fully human antibodies end with the suffix *–umab*, such as adalimumab (anti-TNF) and ipilimumab (anti-CTLA-4).

Further advances in antibody engineering have given rise to therapeutic proteins that only consist of a Fab region, for example ranibizumab (anti-VEGF), antibodies that only have a heavy chain, for example, caplacizumab (anti-vWF), "fusion" antibodies in which the Fab has been replaced by a different protein, such as a "decoy receptor," for example etanercept (another anti-TNF), and "bispecific" antibodies, in which the two arms come from different clones and each bind a different antigen, such as catumaxomab (anti-EpCAM/CD3). Each of these approaches offer certain advantages over traditional antibodies, and as engineering methods continue to evolve, antibody technology will be applied to a broader range of diseases.

FIGURE 61–8

Summary of therapeutic monoclonal antibodies. Chimeric antibodies are generated by fusing DNA encoding mouse variable regions (orange) to DNA encoding human constant regions (green light chain and blue heavy chain). Compared with mouse antibodies, these antibodies (-ximab) have much greater effector function potential because their human Fc fragments bind optimally to human Fc receptors. However, these antibodies are highly immunogenic due to the residual mouse components. Humanized antibodies are generated by replacing all *but* the DNA encoding the hypervariable (antigen-binding) region (orange) with human immunoglobulin gene DNA (green and blue). These antibodies (-zumab) are less immunogenic but may require additional mutation to improve the antigen affinity. Fully human antibodies (-umab) are generated by screening a "phage library" of randomly generated human antigen-binding sites or by immunizing transgenic mice that carry the human immunoglobulin gene loci in place of the mouse genes. These antibodies are the least immunogenic but carry significant technical barriers and cost. Therapeutics that make use of antibody properties but are engineered to have alternative structures include Fab- and Heavy Chain-Only, which can have one or more antigen-binding domains, fusion proteins, and "bispecific" antibodies in which each chain binds a different antigen.



	Chimeric Antibody	Humanized Antibody	Fully Human Antibody
Name Suffix	*-ximab*	*-zumab*	*-umab*
Percent Human	~65%	~95%	100%
Advantages	High affinity for antigen	Less immunogenic	Nonimmunogenic
Disadvantages	Highly immunogenic	May have low antigen affinity	Technically difficult; expensive

TESTS FOR EVALUATION OF B CELLS AND ANTIBODIES

Evaluation of B cell function consists primarily of measuring the amount of each of the three important immunoglobulins (i.e., IgG, IgM, and IgA) in the patient's serum. To evaluate patients suspected of having an immunodeficiency, it may be necessary to count B-cell numbers by flow cytometry, as described in Chapter 60 for T cells. It is also possible to test for B-cell function by comparing antibody titers before vs. after immunization (e.g., with a *S. pneumoniae* vaccine). The absence of a normal rise in IgM and IgG after immunization indicates a defect, either an intrinsic defect in the B cells themselves or an extrinsic defect that, for example, inhibits T cells' capacity to provide help to activate the B cells.

Chapter 62: Major Histocompatibility Complex & Transplantation

INTRODUCTION

In transplantation, an organ or tissue from one person is "grafted" to another person. A major barrier to the success of these life-saving procedures is the immune system, which attacks any cells it sees as foreign. Graft survival is largely determined by the donor's and recipient's **major histocompatibility complex (MHC)** proteins, which present antigens to T cells. In humans, these proteins are encoded by the **human leukocyte antigen (HLA)** genes. (Note that we will use MHC and HLA interchangeably.) Three of these genes (HLA-A, HLA-B, and HLA-C) code for the class I MHC proteins. Several HLA-D loci determine the class II MHC proteins (i.e., DP, DQ, and DR) (Figure 62–1). The features of class I and class II MHC proteins are compared in Table 62–1. If the HLA proteins on the donor's cells differ from those on the recipient's cells, then an immune response occurs in the recipient.

TABLE 62–1

Comparison of Class I and Class II MHC Proteins

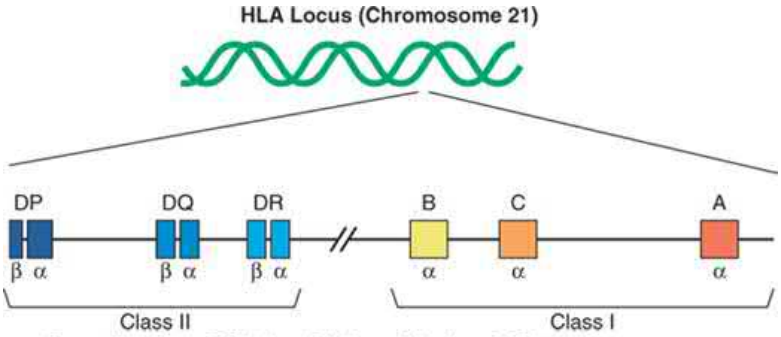
Feature	Class I MHC Proteins	Class II MHC Proteins
Present antigen to CD4-positive cells	No	Yes
Present antigen to CD8-positive cells	Yes	No
Found on surface of all nucleated cells	Yes	No
Found on surface of "professional" antigen-presenting cells, such as dendritic cells, macrophages, and B cells	Yes[1]	Yes
Encoded by genes in the HLA locus	Yes	Yes
Expression of genes is codominant	Yes	Yes
Multiple alleles at each gene locus	Yes	Yes
Composed of two peptides encoded in the HLA locus	No	Yes
Composed of one peptide encoded in the HLA locus and a β_2-microglobulin	Yes	No

[1]Note that class I MHC proteins are found on the surface of all nucleated cells, including those that have class II MHC proteins on their surface. Mature red blood cells are nonnucleated; therefore, they do *not* synthesize class I MHC-proteins.

FIGURE 62–1

The human leukocyte antigen (HLA)–gene complex. A, B, and C are class I loci, and each gene encodes an alpha chain that pairs with the same β2-

microglobulin. DP, DQ, and DR are class II loci, and each gene encodes alpha and beta chains that pair with each other.



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Each person has two HLA **haplotypes** (i.e., two sets of these genes—one on the paternal and the other on the maternal chromosome 6). These genes are highly **polymorphic** (i.e., there are many alleles of the class I and class II genes). For example, as of 2020, there are at least 20,000 HLA Class I alleles and 7700 HLA Class II alleles, and more are being discovered. However, an individual inherits only a single allele at each locus from each parent. Expression of these genes is **codominant** (i.e., the proteins encoded by *both* the paternal and maternal genes are produced).

The class I MHC proteins consist of one HLA-encoded polypeptide (an "alpha" chain), so each individual can have one set encoded by paternal genes and one set encoded by maternal genes. However, the class II MHC proteins consist of two HLA-encoded polypeptides (an "alpha" chain and a "beta" chain), so each individual can have as many as four of each class II MHC proteins because the maternal- and paternal-encoded peptides can mix and match.

HLA gene **polymorphism** causes us each to have somewhat different proteins on the surface of our cells. Each HLA protein can bind certain peptides better than others. This likely explains our HLA diversity: our ability as a population to recognize diverse infectious agents is an evolutionary advantage, ensuring that some individuals would more likely survive an epidemic. But these protein differences can be recognized as nonself when introduced to another person's immune system. The MHC is called **major** because these HLA differences are often responsible for acute rejection of a transplant, and these are the genes most crucial for matching donors and recipients. Another context where MHC genes and proteins are important is in autoimmune diseases, many of which occur more frequently in people who carry certain MHC genes (see Chapter 66).

In addition to the major antigens encoded by the HLA genes, there is a large number of **minor** antigens encoded by genes at sites other than the HLA locus. These minor antigens are various normal body proteins that have one or more amino acid differences from one person to another (i.e., they are "allelic variants"). Like the HLA proteins, these proteins can therefore be recognized as nonself by another person's immune system. Minor antigens induce a weak immune response, which might result in slow rejection of a transplant or rapid rejection if several minor antigens have a cumulative effect. Predicting rejection on the basis of minor antigens is difficult, so donors and recipients are not routinely tested for specific minor histocompatibility antigens. In view of these differences in minor antigens, all recipients routinely receive immunosuppressive drugs, even if their *major* histocompatibility loci are well-matched.

MHC PROTEINS

Class I MHC Proteins

These are found on the **surface of virtually all nucleated cells**. The complete class I protein is composed of a heavy chain (called the alpha chain) bound to a β_2-microglobulin. The alpha heavy chain is **highly polymorphic** and is similar to an immunoglobulin molecule; it has *hypervariable* regions that bind and present short peptides to T cells. The **polymorphism** of these molecules is important in the **recognition of self** and **nonself**. Stated another way, if these molecules were more similar from individual to individual, our ability to accept foreign grafts would be improved but our species might be more susceptible to certain infections. The heavy chain also has a constant region where the **CD8** protein of a cytotoxic T cell binds.

Class II MHC Proteins

These are found only on the surface of **antigen-presenting cells (APCs)**, such as dendritic cells, macrophages, and B cells (see Chapter 60). They

are composed of two **highly polymorphic** chains (called alpha and beta). Like class I proteins, the class II proteins have hypervariable regions that present the short peptides to T cells and provide much of the polymorphism. Unlike class I proteins, which have only one chain encoded by the MHC locus (pairing with β_2-microglobulin), *both* the alpha and beta chains of the class II proteins are encoded by the MHC locus. The two peptides also have a constant region where the **CD4** protein of a helper T cell binds.

BIOLOGIC IMPORTANCE OF MHC

The ability of T cells to recognize antigen depends on association of the antigen with either class I or class II proteins (see Chapter 60). For example, CD8-positive cytotoxic T cells only respond to antigen *in association with class I MHC proteins.* Thus, a cytotoxic T cell that is activated to kill a virus-infected cell will *only* kill a cell infected with the same virus and presenting antigen with the appropriate class I proteins. (This was determined by mixing cytotoxic T cells from individual "A," bearing one set of class I MHC proteins, with virus-infected cells bearing a set of class I MHC proteins from individual "B." Because of the class I MHC mismatch, no killing of the virus-infected "B" cells occurred.) The requirement that antigen recognition occurs in association with a "self" MHC protein is called **MHC restriction** and is a result of **positive thymic selection** (see Chapter 59).

TRANSPLANTATION

The likelihood that a transplanted organ, or *graft*, is accepted by the recipient's immune system depends on the genetic similarity between the recipient and the donor. On one end of the spectrum, an **autograft** (transfer of an individual's own tissue to another site in the body) is always permanently accepted (i.e., it always "takes"). A **syngeneic graft** is a transfer of tissue between genetically identical individuals (i.e., identical twins) and almost always "takes." On the other end of the spectrum, a **xenograft**, a transfer of tissue between different species, is the least likely to succeed except under certain unusual circumstances.

An **allograft** is a graft between genetically different members of the same species (e.g., from one human to another). Allografts are usually rejected unless the recipient is given immunosuppressive drugs. The rapidity of the rejection will vary depending on the degree of difference between the donor and the recipient at the MHC loci.

Solid Organ Rejection

Even with perfect HLA matching, the presence of minor antigens means that immunosuppression is required after a transplant to prevent **allograft rejection**. As HLA mismatching increases, more immunosuppression is needed. In **acute allograft rejection**, vascularization of the graft is normal initially, but, in 11 to 14 days, there is marked reduction in blood flow and immune cells infiltrate the graft, with eventual necrosis. A **T-cell–mediated reaction is the main cause of acute rejection** of many types of grafts, but antibodies may contribute to the rejection of certain transplants.

A graft that survives an acute allograft reaction can undergo **chronic rejection**. This causes gradual loss of graft function and can occur months to years after engraftment. The main pathologic finding in chronic rejection is atherosclerosis of the vascular endothelium. The immunologic stimulus that causes chronic rejection is complex and multifactorial and can occur even in HLA-matched donor–recipient pairs due to the presence of *minor* histocompatibility antigens. The adverse effects of long-term use of immunosuppressive drugs may also play a role in chronic rejection. Chronic rejection generally does not respond to treatment, and it carries a poor prognosis.

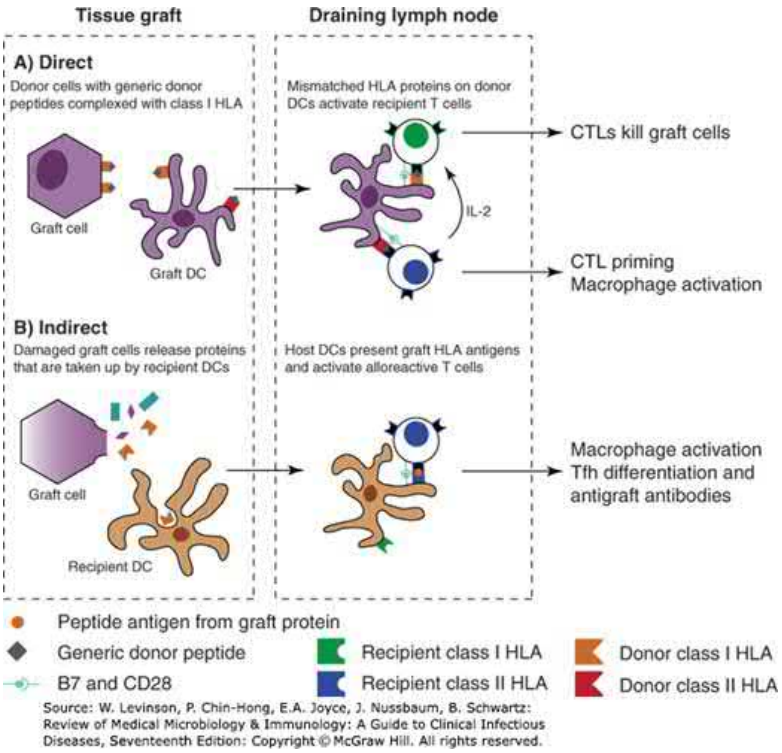
In addition to acute and chronic rejection, a third type called **hyperacute rejection** can occur. Hyperacute rejection typically occurs within minutes of a solid organ transplant graft and is due to the reaction of **preformed** anti-ABO antibodies in the recipient with ABO antigens on the surface of the endothelium of the graft. To prevent this severe rejection reaction, the ABO blood group of donors and recipients must be matched, and a cross-matching test must be done (see Chapter 64).

Depending on the type of graft and the type of rejection, *mismatching* of the HLA-A, HLA-B, and HLA-DR alleles is the most predictive factor in solid organ transplant rejection. The strength of the recipient's T-cell response to alloantigens encoded by these donor MHC alleles can be explained by the observation that there are two immune pathways by which the immune response is triggered (Figure 62–2).

FIGURE 62–2

The direct and indirect pathways of solid organ rejection. **A: Direct Pathway.** In the **direct pathway,** *donor* dendritic cells (DCs) migrate from the

graft to the draining lymph node and interact with recipient CD4-positive **T helper** cells (Th cells, blue) and CD8-positive **cytotoxic T** cells (CTLs, green). Because of the human leukocyte antigen (HLA) mismatch (green ≠ orange and red ≠ blue), the generic peptide presented by the DC is irrelevant! The Th cells help activate the CTLs (see Chapter 60), which go into the graft and kill donor cells that express the mismatched class I HLA protein (orange). The Th cells also migrate into the graft and interact with mismatched class II HLA proteins (red), releasing cytokines that activate macrophages to enhance inflammation. **B: Indirect Pathway.** In the **indirect pathway,** *recipient* DCs take up proteins released by damaged graft cells. (This could be any mechanism of damage, including that caused by the direct rejection pathway described above.) The DCs process the antigens into peptides and carry them to the draining lymph node where they interact with recipient Th cells (blue). The Th cells can differentiate into T follicular helper (Tfh) cells that activate B cells to make antibody (see Chapter 61), or they can migrate into the graft and activate recipient macrophages to enhance inflammation. (If there is HLA mismatch, then CTLs might be activated, but they will not be able to kill donor cells.) Note that both pathways require co-stimulatory signals, such as B7 interacting with CD28, and therefore can only occur in inflammatory settings.



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These pathways are summarized as follows and are differentiated by whether the *sensitizing APC* is **donor-derived** or **recipient-derived**:

1. In the **direct pathway** of allograft recognition, there must be donor–recipient HLA mismatch. In this pathway, the *donor's* APCs contained within the grafted organ migrate to a nearby secondary lymphoid tissue and present peptides in association with their class I and class II MHC proteins. The mere presence of the donor HLA protein that is *presenting* the peptide is enough to make the peptide–MHC complex **appear to be nonself** to the recipient's T cells, *regardless of the peptide*. Unlike the conventional activation of T cells by cognate peptides complexed with MHC (see Chapter 60), "direct" recognition of these nonself HLA proteins triggers a **polyclonal activation of a much larger percentage of recipient T-cell clones**, by some estimates up to 10% of the recipient T cells. This is likely because the diversity of peptides complexed to the donor MHC proteins can trigger a similarly diverse array of T-cell clones.

   If the nonself HLA proteins are class I, they will **activate CD8-positive T cells** to become cytotoxic T lymphocytes (CTLs), which infiltrate the graft and kill the graft cells because they express the same class I proteins. "Direct" recognition of class II HLA proteins can also trigger **activation of the recipient's CD4-positive T cells**, which can provide the cytokine "help" that enhances CD8-positive T-cell activation, as described in Chapter 60.

2. In the **indirect pathway** of allograft recognition, the *recipient's* APCs present the donor's proteins. The donor's proteins that are shed by damaged cells of the graft are taken up by recipient dendritic cells, processed, and presented to T cells as "foreign" proteins in a draining lymph node. (If there is HLA mismatch, the donor HLA proteins are often the antigens responsible for this pathway because HLA proteins are highly polymorphic and immunogenic. But even without HLA mismatch, there are minor antigens that can bring about the indirect pathway of rejection.)

This results in activation of CD4-positive helper T cells (see Chapter 60). The newly activated T helper cells can (a) migrate back to the graft and **activate macrophages** and (b) **recruit neutrophils**, or (c) migrate to the B-cell follicle and **induce antibodies** against the graft cells.

Compared with the *direct pathway*, the *indirect pathway* takes longer because the recipient dendritic cells have to enter the graft, take up nonself proteins, and migrate to the draining lymph node to activate the adaptive immune response. As time passes, the donor APCs in the graft are replaced by recipient APCs, and the risk of *direct recognition* being a mechanism for rejection declines. Therefore, whereas both the *direct* and *indirect* pathways can contribute to **acute rejection**, the *indirect* pathway is primarily responsible for **chronic rejection**.

In all rejection scenarios, the activation of T cells is accompanied by inflammatory stimuli, such as pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). These are necessary to induce the co-stimulatory signals, such as B7 molecules, on the APCs in order to fully activate the T cells (see Chapter 60). This is clinically relevant because limiting inflammation and tissue damage at the time of transplantation significantly limits the likelihood of graft rejection and improves outcomes. This explains why, given a choice, surgeons prefer to transplant organs from live donors whenever possible.

Hematopoietic Stem Cell Transplants

Malignancies of the hematologic system, particularly leukemia, are often treated with transplant of **hematopoietic stem cells**. The principle of this approach is to use aggressive chemotherapy to ablate all of the patient's hematopoietic cells, which includes most of the malignant cells, and then replace them with healthy stem cells that can repopulate the hematopoietic system (see Figure 58–1). The stem cells can be obtained from *leukopheresis of peripheral blood* or from a sample of *banked umbilical cord blood*.

Unlike most solid organ transplants, transplanted hematopoietic stem cells can be *autologous* (from the patient's own stem cell pool) or *allogeneic* (from a donor). Autologous cell transplants are safer and avoid the need to find a matched donor, so you might think this would be the preferred approach in all cases. However, the main advantage to using allogeneic cells is that once these cells engraft, the T cells will actually *attack any surviving malignant cells*. This **graft-versus-malignancy** effect can occur with HLA-matched or -unmatched stem cells because of the minor antigens recognized by the donor cells. Without this effect, autologous transplants have higher relapse rates.

Graft-Versus-Host Reaction

While it is advantageous to transplant cells that attack the remaining malignant cells, an unfortunate adverse effect in autologous transplants is that the transplanted cells may *attack healthy host cells*. This **graft-versus-host (GVH)** reaction develops in 30% to 70% of recipients, depending on the type of donor cells and other factors. The reaction occurs because grafted immunocompetent T cells proliferate in the immunocompromised host and reject host cells with "foreign" proteins, resulting in severe organ dysfunction. The *donor's* cytotoxic T cells play a major role in destroying the *recipient's* cells. These reactions tend to occur in the skin and gastrointestinal system, causing severe rash, oral ulcers, diarrhea, and hepatitis.

There are three requirements for a GVH reaction to occur:

1. The graft must contain immunocompetent T cells
2. The recipient must be immunocompromised
3. The recipient must express antigens (e.g., MHC proteins) foreign to the donor (i.e., the donor T cells recognize the recipient cells as foreign).

Risk of GVH reactions can be reduced by depleting the donor cell pool of T cells before the transplant, but this also reduces the graft-versus-malignancy effect and therefore increases relapse rates. Once it occurs, patients with GVH disease are treated with immunosuppressive agents, often for their entire lives. Immunosuppression increases the risk of disease relapse (by limiting graft-versus-malignancy effect) and also increases the risk of developing other malignancies as well as opportunistic infections.

HLA Typing in the Laboratory

Prior to transplantation, laboratory tests, commonly called **HLA typing** or **tissue typing**, are performed to match the donor and the recipient. The most important alleles to match are HLA-A, HLA-B, HLA-C, HLA-DR, and HLA-DQ, and a donor–recipient pair in which all 10 of the maternal and paternal alleles of these five genes match is called a "10/10 match." In the past, serologic assays were used to determine the Class 1 and Class II MHC proteins of

the donor and recipient. However, serologic assays have now been largely replaced by **DNA sequencing** using polymerase chain reaction (PCR) amplification.

In addition to the tests used for matching, preformed cytotoxic antibodies in the recipient's serum reactive against the graft are detected by observing the lysis of donor lymphocytes by the recipient's serum. This is called **cross-matching** and is done to prevent hyperacute rejections from occurring. In solid organ transplants, the donor and recipient are also matched for the compatibility of their ABO blood groups (see Chapter 64).

Among siblings, there is a 25% chance for both haplotypes to be shared, a 50% chance for one haplotype to be shared, and a 25% chance for no haplotypes to be shared. For example, if the father is haplotype AB, the mother is CD, and the recipient child is AC, there is a 25% chance for a sibling to be AC (i.e., a two-haplotype match), a 50% chance for a sibling to be either BC or AD (i.e., a one-haplotype match), and a 25% chance for a sibling to be BD (i.e., a zero-haplotype match).

The Fetus Is an Allograft that Is Not Rejected

A fetus has MHC genes inherited from the father that are foreign to the mother, yet allograft rejection of the fetus does not occur. The reason that the mother's immune system does not reject the fetus as foreign is not fully understood. The mother can form antibodies against paternal MHC proteins; therefore, the reason is not that the mother is not exposed to fetal antigens. Some possible explanations are (1) that the placenta does not allow maternal T cells to enter the fetus and (2) the maternal T cells within the placenta are biased toward a T-regulatory subset, which promotes tolerance of fetal antigens (see Chapter 60).

EFFECT OF IMMUNOSUPPRESSION ON GRAFT REJECTION

To reduce the rejection of transplanted cells or to treat GVH disease, immunosuppressive measures are generally required (Table 62–2 and Figure 62–3). These fall under the categories of corticosteroids (prednisone), DNA synthesis inhibitors (azathioprine, methotrexate, mycophenolate), calcineurin inhibitors (cyclosporine and tacrolimus), mammalian target of rapamycin (mTOR) inhibitors (sirolimus), signaling blockade (belatacept, basiliximab, etc.), and cell-depleting antibodies (antithymocyte globulin [ATG]).

TABLE 62–2

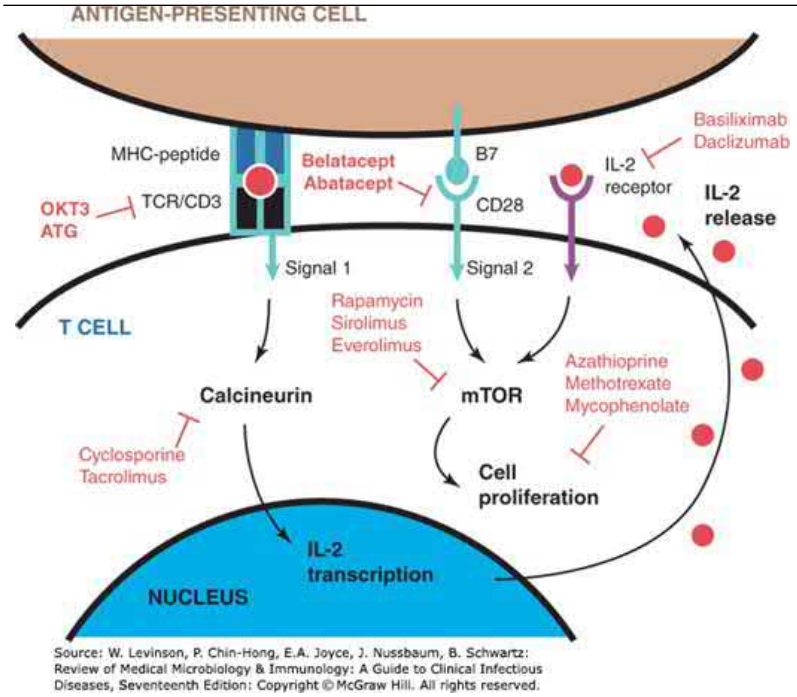
Immunosuppressive Therapies Used in Transplantation

Category	Example(s)	Mechanism of Action
Corticosteroids	Prednisone Methylprednisolone	Bind to glucocorticoid receptor, causing many changes in gene transcription. Suppress production of inflammatory mediators (including leukotrienes and prostaglandins) and cytokines (including IL-1 and TNF). Induce apoptosis in T cells and B cells.
DNA synthesis inhibitors	Azathioprine Methotrexate Mycophenolate	Inhibit purine synthesis or purine metabolism, preventing DNA synthesis, which leads to apoptosis in rapidly dividing lymphocytes. Azathioprine is converted to 6-mercaptopurine. Methotrexate may also directly impair T-cell and B-cell function through its effects on various signaling enzymes.
Calcineurin inhibitors	Cyclosporine Tacrolimus	Cyclosporine binds to cyclophilin; tacrolimus binds to FKBP1A. Inhibition of calcineurin blocks calcium-dependent dephosphorylation (activation) of NFAT, a transcription factor required for the **first phase** of T-cell activation. Reduces synthesis of IL-2 and IL-2 receptor.
mTOR inhibitors	Sirolimus/rapamycin Everolimus	Binds to FKBP1A. Inhibits mTOR, which is involved in the **second phase** of T-cell activation. Blocks IL-2 signal transduction and clonal proliferation.
Signaling blockade	Belatacept Abatacept	These are CTLA-4 proteins fused with an Fc region of an immunoglobulin. They act as “mimics” that bind B7 molecules on APCs with high affinity, competing with CD28 and preventing them from providing co-stimulatory signals to T cells.
	Basiliximab Daclizumab	Monoclonal mouse/human mixture antibodies that bind and block the IL-2 receptor on T cells, inhibiting proliferation.
	Muromonab (OKT3)	Monoclonal mouse antibody that binds and blocks CD3, inhibiting T-cell receptor signaling.
Cell-depleting antibodies	Antithymocyte globulin	Polyclonal horse or rabbit antibodies prepared by immunizing animals with human T cells. Antibodies bind to multiple targets (CD3, CD4, CD8, etc.) and cause complement-mediated cell lysis; binding also inhibits signaling to the T cells, suppressing their survival and activation.

APC = antigen-presenting cell; IL = interleukin; CTLA = cytotoxic T-cell associated protein; mTOR = mammalian target of rapamycin; NFAT = nuclear factor of activated T cells; TNF = tumor necrosis factor.

FIGURE 62–3

Mechanism of action of important immunosuppressive drugs, depicted on a schematic of an interaction in which an antigen-presenting cell activates a T cell. Corticosteroids have varied effects and are not described in the figure. ATG = antithymocyte globulin.



Corticosteroids bind to glucocorticoid receptors that result in altered gene transcription in a variety of cell types. In immune cells, steroids act by inhibiting synthesis of leukotrienes, prostaglandins, and cytokines (e.g., IL-2) and by inducing apoptosis of rapidly dividing T cells. Corticosteroids inhibit cytokine production by blocking transcription factors, such as nuclear factor-κB and AP-1, which prevents the mRNA for these cytokines from being synthesized. Glucocorticoid receptors are found in nearly every cell in the body, causing widespread off-target changes in gene transcription. Therefore, the major disadvantage of corticosteroids that limits their chronic use is that they have numerous adverse endocrine, neuropsychiatric, metabolic, and cardiovascular side effects.

Azathioprine (which is converted to 6-mercaptopurine in the body), methotrexate, and mycophenolate mofetil inhibit different aspects of the nucleotide synthesis and metabolism, **shutting down DNA synthesis** and thereby blocking T-cell proliferation. Methotrexate may also have a variety of other effects on cell signaling through inhibition of signaling enzymes.

Cyclosporine prevents the activation of T lymphocytes by inhibiting the synthesis of interleukin (IL)-2 and IL-2 receptor. It does so by **inhibiting calcineurin**—a phosphatase enzyme that is activated by calcium flux following binding of the T-cell receptor, the first step in the cascade that ultimately leads to transcription of the genes encoding IL-2 and the IL-2 receptor. Tacrolimus binds a different protein (FKBP1A) but has a similar effect on calcineurin. Thus, cyclosporine and tacrolimus inhibit one of the earliest steps in the **first phase** of T-cell activation.

Sirolimus inhibits signal transduction through **mTOR**, which is primarily involved in signal transduction *downstream* of IL-2. Therefore, sirolimus inhibits later steps in the **second phase** of T-cell activation, in a pathway different from that of cyclosporine and tacrolimus. These drugs are more selective than steroids and therefore have fewer toxicities.

Belatacept is a fusion protein consisting of cytotoxic T lymphocyte antigen-4 (CTLA-4) fused to the Fc fragment of human IgG. CTLA-4 competes with CD28 for binding to B7 proteins, but does so with higher affinity, thereby **blocking co-stimulation of T cells** and preventing graft rejection. Muromonab (OKT3) was the first approved monoclonal antibody. It is a mouse antibody against CD3 that **blocks signal transduction through the T-cell receptor**. Basiliximab is a chimeric monoclonal antibody that **blocks the IL-2 receptor**, preventing T-cell proliferation.

ATG is a polyclonal cocktail of horse (Atgam) or rabbit (Thymoglobulin) antibodies against human thymocytes. ATG contains antibodies against many lymphocyte antigens (e.g., CD3, CD4, CD8, and others). After binding to their targets on the surfaces of T cells, these antibodies **kill T cells** through complement-mediated lysis of the cell (among other potential mechanisms). As a consequence, ATG has a broader immunosuppressive effect than do the more targeted monoclonal antibodies described in the previous paragraph.

Unfortunately, immunosuppression greatly enhances the recipient's susceptibility to opportunistic infections and neoplasms. For example, some patients undergoing treatment for multiple sclerosis with the monoclonal antibody natalizumab developed progressive multifocal

leukoencephalopathy (see Chapter 44 for a description of this viral disease). The incidence of cancer is increased as much as 100-fold in transplant recipients who have been immunosuppressed for a long time. Common cancers in these patients include squamous cell carcinoma of the skin, adenocarcinoma of the colon and the lung, and lymphoma.

Chapter 63: Complement

INTRODUCTION

The complement system consists of approximately 20 proteins that are present in normal human (and other animal) serum. The term *complement* refers to the ability of these proteins to complement (i.e., augment) the effects of other components of the immune system (e.g., antibody). Complement is an important component of our innate host defenses.

There are three main effects of complement: (1) **lysis** of cells such as bacteria, allografts, and tumor cells; (2) **generation of mediators** that participate in inflammation and attract neutrophils; and (3) **opsonization** (i.e., enhancement of phagocytosis). Complement proteins are synthesized mainly by the liver.

ACTIVATION OF COMPLEMENT

Several complement components are proenzymes that must be cleaved to form active enzymes. Activation of the complement system can be initiated either by antigen–antibody complexes or by a variety of nonimmunologic molecules (e.g., endotoxin).

Sequential activation of complement components (Figure 63–1) occurs via one of three pathways: the *classical* pathway, the *lectin* pathway, and the *alternative* pathway (see later). Of these pathways, the **lectin and the alternative pathways are more important the first time** we are infected by a microorganism because the antibody required to trigger the classical pathway is not present.

FIGURE 63–1

The classical and alternative pathways of the complement system indicate that proteolytic cleavage of the molecule at the tip of the arrow has occurred; a line over a complex indicates that it is enzymatically active. Note that all small fragments are labeled “a,” and all large fragments are labeled “b.” Hence, the C3 convertase is depicted as C4b,2b. Note that proteases associated with the mannan-binding lectin cleave C4 as well as C2.

Structure and Function of the Genetic Material

LEARNING OBJECTIVES

- **8-1** Define *genetics, genome, chromosome, gene, genetic code, genotype, phenotype,* and *genomics.*
- **8-2** Describe how DNA serves as genetic information.
- **8-3** Describe the process of DNA replication.
- **8-4** Describe protein synthesis, including transcription, RNA processing, and translation.
- **8-5** Compare protein synthesis in prokaryotes and eukaryotes.

Genetics is the science of heredity; it includes the study of what genes are, how they carry information, how they are replicated and passed to subsequent generations of cells or passed between organisms, and how the expression of their information within an organism determines the particular characteristics of that organism. The genetic information in a cell is called the **genome.** A cell's genome includes its chromosomes and plasmids. **Chromosomes** are structures containing DNA that physically carry hereditary information; the chromosomes contain the genes. **Genes** are segments of DNA (except in some viruses, in which they are made of RNA) that code for functional products. We saw in Chapter 2 that DNA is a macromolecule composed of repeating units called *nucleotides.* Recall that each nucleotide consists of a nucleobase (adenine, thymine, cytosine, or guanine), deoxyribose (a pentose sugar), and a phosphate group (see Figure 2.16, page 48). The DNA within a cell exists as long strands of nucleotides twisted together in pairs to form a double helix. Each strand has a string of alternating sugar and phosphate groups (its *sugar-phosphate backbone*), and a nitrogenous base is attached to each sugar in the backbone. The two strands are held together by hydrogen bonds between their nitrogenous bases. The **base pairs** always occur in a specific way: adenine always pairs with thymine, and cytosine always pairs with guanine. Because of this specific base pairing, the base sequence of one DNA strand determines the base sequence of the other strand. The two strands of DNA are thus *complementary.*

The structure of DNA helps explain two primary features of biological information storage. First, the linear sequence of bases provides the actual information. Genetic information is encoded by the sequence of bases along a strand of DNA, in much the same way as our written language uses a linear sequence of letters to form words and sentences. The genetic language, however, uses an alphabet with only four letters—the four kinds of nucleobases in DNA (or RNA). But 1000 of these four bases, the number contained in an average-sized gene, can be arranged in 4^{1000} different ways. This astronomically large number explains how genes can be varied enough to provide all the information a cell needs to grow and perform its functions. The **genetic code,** the set of rules that determines how a nucleotide sequence is converted into the amino acid sequence of a protein, is discussed in more detail later in the chapter.

Second, the complementary structure allows for the precise duplication of DNA during cell division. Each daughter cell receives one of the original strands from the parent; thus ensuring one strand that functions correctly.

Much of cellular metabolism is concerned with translating the genetic message of genes into specific proteins. A gene usually codes for a messenger RNA (mRNA) molecule, which ultimately results in the formation of a protein. Alternatively, the gene product can be a ribosomal RNA (rRNA) or a transfer RNA (tRNA). As we will see, all of these types of RNA are involved in the process of protein synthesis. When the ultimate molecule for which a gene codes (a protein, for example) has been produced, we say that the gene has been *expressed.*

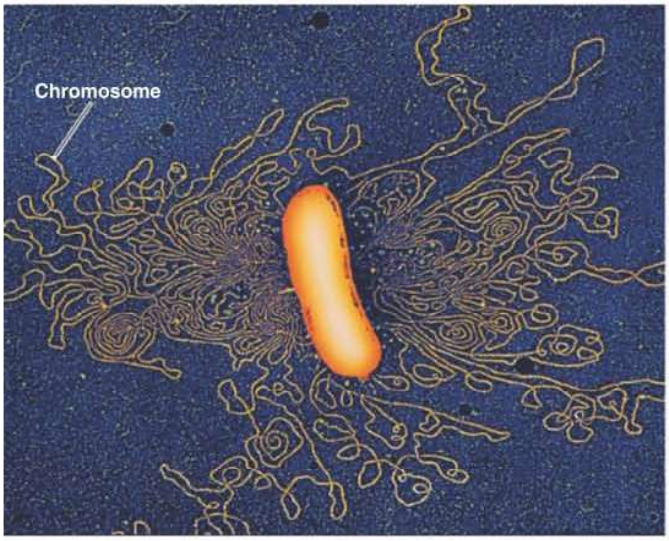
Genotype and Phenotype

The **genotype** of an organism is its genetic makeup, the information that codes for all the particular characteristics of the organism. The genotype represents *potential* properties, but not the properties themselves. **Phenotype** refers to *actual, expressed* properties, such as the organism's ability to perform a particular chemical reaction. Phenotype, then, is the manifestation of genotype.

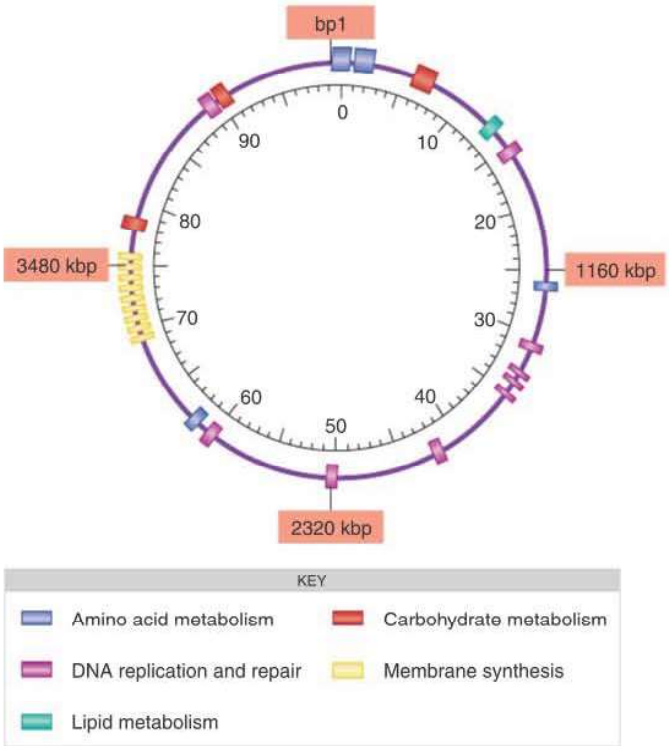
In molecular terms, an organism's genotype is its collection of genes, its entire DNA. What constitutes the organism's phenotype in molecular terms? In a sense, an organism's phenotype is its collection of proteins. Most of a cell's properties derive from the structures and functions of its proteins. In microbes, most proteins are either *enzymatic* (catalyze particular reactions) or *structural* (participate in large functional complexes such as membranes or flagella). Even phenotypes that depend on structural macromolecules other than proteins (such as lipids or polysaccharides) rely indirectly on proteins. For instance, the structure of a complex lipid or polysaccharide molecule results from the catalytic activities of enzymes that synthesize, process, and degrade those molecules. Thus, although it is not completely accurate to say that phenotypes are due only to proteins, it is a useful simplification.

DNA and Chromosomes

Bacteria typically have a single circular chromosome consisting of a single circular molecule of DNA with associated proteins. The chromosome is looped and folded and attached at one or several points to the plasma membrane. The DNA of *E. coli,* the most-studied bacterial species, has about 4.6 million base pairs and is about 1 mm long—1000 times longer than the entire cell



(a) The tangled mass and looping strands of DNA emerging from this disrupted *E. coli* cell are part of its single chromosome.



(b) A genetic map of the chromosome of *E. coli*. The numbers inside the circle indicate the number of minutes it takes to transfer the genes during mating between two cells; the numbers in colored boxes indicate the number of base pairs.

Figure 8.1 A prokaryotic chromosome.

Q **What is a gene? What is an open-reading frame?**

(Figure 8.1a). However, the chromosome takes up only about 10% of the cell's volume because the DNA is twisted, or *supercoiled*—much like a telephone cord when you put the handset back on the receiver.

The location of genes on a bacterial chromosome can be determined by experiments on the transfer of genes from one cell to another. These processes will be discussed later in this chapter. The bacterial chromosome map that results is marked in minutes corresponding to when the genes are transferred from a donor cell to a recipient cell (Figure 8.1b).

In recent years, the complete base sequences of several bacterial chromosomes have been determined. Computers are used to search for *open-reading frames,* that is, regions of DNA that are likely to encode a protein. As you will see later, these are base sequences between start and stop codons. The sequencing and molecular characterization of genomes is called **genomics.** The use of genomics to track West Nile virus is described in the box on page 223.

The Flow of Genetic Information

DNA replication makes possible the flow of genetic information from one generation to the next. As shown in Figure 8.2, the DNA of a cell replicates before cell division so that each offspring cell receives a chromosome identical to the parent's. Within each metabolizing cell, the genetic information contained in DNA also flows in another way: it is transcribed into mRNA and then translated into protein. We describe the processes of transcription and translation later in this chapter.

CHECK YOUR UNDERSTANDING

- Give a clinical application of genomics. 8-1
- Why is the base pairing in DNA important? 8-2

DNA Replication

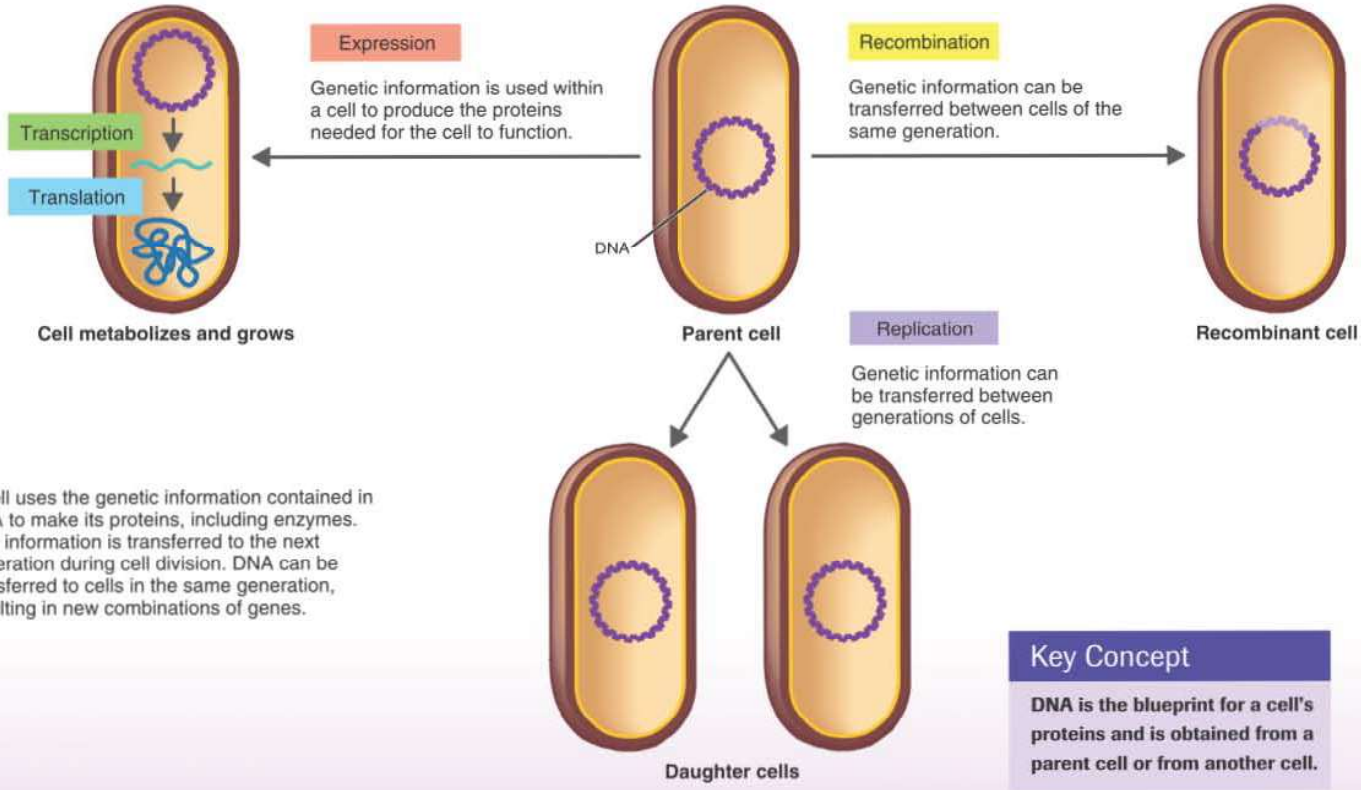
In DNA replication, one "parental" double-stranded DNA molecule is converted to two identical "daughter" molecules. The complementary structure of the nitrogenous base sequences in the DNA molecule is the key to understanding DNA replication. Because the bases along the two strands of double-helical DNA are complementary, one strand can act as a template for the production of the other strand (Figure 8.3a).

DNA replication requires the presence of several cellular proteins that direct a particular sequence of events. Enzymes involved in DNA replication and other processes are listed in Table 8.1 on page 215. When replication begins, the supercoiling is relaxed by *topoisomerase* or *gyrase*, and the two strands of parental DNA are unwound by *helicase* and separated from

Figure 8.2

FOUNDATION FIGURE The Flow of Genetic Information

Using the example of a bacterium with a single circular chromosome, this figure summarizes how genetic information is used within and passed between cells. Small versions of the relevant portions of this overview figure will appear in other figures throughout the chapter to indicate the relationships of different processes.



A cell uses the genetic information contained in DNA to make its proteins, including enzymes. This information is transferred to the next generation during cell division. DNA can be transferred to cells in the same generation, resulting in new combinations of genes.

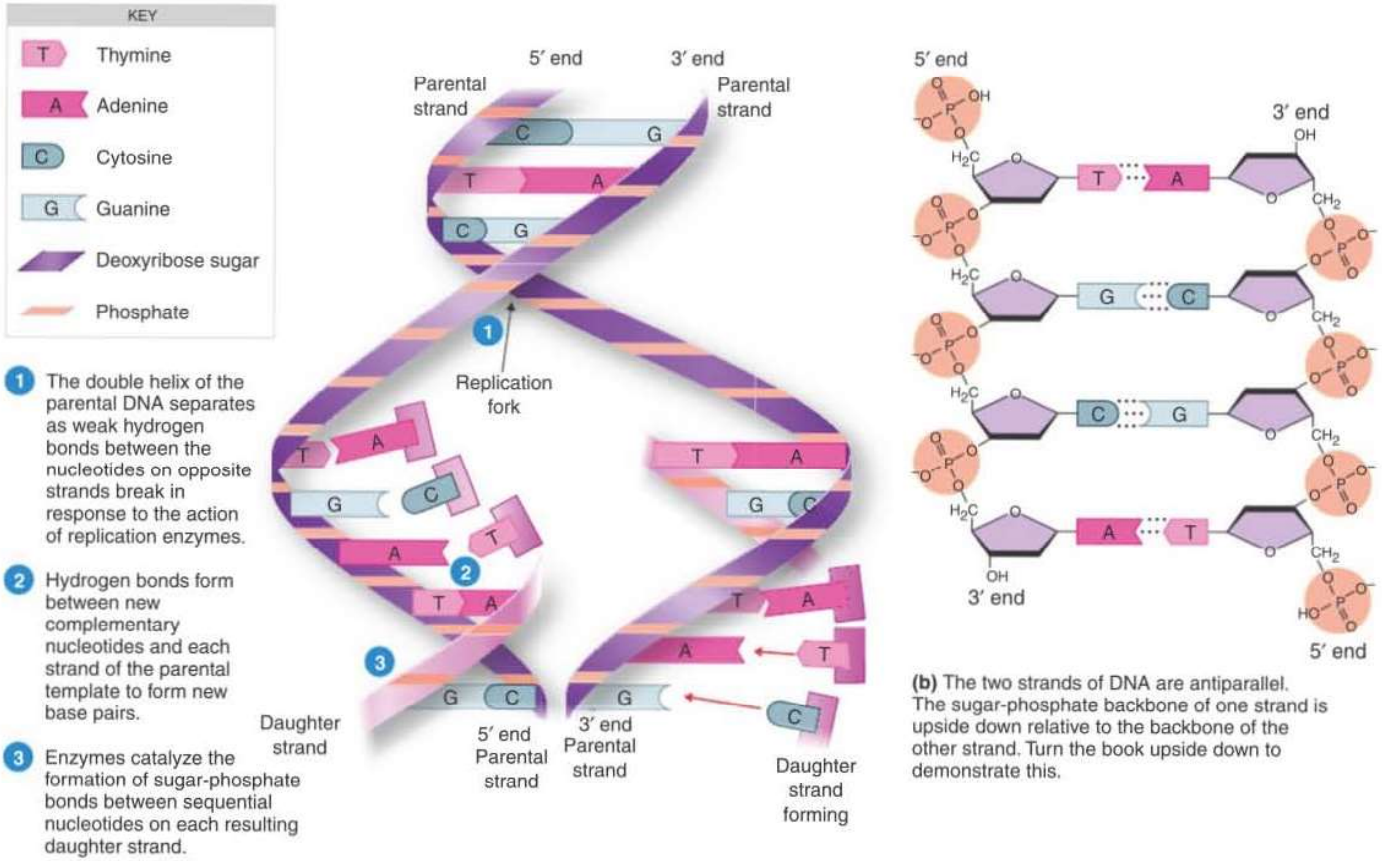
Key Concept

DNA is the blueprint for a cell's proteins and is obtained from a parent cell or from another cell.

each other in one small DNA segment after another. Free nucleotides present in the cytoplasm of the cell are matched up to the exposed bases of the single-stranded parental DNA. Where thymine is present on the original strand, only adenine can fit into place on the new strand; where guanine is present on the original strand, only cytosine can fit into place, and so on. Any bases that are improperly base-paired are removed and replaced by replication enzymes. Once aligned, the newly added nucleotide is joined to the growing DNA strand by an enzyme called **DNA polymerase.** Then the parental DNA is unwound a bit further to allow the addition of the next nucleotides. The point at which replication occurs is called the *replication fork.*

As the replication fork moves along the parental DNA, each of the unwound single strands combines with new nucleotides. The original strand and this newly synthesized daughter strand then rewind. Because each new double-stranded DNA molecule contains one original (conserved) strand and one new strand, the process of replication is referred to as **semiconservative replication.**

Before looking at DNA replication in more detail, let's take a closer look at the structure of DNA (see Figure 2.16, on page 48). It is important to understand the concept that the paired DNA strands are oriented in opposite directions relative to each other. Notice in Figure 2.16 that the carbon atoms of the sugar component of each nucleotide are numbered 1′ (pronounced "one prime") to 5′. For the paired bases to be next to each other, the sugar components in one strand are upside down relative to the other. The end with the hydroxyl attached to the 3′ carbon is called the 3′ end of the DNA strand; the end having a phosphate attached



(a) The replication fork.

(b) The two strands of DNA are antiparallel. The sugar-phosphate backbone of one strand is upside down relative to the backbone of the other strand. Turn the book upside down to demonstrate this.

Figure 8.3 DNA replication.

Q **What is meant by semiconservative replication?**

to the 5′ carbon is called the 5′ end. The way in which the two strands fit together dictates that the 5′ → 3′ direction of one strand runs counter to the 5′ → 3′ direction of the other strand (**Figure 8.3b**). This structure of DNA affects the replication process because DNA polymerases can add new nucleotides to the 3′ end only. Therefore, as the replication fork moves along the parental DNA, the two new strands must grow in different directions.

DNA replication requires a great deal of energy. The energy is supplied from the nucleotides, which are actually nucleoside triphosphates. You already know about ATP; the only difference between ATP and the adenine nucleotide in DNA is the sugar component. Deoxyribose is the sugar in the nucleosides used to synthesize DNA, and nucleoside triphosphates with ribose are used to synthesize RNA. Two phosphate groups are removed to add the nucleotide to a growing strand of DNA; hydrolysis of the nucleoside is exergonic and provides energy to make the new bonds in the DNA strand (**Figure 8.4**).

Figure 8.5 provides more detail about the many steps that go into this complex process.

DNA replication by some bacteria, such as *E. coli*, goes *bidirectionally* around the chromosome (**Figure 8.6**). Two replication forks move in opposite directions away from the origin of replication. Because the bacterial chromosome is a closed loop, the replication forks eventually meet when replication is completed. The two loops must be separated by a topoisomerase. Much evidence shows an association between the bacterial plasma membrane and the origin of replication. After duplication, if each copy of the origin binds to the membrane at opposite poles, then each daughter cell receives one copy of the DNA molecule—that is, one complete chromosome.

DNA replication is an amazingly accurate process. Typically, mistakes are made at a rate of only 1 in every 10^{10} bases incorporated. Such accuracy is largely due to the *proofreading* capability of DNA polymerase. As each new base is added, the enzyme evaluates whether it forms the proper complementary base-pairing structure. If not, the enzyme excises the improper

Table 8.1 Important Enzymes in DNA Replication, Expression, and Repair

DNA Gyrase	Relaxes supercoiling ahead of the replication fork
DNA Ligase	Makes covalent bonds to join DNA strands; joins Okazaki fragments and new segments in excision repair
DNA Polymerase	Synthesizes DNA; proofreads and repairs DNA
Endonucleases	Cut DNA backbone in a strand of DNA; facilitate repair and insertions
Exonucleases	Cut DNA from an exposed end of DNA; facilitate repair
Helicase	Unwinds double-stranded DNA
Methylase	Adds methyl group to selected bases in newly made DNA
Photolyase	Uses visible light energy to separate UV-induced pyrimidine dimers
Primase	Makes RNA primers from a DNA template
Ribozyme	RNA enzyme that removes introns and splices exons together
RNA Polymerase	Copies RNA from a DNA template
snRNP	RNA-protein complex that removes introns and splices exons together
Topoisomerase	Relaxes supercoiling ahead of the replication fork; separates DNA circles at the end of DNA replication
Transposase	Cuts DNA backbone leaving single-stranded "sticky ends"

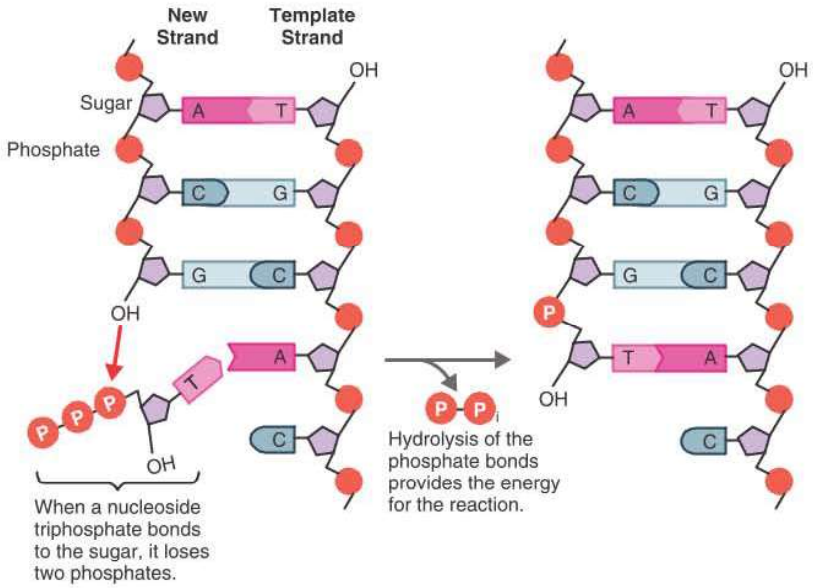
base and replaces it with the correct one. In this way, DNA can be replicated very accurately, allowing each daughter chromosome to be virtually identical to the parental DNA. **Animations** DNA Replication: Overview, Forming the Replication Fork, Replication Proteins, Synthesis. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

✓ Describe DNA replication, including the functions of DNA gyrase, DNA ligase, and DNA polymerase. **8-3**

Figure 8.4 Adding a nucleotide to DNA.

Q Why is one strand "upside down" relative to the other strand? Why can't both strands "face" the same way?



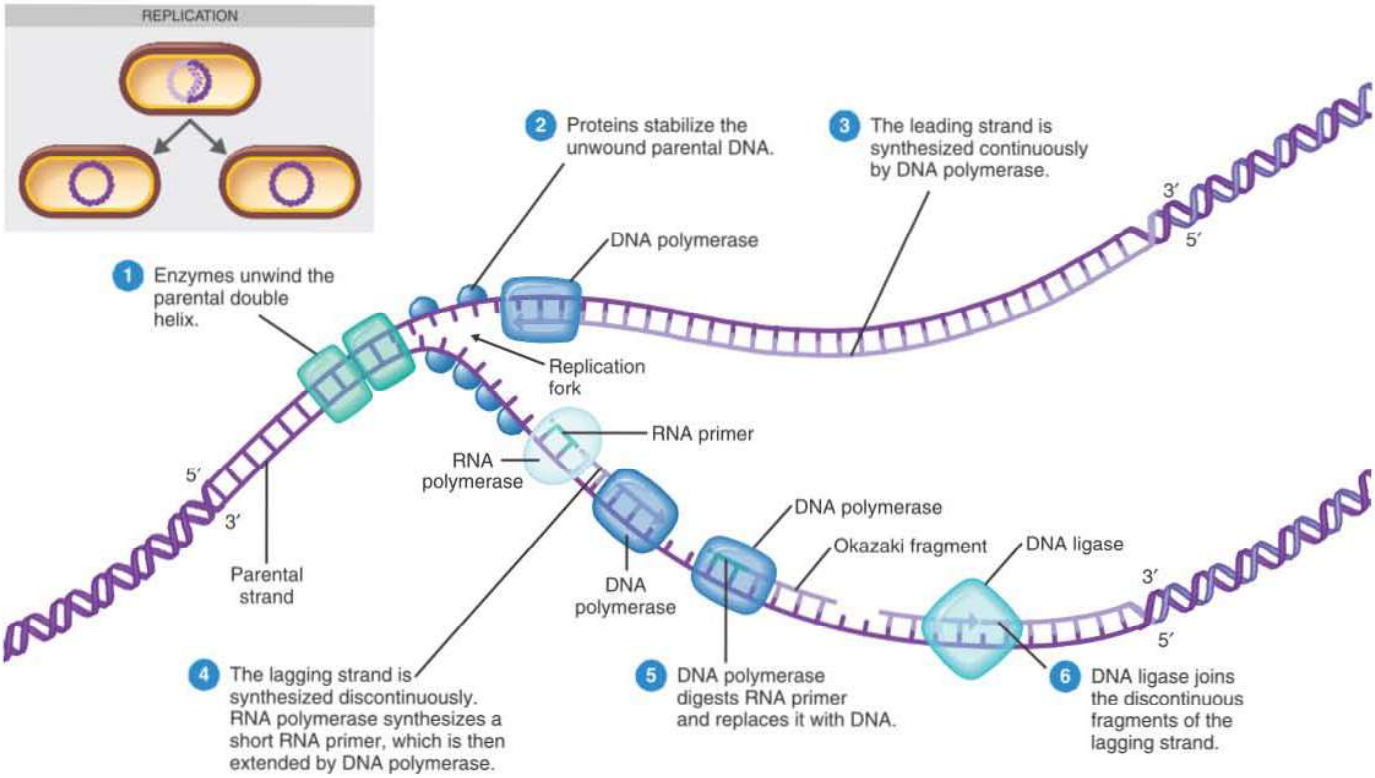


Figure 8.5 A summary of events at the DNA replication fork.

Q **Why is one strand of DNA synthesized discontinuously?**

RNA and Protein Synthesis

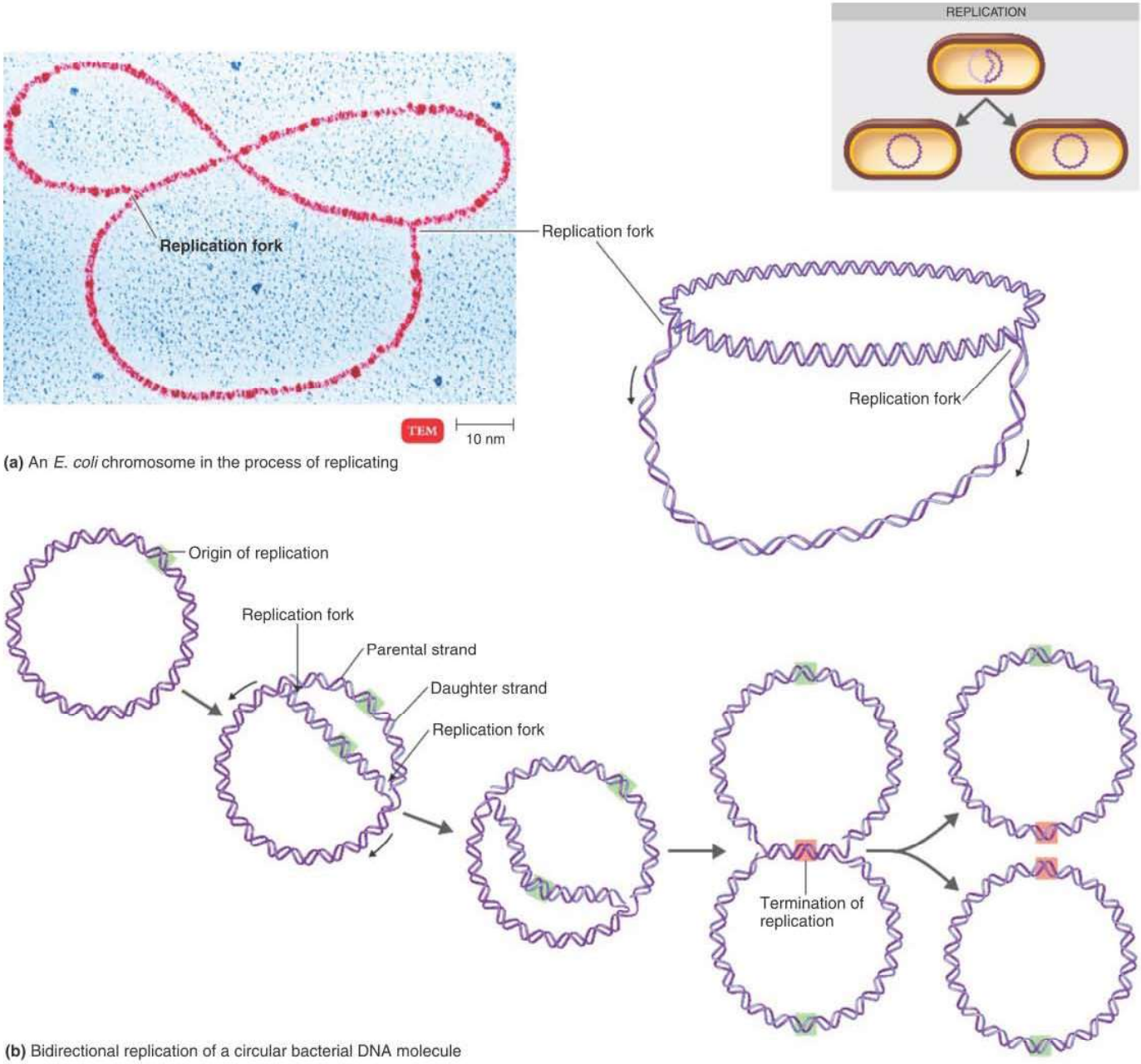
How is the information in DNA used to make the proteins that control cell activities? In the process of *transcription,* genetic information in DNA is copied, or transcribed, into a complementary base sequence of RNA. The cell then uses the information encoded in this RNA to synthesize specific proteins through the process of *translation.* We now take a closer look at these two processes as they occur in a bacterial cell.

Transcription

Transcription is the synthesis of a complementary strand of RNA from a DNA template. We will discuss transcription in prokaryotic cells here. Transcription in eukaryotes is discussed on page 220. As mentioned earlier, there are three kinds of RNA in bacterial cells: messenger RNA, ribosomal RNA, and transfer RNA. Ribosomal RNA forms an integral part of ribosomes, the cellular machinery for protein synthesis. Transfer RNA is also involved in protein synthesis, as we will see. **Messenger RNA (mRNA)** carries the coded information for making specific proteins from DNA to ribosomes, where proteins are synthesized.

During transcription, a strand of mRNA is synthesized using a specific portion of the cell's DNA as a template. In other words, the genetic information stored in the sequence of nitrogenous bases of DNA is rewritten so that the same information appears in the base sequence of mRNA. As in DNA replication, a G in the DNA template dictates a C in the mRNA being made, a C in the DNA template dictates a G in the mRNA, and a T in the DNA template dictates an A in the mRNA. However, an A in the DNA template dictates a uracil (U) in the mRNA, because RNA contains U instead of T. (U has a chemical structure slightly different from T, but it base-pairs in the same way.) If, for example, the template portion of DNA has the base sequence 3′-ATGCAT, the newly synthesized mRNA strand will have the complementary base sequence 5′-UACGUA.

The process of transcription requires both an enzyme called *RNA polymerase* and a supply of RNA nucleotides (**Figure 8.7**). Transcription begins when RNA polymerase binds to the DNA at a site called the **promoter.** Only one of the two DNA strands serves as the template for RNA synthesis for a given gene. Like DNA, RNA is synthesized in the 5′ → 3′ direction. RNA



(a) An *E. coli* chromosome in the process of replicating

(b) Bidirectional replication of a circular bacterial DNA molecule

Figure 8.6 Replication of bacterial DNA.

Q **What is the origin of replication?**

synthesis continues until RNA polymerase reaches a site on the DNA called the **terminator.**

The process of transcription allows the cell to produce short-term copies of genes that can be used as the direct source of information for protein synthesis. Messenger RNA acts as an intermediate between the permanent storage form, DNA, and the process that uses the information, translation. **Animations** Transcription: Overview, Process. **www.microbiologyplace.com**

Translation

We have seen how the genetic information in DNA is transferred to mRNA during transcription. Now we will see how mRNA

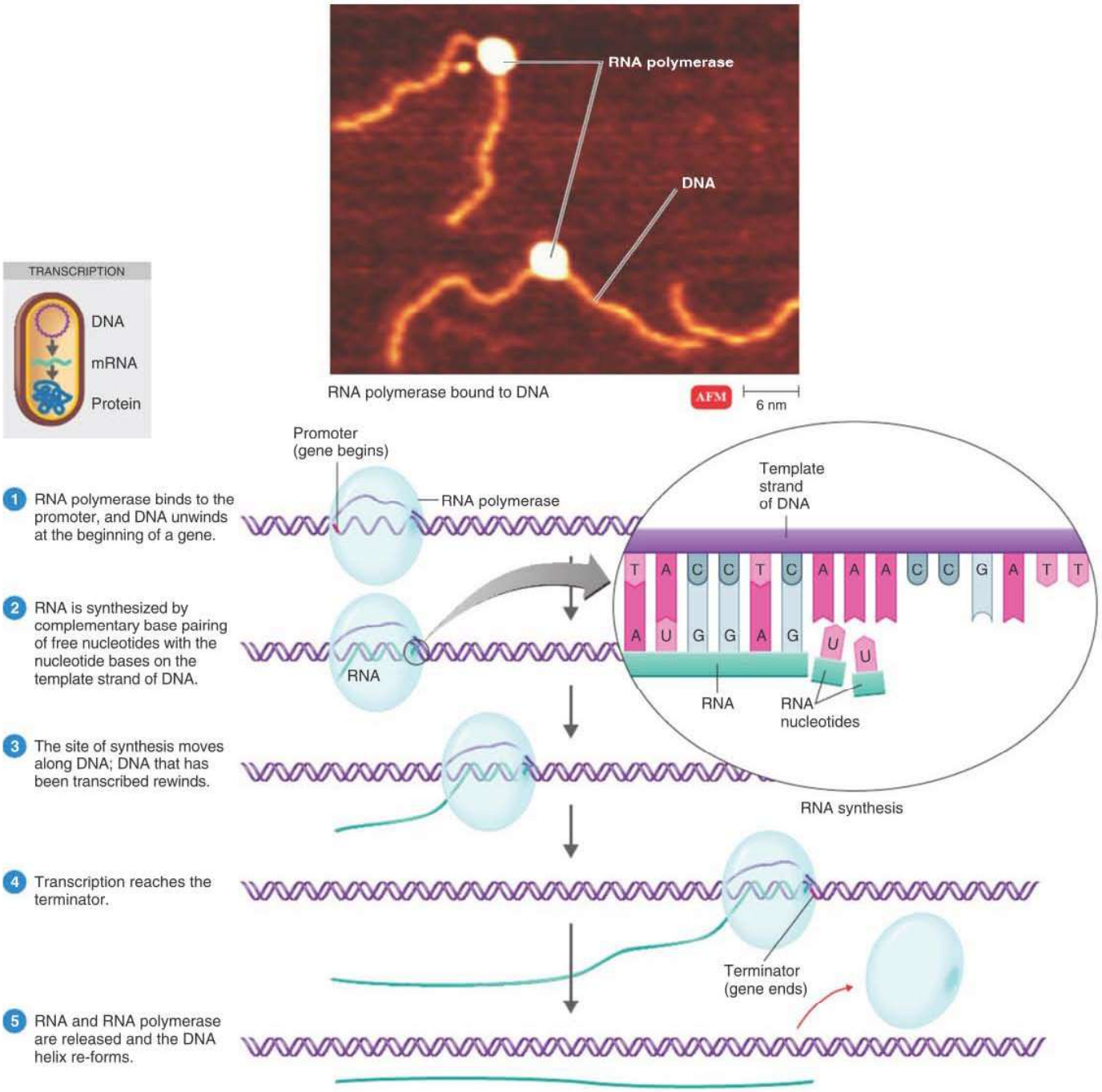


Figure 8.7 The process of transcription. The orienting diagram indicates the relationship of transcription to the overall flow of genetic information within a cell.

Q In transcription, what is copied, and what is made?

serves as the source of information for the synthesis of proteins. Protein synthesis is called **translation** because it involves decoding the "language" of nucleic acids and converting that information into the "language" of proteins.

The language of mRNA is in the form of **codons,** groups of three nucleotides, such as AUG, GGC, or AAA. The sequence of codons on an mRNA molecule determines the sequence of amino acids that will be in the protein being synthesized. Each codon "codes" for a particular amino acid. This is the genetic code (Figure 8.8).

Codons are written in terms of their base sequence in mRNA. Notice that there are 64 possible codons but only 20 amino acids. This means that most amino acids are signaled by several alternative codons, a situation referred to as the **degeneracy** of the code. For example, leucine has six codons, and alanine has four codons. Degeneracy allows for a certain amount of change, or mutation, in the DNA without affecting the protein ultimately produced.

Of the 64 codons, 61 are sense codons, and 3 are nonsense codons. **Sense codons** code for amino acids, and **nonsense codons** (also called *stop codons*) do not. Rather, the nonsense codons—UAA, UAG, and UGA—signal the end of the protein molecule's synthesis. The start codon that initiates the synthesis of the protein molecule is AUG, which is also the codon for methionine. In bacteria, the start AUG codes for formylmethionine rather than the methionine found in other parts of the protein. The initiating methionine is often removed later, so not all proteins begin with methionine.

The codons of mRNA are converted into protein through the process of translation. The codons of an mRNA are "read" sequentially; and, in response to each codon, the appropriate amino acid is assembled into a growing chain. The site of translation is the ribosome, and **transfer RNA (tRNA)** molecules both recognize the specific codons and transport the required amino acids.

Each tRNA molecule has an **anticodon,** a sequence of three bases that is complementary to a codon. In this way, a tRNA molecule can base-pair with its associated codon. Each tRNA can also carry on its other end the amino acid encoded by the codon that the tRNA recognizes. The functions of the ribosome are to direct the orderly binding of tRNAs to codons and to assemble the amino acids brought there into a chain, ultimately producing a protein.

Figure 8.9 shows the details of translation. The necessary components assemble: the two ribosomal subunits, a tRNA with the anticodon UAC, and the mRNA molecule to be translated, along with several additional protein factors. This sets up the start codon (AUG) in the proper position to allow translation to begin. After the ribosome joins the first two amino acids with a peptide bond, the first tRNA molecule leaves the ribosome. The ribosome then moves along the mRNA to the next codon. As the proper amino acids are brought into line one by one, peptide bonds are formed between them, and a polypeptide chain results. (See Figure 2.14, page 45.) Translation ends when one of the three nonsense codons in the mRNA is reached. The ribosome then comes apart into its two subunits, and the mRNA and newly synthesized polypeptide chain are released. The ribosome, the mRNA, and the tRNAs are then available to be used again.

The ribosome moves along the mRNA in the 5′ → 3′ direction. As a ribosome moves along the mRNA, it will soon allow the start codon to be exposed. Additional ribosomes can then assemble and begin synthesizing protein. In this way, there are usually a number of ribosomes attached to a single mRNA, all at various stages of protein synthesis. In prokaryotic cells, the

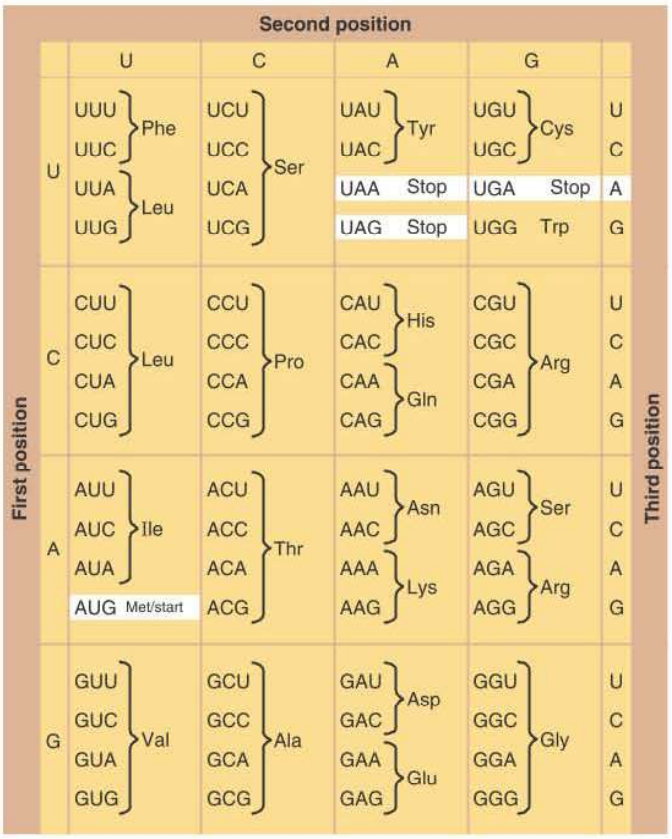


Figure 8.8 The genetic code. The three nucleotides in an mRNA codon are designated, respectively, as the first position, second position, and third position of the codon on the mRNA. Each set of three nucleotides specifies a particular amino acid, represented by a three-letter abbreviation (see Table 2.4, page 44). The codon AUG, which specifies the amino acid methionine, is also the start of protein synthesis. The word *Stop* identifies the nonsense codons that signal the termination of protein synthesis.

Q Why is the genetic code described as degenerate?

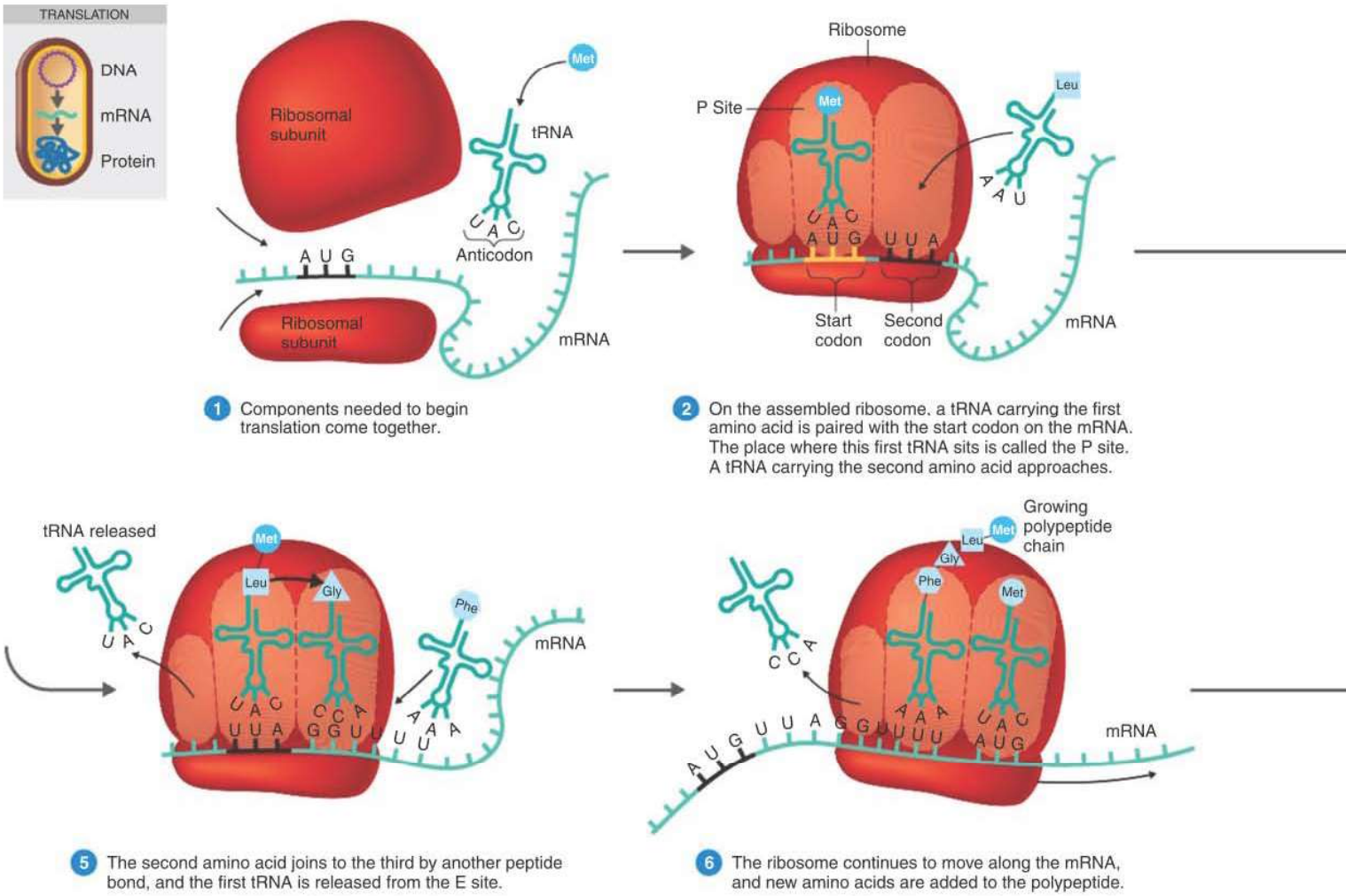


Figure 8.9 The process of translation. The overall goal of translation is to produce proteins using mRNAs as the source of biological information. The complex cycle of events illustrated here shows the primary role of tRNA and ribosomes in the decoding of this information. The ribosome acts as the site where the mRNA-encoded information is decoded, as well as the site where individual amino acids are connected into polypeptide chains. The tRNA molecules act as the actual "translators"—one end of each tRNA recognizes a specific mRNA codon, while the other end carries the amino acid coded for by that codon.

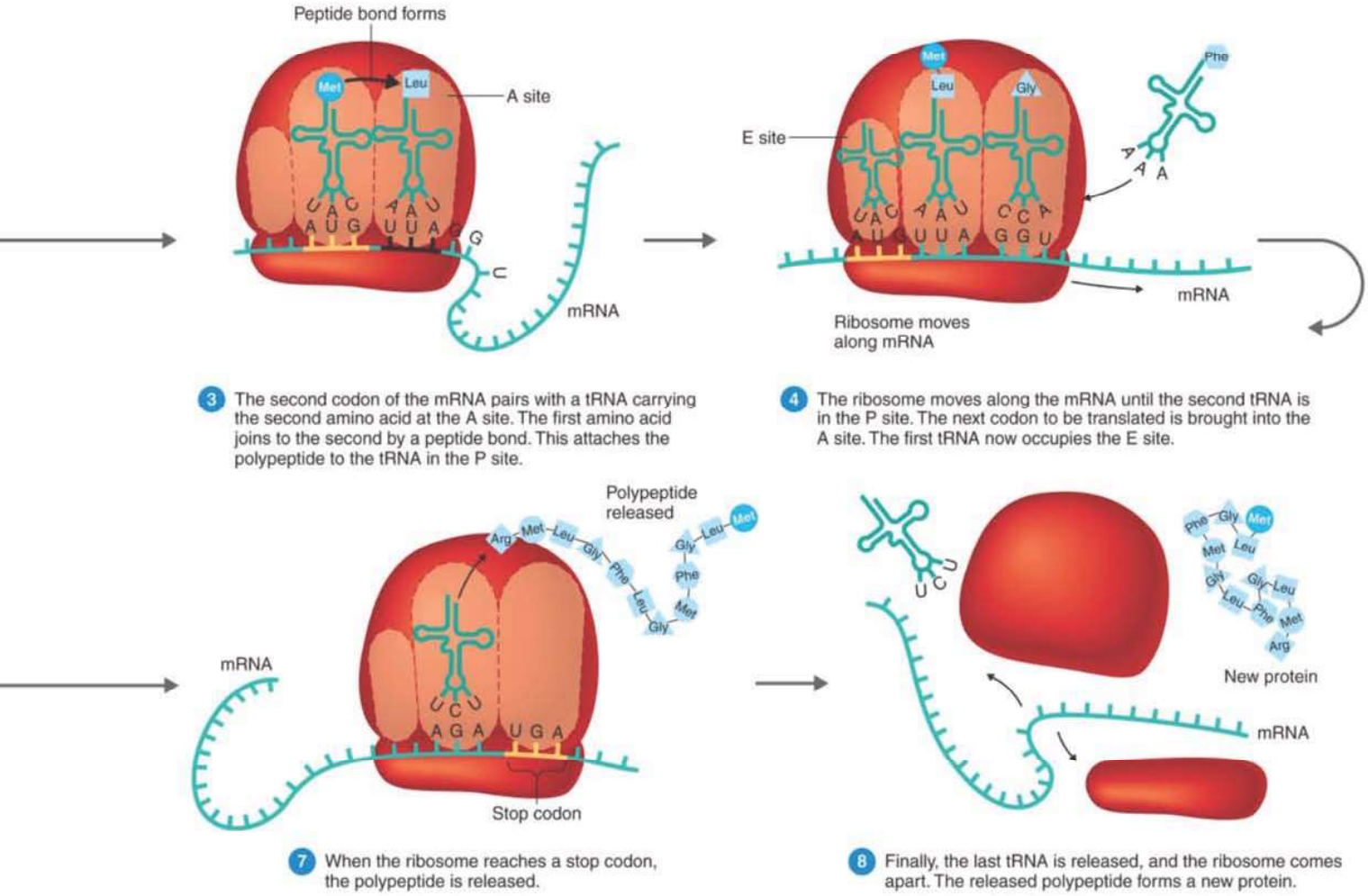
Q **Why is this process called translation?**

translation of mRNA into protein can begin even before transcription is complete (**Figure 8.10**). Because mRNA is produced in the cytoplasm, the start codons of an mRNA being transcribed are available to ribosomes before the entire mRNA molecule is even made.

In eukaryotic cells, transcription takes place in the nucleus. The mRNA must be completely synthesized and moved through the nuclear membrane to the cytoplasm before translation can begin. In addition, the RNA undergoes processing before it leaves the nucleus. In eukaryotic cells, the regions of genes that code for proteins are often interrupted by noncoding DNA. Thus, eukaryotic genes are composed of **exons,** the regions of DNA *expressed*, and **introns,** the *intervening* regions of DNA that do not encode protein. In the nucleus, RNA polymerase synthesizes a molecule called an RNA transcript that contains copies of the introns. Particles called **small nuclear ribonucleoproteins,** abbreviated **snRNPs** and pronounced "snurps," remove the introns and splice the exons together. In some organisms, the introns act as ribozymes to catalyze their own removal (**Figure 8.11**).

* * *

To summarize, genes are the units of biological information encoded by the sequence of nucleotide bases in DNA. A gene is



3 The second codon of the mRNA pairs with a tRNA carrying the second amino acid at the A site. The first amino acid joins to the second by a peptide bond. This attaches the polypeptide to the tRNA in the P site.

4 The ribosome moves along the mRNA until the second tRNA is in the P site. The next codon to be translated is brought into the A site. The first tRNA now occupies the E site.

7 When the ribosome reaches a stop codon, the polypeptide is released.

8 Finally, the last tRNA is released, and the ribosome comes apart. The released polypeptide forms a new protein.

Figure 8.9 The process of translation. (continued)

expressed, or turned into a product within the cell, through the processes of transcription and translation. The genetic information carried in DNA is transferred to a temporary mRNA molecule by transcription. Then, during translation, the mRNA directs the assembly of amino acids into a polypeptide chain: a ribosome attaches to mRNA, tRNAs deliver the amino acids to the ribosome as directed by the mRNA codon sequence, and the ribosome assembles the amino acids into the chain that will be the newly synthesized protein. **Animations** Translation: Overview, Genetic Code, Process. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

- What is the role of the promoter, terminator, and mRNA in transcription? **8-4**
- How does mRNA production in eukaryotes differ from the process in prokaryotes? **8-5**

The Regulation of Bacterial Gene Expression

LEARNING OBJECTIVE

8-6 Define *operon*.

8-7 Explain the regulation of gene expression in bacteria by induction, repression, and catabolite repression.

A cell's genetic machinery and its metabolic machinery are integrated and interdependent. Recall from Chapter 5 that the bacterial cell carries out an enormous number of metabolic reactions. The common feature of all metabolic reactions is that they are catalyzed by enzymes. Also recall from Chapter 5 (page 120) that feedback inhibition stops a cell from performing unneeded chemical reactions. Feedback inhibition stops enzymes that have already been synthesized. We will now look at mechanisms to prevent synthesis of enzymes that are not needed.

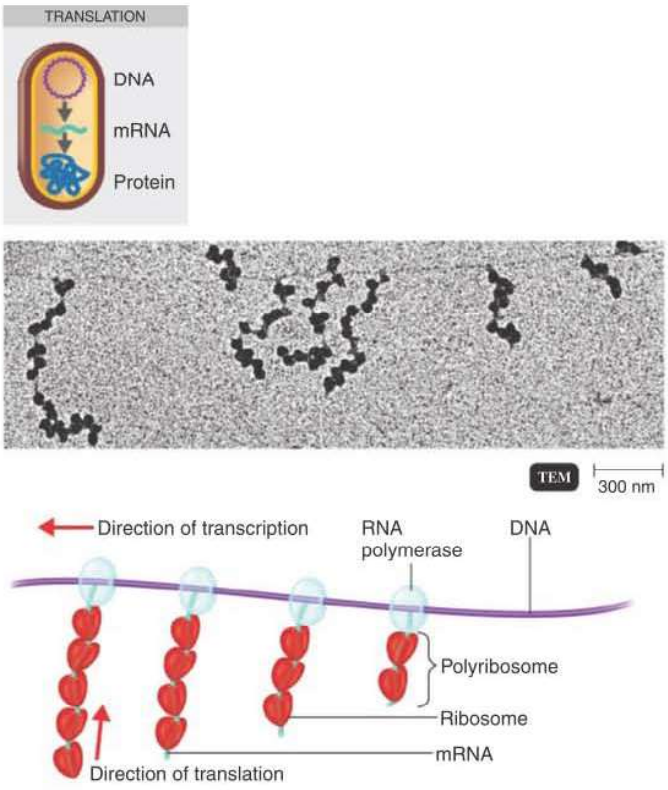


Figure 8.10 Simultaneous transcription and translation in bacteria. The micrograph and diagram show these processes in a single bacterial gene. Many molecules of mRNA are being synthesized simultaneously. The longest mRNA molecules were the first to be transcribed at the promoter. Note the ribosomes attached to the newly forming mRNA. The newly synthesized polypeptides are not shown.

Q Why can translation begin before transcription is complete in prokaryotes but not in eukaryotes?

We have seen that genes, through transcription and translation, direct the synthesis of proteins, many of which serve as enzymes—the very enzymes used for cellular metabolism. Because protein synthesis requires a huge amount of energy, regulation of protein synthesis is important to the cell's energy economy. Cells save energy by making only those proteins needed at a particular time. Next we look at how chemical reactions are regulated by controlling the synthesis of the enzymes.

Many genes, perhaps 60–80%, are not regulated but are instead *constitutive,* meaning that their products are constantly produced at a fixed rate. Usually these genes, which are effectively turned on all the time, code for enzymes that the cell needs in fairly large amounts for its major life processes; the enzymes of glycolysis are examples. The production of other enzymes is regulated so that they are present only when needed. *Trypanosoma,* the protozoan parasite that causes African sleeping sickness, has hundreds of genes coding for surface glycoproteins. Each protozoan cell turns on only one glycoprotein gene at a time. As the host's immune system kills parasites with one type of surface molecule, parasites expressing a different surface glycoprotein can continue to grow.

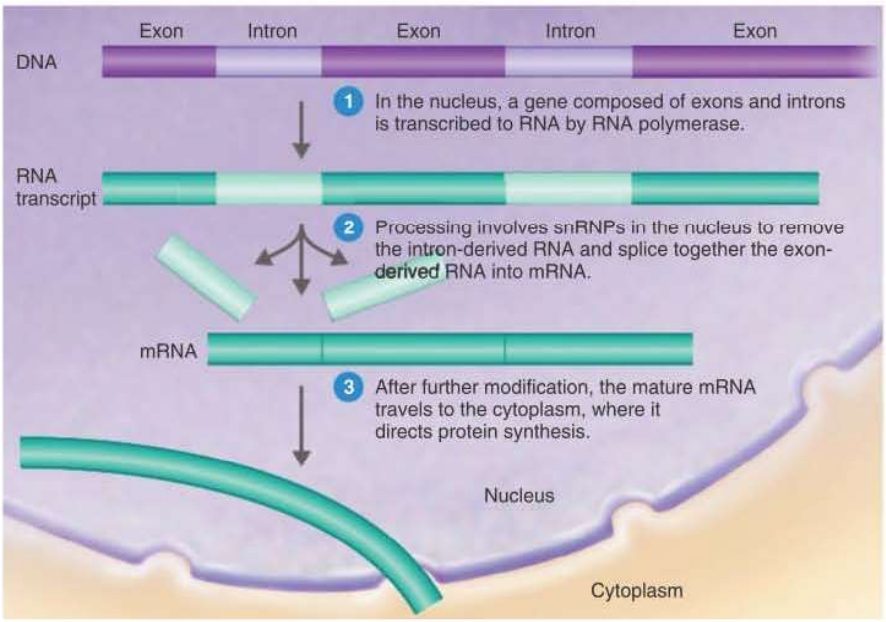


Figure 8.11 RNA processing in eukaryotic cells.

Q Why can't the RNA transcript be used for translation?

CLINICAL FOCUS

Tracking West Nile Virus

On August 23, 1999, an infectious disease physician from a hospital in northern Queens contacted the New York City Department of Health (NYCDOH) to report two patients with encephalitis. On investigation, NYCDOH initially identified a cluster of six patients with encephalitis, five of whom had profound muscle weakness and required respiratory support. No bacteria were cultured from the patients' blood or cerebrospinal fluid. Viruses transmitted by mosquitoes are a likely cause of aseptic encephalitis during the summer months. These viruses are called arboviruses. Arboviruses, *ar*thropod-*bo*rne, are viruses that are maintained in nature through biological transmission between susceptible vertebrate hosts by blood-feeding arthropods such as mosquitoes.

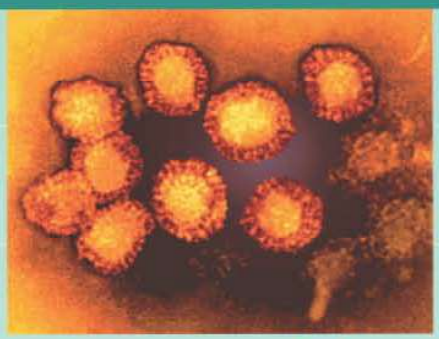
Nucleic acid sequencing of these isolates was performed at the CDC on September 23. Comparison of the nucleic acid sequences to databases indicated that the viruses were closely related to West Nile virus (WNV, see the photo), which had never been isolated in the Western Hemisphere.

By 2007, WNV had been found in birds in all states except Alaska and Hawaii. The recognition of WNV in the Western Hemisphere in the summer of 1999 marked the first introduction in recent history of an Old World flavivirus into the New World. The United States is not alone, however, in reporting new or heightened activity in humans and other animals. As of 2007, WNV caused human encephalitis in Mexico and in Canada, and incursions of flaviviruses into new areas are likely to continue through increasing global commerce and travel.

West Nile virus was first isolated in 1937 in the West Nile district of Uganda. In the early 1950s, scientists recognized WNV encephalitis outbreaks in humans in Egypt and Israel. Initially considered a minor arbovirus, WNV has emerged as a major public health and veterinary concern in southern Europe, the Mediterranean basin, and North America.

Currently, researchers are looking at the virus's genome for clues about its path around the world. The flavivirus genome consists of a positive, single-stranded RNA 11,000 to 12,000 nucleotides long. (Positive RNA can act as mRNA and be translated.) The virus has acquired several mutations, and researchers are looking for clues in these mutations to determine the virus's journey.

1. Using the portions of the genomes (shown below) that encode viral proteins, can you determine how similar are these viruses? Can you figure out its movement around the world?
   Determine the amino acids encoded, and divide the viruses based on percentage of similarity to the Uganda strain.
2. Based on amino acids, there are two groups called clades.
   Which group is the older?
3. The North American and Australian strains have accumulated more mutations, so these should be more recent.
   Calculate the percentage of difference between nucleotides to see how the viruses are related within their clade.
4. Although genetically related groups or clades can be seen, the actual journey of the virus remains elusive.



West Nile virus

SEM

50 nm

Source: Adapted from CDC data.

Australia	A	C	C	C	C	G	T	C	C	A	C	C	C	T	T	T	C	A	A	T	T
Egypt	A	A	T	C	G	A	T	C	A	T	C	T	T	C	G	T	C	G	A	T	C
France	A	A	T	C	G	A	T	C	A	T	C	G	T	C	G	T	C	G	A	T	C
Israel	A	T	C	C	A	T	T	C	A	T	C	C	T	C	A	T	C	G	A	T	T
Italy	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Kenya	A	T	C	C	A	C	T	C	A	T	C	C	T	C	G	T	C	G	A	T	T
Mexico	A	A	C	C	C	T	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
United States	A	A	C	C	C	C	T	C	C	T	C	C	C	C	T	T	C	G	A	T	T
Uganda	A	T	A	C	G	A	T	C	A	T	G	C	T	C	G	T	C	C	A	T	C

Repression and Induction

Two genetic control mechanisms known as repression and induction regulate the transcription of mRNA and consequently the synthesis of enzymes from them. These mechanisms control the formation and amounts of enzymes in the cell, not the activities of the enzymes.

Repression

The regulatory mechanism that inhibits gene expression and decreases the synthesis of enzymes is called **repression.** Repression is usually a response to the overabundance of an end-product of a metabolic pathway; it causes a decrease in the rate of synthesis of the enzymes leading to the formation of that product. Repression is mediated by regulatory proteins called **repressors,** which block the ability of RNA polymerase to initiate transcription from the repressed genes. The default position of a repressible gene is *on.*

Induction

The process that turns on the transcription of a gene or genes is **induction.** A substance that acts to induce transcription of a gene is called an **inducer,** and enzymes that are synthesized in the presence of inducers are *inducible enzymes.* The genes required for lactose metabolism in *E. coli* are a well-known example of an inducible system. One of these genes codes for the enzyme β-galactosidase, which splits the substrate lactose into two simple sugars, glucose and galactose. (β refers to the type of linkage that joins the glucose and galactose.) If *E. coli* is placed into a medium in which no lactose is present, the organisms contain almost no β-galactosidase; however, when lactose is added to the medium, the bacterial cells produce a large quantity of the enzyme. Lactose is converted in the cell to the related compound allolactose, which is the inducer for these genes; the presence of lactose thus indirectly induces the cells to synthesize more enzyme. The default position of an inducible gene is *off.* **Animations** Operons: Induction, Repression. **www.microbiologyplace.com**

The Operon Model of Gene Expression

Details of the control of gene expression by induction and repression are described by the operon model. François Jacob and Jacques Monod formulated this general model in 1961 to account for the regulation of protein synthesis. They based their model on studies of the induction of the enzymes of lactose catabolism in *E. coli.* In addition to β-galactosidase, these enzymes include lac permease, which is involved in the transport of lactose into the cell, and transacetylase, which metabolizes certain disaccharides other than lactose.

The genes for the three enzymes involved in lactose uptake and utilization are next to each other on the bacterial chromosome and are regulated together (**Figure 8.12**). These genes,

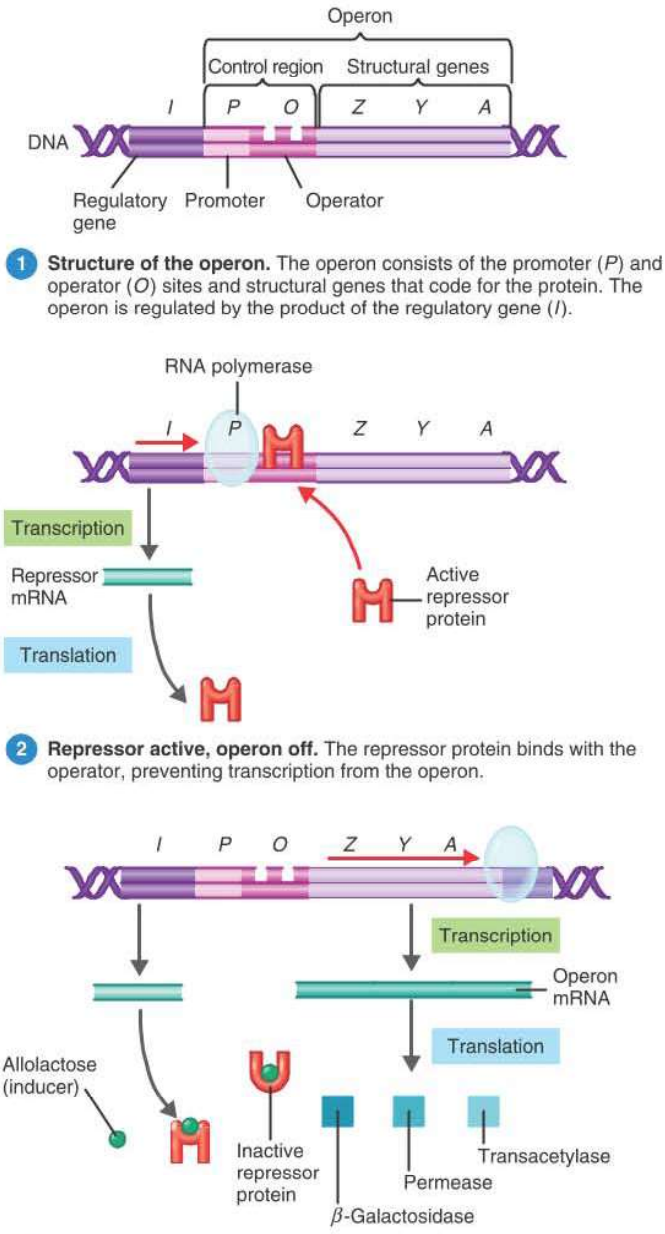


Figure 8.12 An inducible operon. Lactose-digesting enzymes are produced in the presence of lactose. In *E. coli,* the genes for the three enzymes are in the *lac* operon. β-galactosidase is encoded by *lacZ.* The *lacY* gene encodes the lac permease, and *lacA* encodes transacetylase, whose function in lactose metabolism is still unclear.

Q What causes transcription of an inducible enzyme?

which determine the structures of proteins, are called *structural genes* to distinguish them from an adjoining control region on the DNA. When lactose is introduced into the culture medium, the *lac* structural genes are all transcribed and translated rapidly and simultaneously. We will now see how this regulation occurs.

In the control region of the *lac* operon are two relatively short segments of DNA. One, the *promoter,* is the region of DNA where RNA polymerase initiates transcription. The other is the **operator,** which is like a traffic light that acts as a go or stop signal for transcription of the structural genes. A set of operator and promoter sites and the structural genes they control define an **operon;** thus, the combination of the three *lac* structural genes and the adjoining control regions is called the *lac* operon.

A regulatory gene called the *I gene* encodes a **repressor** protein that switches inducible and repressible operons on or off. The *lac* operon is an **inducible operon** (see Figure 8.12). In the absence of lactose, the repressor binds to the operator site, thus preventing transcription. If lactose is present, the repressor binds to a metabolite of lactose instead of to the operator, and lactose-digesting enzymes are transcribed.

In **repressible operons,** the structural genes are transcribed until they are turned off, or *repressed* (**Figure 8.13**). The genes for the enzymes involved in the synthesis of tryptophan are regulated in this manner. The structural genes are transcribed and translated, leading to tryptophan synthesis. When excess tryptophan is present, the tryptophan acts as a **corepressor** binding to the repressor protein. The repressor protein can now bind to the operator, stopping further tryptophan synthesis.

Animation Operons: Overview. **www.microbiologyplace.com**

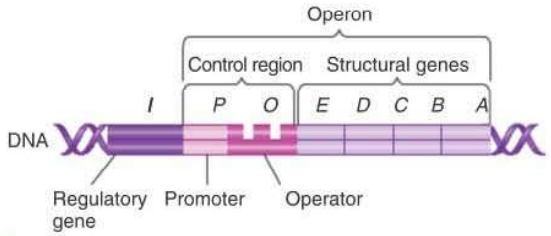
CHECK YOUR UNDERSTANDING

✓ What is an operon? **8-6**

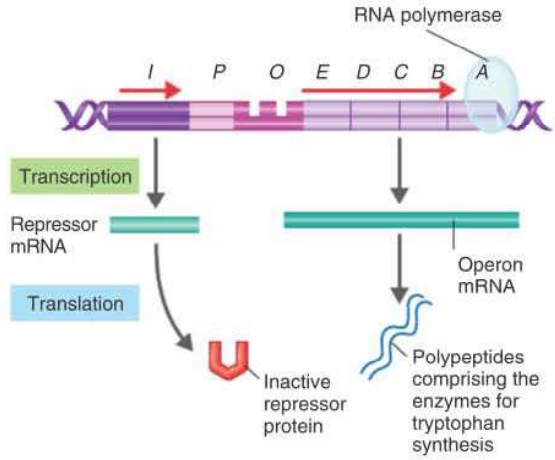
Positive Regulation

Regulation of the lactose operon also depends on the level of glucose in the medium, which in turn controls the intracellular level of the small molecule **cyclic AMP (cAMP),** a substance derived from ATP that serves as a cellular alarm signal. Enzymes that metabolize glucose are constitutive, and cells grow at their maximal rate with glucose as their carbon source because they can use it most efficiently (**Figure 8.14**). When glucose is no longer available, cAMP accumulates in the cell. The cAMP binds to the allosteric site of *catabolic activator protein (CAP).* CAP then binds to the *lac* promoter, which initiates transcription by making it easier for RNA polymerase to bind to the promoter. Thus transcription of the *lac* operon requires both the presence of lactose and the absence of glucose (**Figure 8.15**).

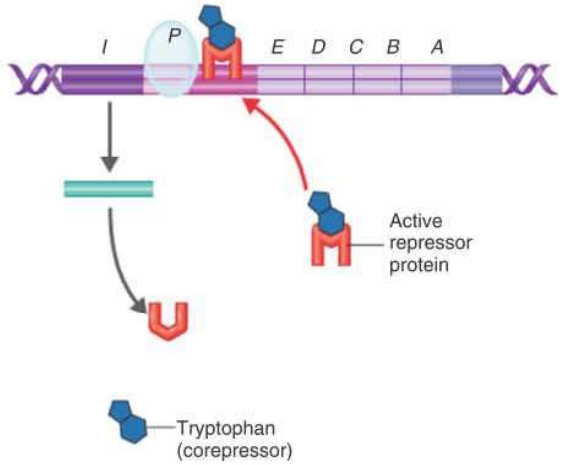
Cyclic AMP is an example of an *alarmone,* a chemical alarm signal that promotes a cell's response to environmental or nutritional stress. (In this case, the stress is the lack of glucose.) The



1 Structure of the operon. The operon consists of the promoter (*P*) and operator (*O*) sites and structural genes that code for the protein. The operon is regulated by the product of the regulatory gene (*I*).



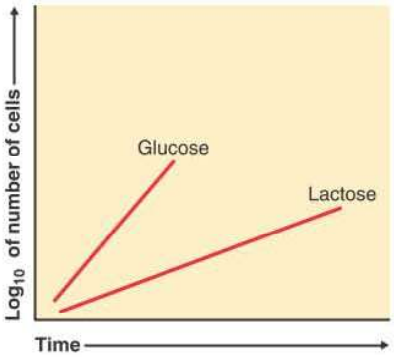
2 Repressor inactive, operon on. The repressor is inactive, and transcription and translation proceed, leading to the synthesis of tryptophan.



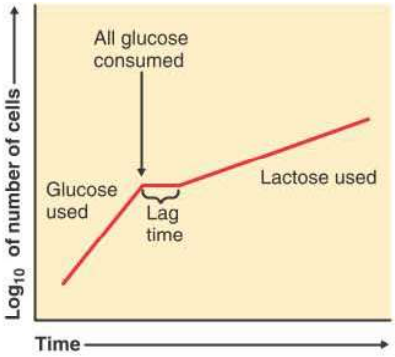
3 Repressor active, operon off. When the corepressor tryptophan binds to the repressor protein, the activated repressor binds with the operator, preventing transcription from the operon.

Figure 8.13 A repressible operon. Tryptophan, an amino acid, is produced by anabolic enzymes encoded by five structural genes. Accumulation of tryptophan represses transcription of these genes, preventing further synthesis of tryptophan. The *E. coli trp* operon is shown here.

Q What causes transcription of a repressible enzyme?



(a) Bacteria growing on glucose as the sole carbon source grow faster than on lactose.



(b) Bacteria growing in a medium containing glucose and lactose first consume the glucose and then, after a short lag time, the lactose. During the lag time, intracellular cAMP increases, the *lac* operon is transcribed, more lactose is transported into the cell, and β-galactosidase is synthesized to break down lactose.

Figure 8.14 The growth rate of *E. coli* on glucose and lactose.

Q When both glucose and lactose are present, why will cells use glucose first?

same mechanism involving cAMP allows the cell to grow on other sugars. Inhibition of the metabolism of alternative carbon sources by glucose is termed **catabolite repression** (or the *glucose effect*). When glucose is available, the level of cAMP in the cell is low, and consequently CAP is not bound.

CHECK YOUR UNDERSTANDING

✓ What is the role of cAMP in catabolite repression? 8-7

Mutation: Change in the Genetic Material

LEARNING OBJECTIVES

8-8 Classify mutations by type.

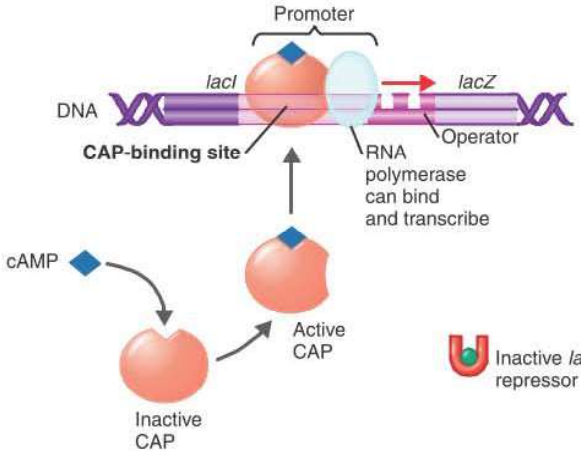
8-9 Define *mutagen*.

8-10 Describe two ways mutations can be repaired.

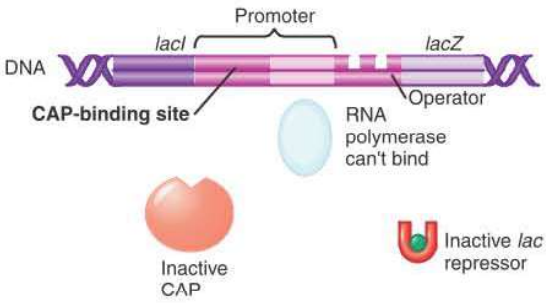
8-11 Describe the effect of mutagens on the mutation rate.

8-12 Outline the methods of direct and indirect selection of mutants.

8-13 Identify the purpose of and outline the procedure for the Ames test.



(a) Lactose present, glucose scarce (cAMP level high). If glucose is scarce, the high level of cAMP activates CAP, and the *lac* operon produces large amounts of mRNA for lactose digestion.



(b) Lactose present, glucose present (cAMP level low). When glucose is present, cAMP is scarce, and CAP is unable to stimulate transcription.

Figure 8.15 Positive regulation of the *lac* operon.

Q Will transcription of the *lac* operon occur in the presence of lactose and glucose? In the presence of lactose and the absence of glucose? In the presence of glucose and the absence of lactose?

A **mutation** is a change in the base sequence of DNA. Such a change in the base sequence of a gene will sometimes cause a change in the product encoded by that gene. For example, when the gene for an enzyme mutates, the enzyme encoded by the gene may become inactive or less active because its amino acid sequence has changed. Such a change in genotype may be disadvantageous, or even lethal, if the cell loses a phenotypic trait it needs. However, a mutation can be beneficial if, for instance, the altered enzyme encoded by the mutant gene has a new or enhanced activity that benefits the cell.

Many simple mutations are silent (neutral); the change in DNA base sequence causes no change in the activity of the product encoded by the gene. Silent mutations commonly occur when one nucleotide is substituted for another in the DNA, especially at a location corresponding to the third position of the mRNA codon. Because of the degeneracy of the genetic code, the resulting new codon might still code for the

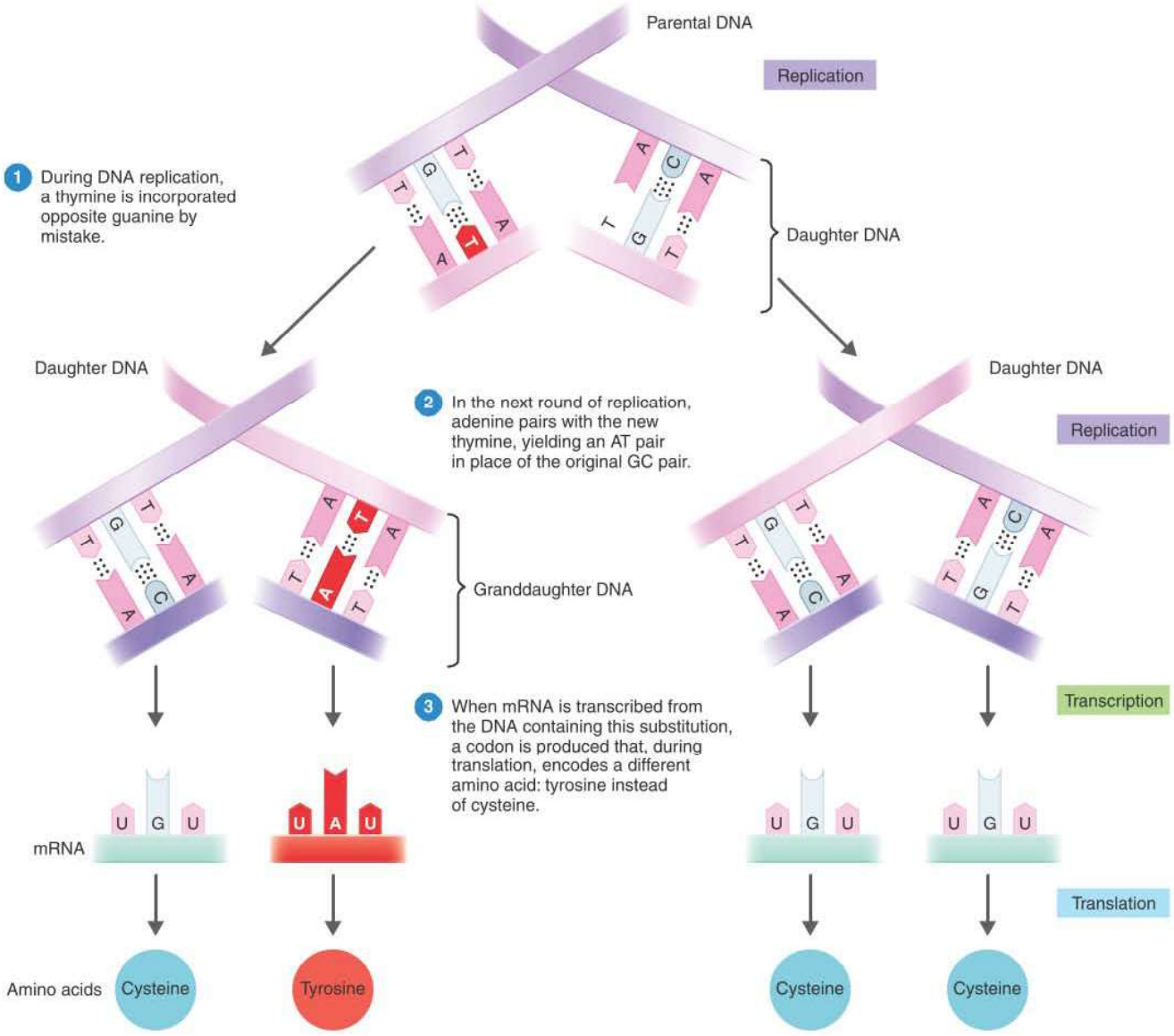


Figure 8.16 Base substitution. This mutation leads to an altered protein in a granddaughter cell.

Q Does a base substitution always result in a different amino acid?

same amino acid. Even if the amino acid is changed, the function of the protein may not change if the amino acid is in a nonvital portion of the protein, or is chemically very similar to the original amino acid.

Types of Mutations

The most common type of mutation involving single base pairs is **base substitution** (or *point mutation*), in which a single base at one point in the DNA sequence is replaced with a different base. When the DNA replicates, the result is a substituted base pair (Figure 8.16). For example, AT might be substituted for GC, or CG for GC. If a base substitution occurs within a gene that codes for a protein, the mRNA transcribed from the gene will carry an incorrect base at that position. When the mRNA is translated into protein, the incorrect base may cause the insertion of an incorrect amino acid in the protein. If the base substitution results in an amino acid substitution in the synthesized protein, this change in the DNA is known as a **missense mutation** (Figure 8.17a and Figure 8.17b).

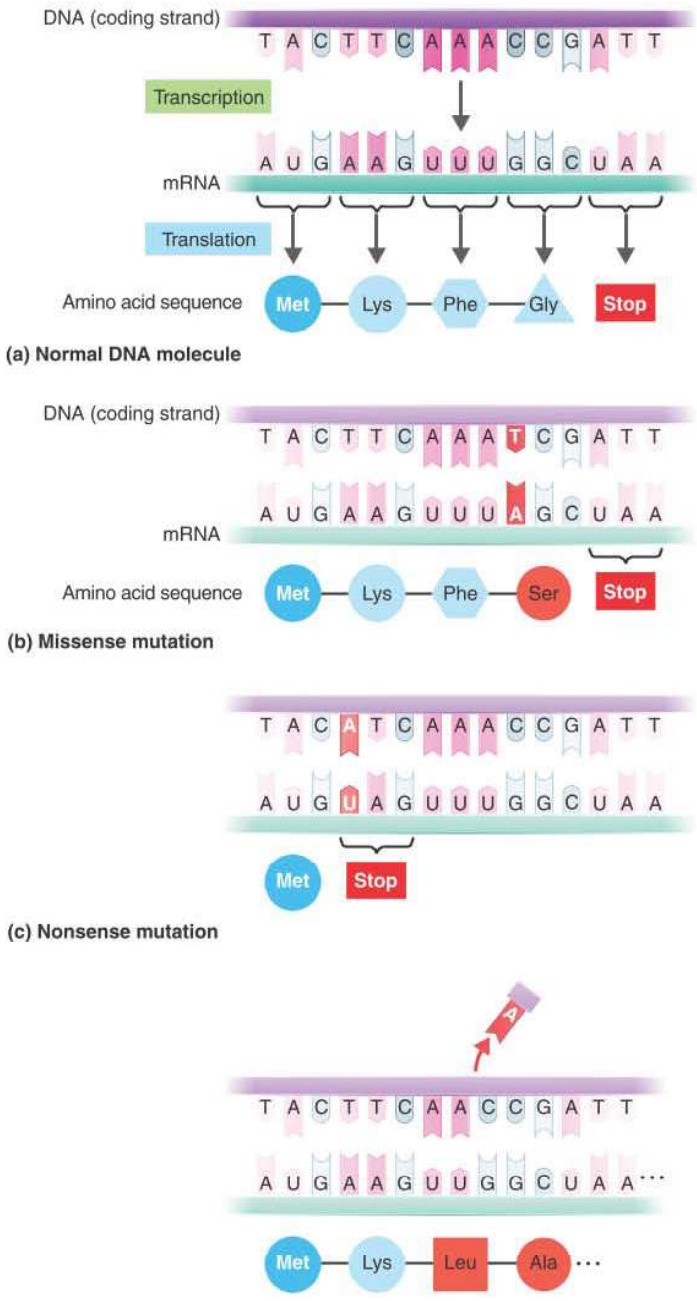


Figure 8.17 Types of mutations and their effects on the amino acid sequences of proteins.

Q **On what basis are missense, nonsense, and frameshift mutations distinguished?**

The effects of such mutations can be dramatic. For example, sickle cell disease is caused by a single change in the gene for globin, the protein component of hemoglobin. Hemoglobin is primarily responsible for transporting oxygen from the lungs to the tissues. A single missense mutation, a change from an A to a T at a specific site, results in the change from glutamic acid to valine in the protein. The effect of this change is that the shape of the hemoglobin molecule changes under conditions of low oxygen, altering the shape of the red blood cells such that movement of the cells through small capillaries is greatly impeded.

By creating a nonsense (stop) codon in the middle of an mRNA molecule, some base substitutions effectively prevent the synthesis of a complete functional protein; only a fragment is synthesized. A base substitution resulting in a nonsense codon is thus called a **nonsense mutation** (Figure 8.17c).

Besides base-pair mutations, there are also changes in DNA called **frameshift mutations,** in which one or a few nucleotide pairs are deleted or inserted in the DNA (Figure 8.17d). This mutation can shift the "translational reading frame"—that is, the three-by-three grouping of nucleotides recognized as codons by the tRNAs during translation. For example, deleting one nucleotide pair in the middle of a gene causes changes in many amino acids downstream from the site of the original mutation. Frameshift mutations almost always result in a long stretch of altered amino acids and the production of an inactive protein from the mutated gene. In most cases, a nonsense codon will eventually be encountered and thereby terminate translation.

Occasionally, mutations occur where significant numbers of bases are added to (inserted into) a gene. Huntington's disease, for example, is a progressive neurological disorder caused by extra bases inserted into a particular gene. The reason these insertions occur in this particular gene is still being studied.

Base substitutions and frameshift mutations may occur spontaneously because of occasional mistakes made during DNA replication. These **spontaneous mutations** apparently occur in the absence of any mutation-causing agents. Agents in the environment, such as certain chemicals and radiation, that directly or indirectly bring about mutations are called **mutagens.** Almost any agent that can chemically or physically react with DNA can potentially cause mutations. A wide variety of chemicals, many of which are common in nature or in households, are known to be mutagens. Many forms of radiation, including X rays and ultraviolet light, are also mutagenic, as discussed shortly.

In the microbial world, certain mutations result in resistance to antibiotics (see the box in Chapter 26, page 751) or altered pathogenicity. A mutation in a gene encoding the outer membrane may increase pathogenicity; for example, *Salmonella enterica* with an altered outer membrane can survive in phagocytes. A mutation in a capsule-encoding gene may result in decreased pathogenicity because phagocytes can destroy the bacteria, as in the cases of *Streptococcus pneumoniae, Haemophilus influenzae,* and *Neisseria meningitidis.*

CHECK YOUR UNDERSTANDING

✓ How can a mutation be beneficial? 8-8

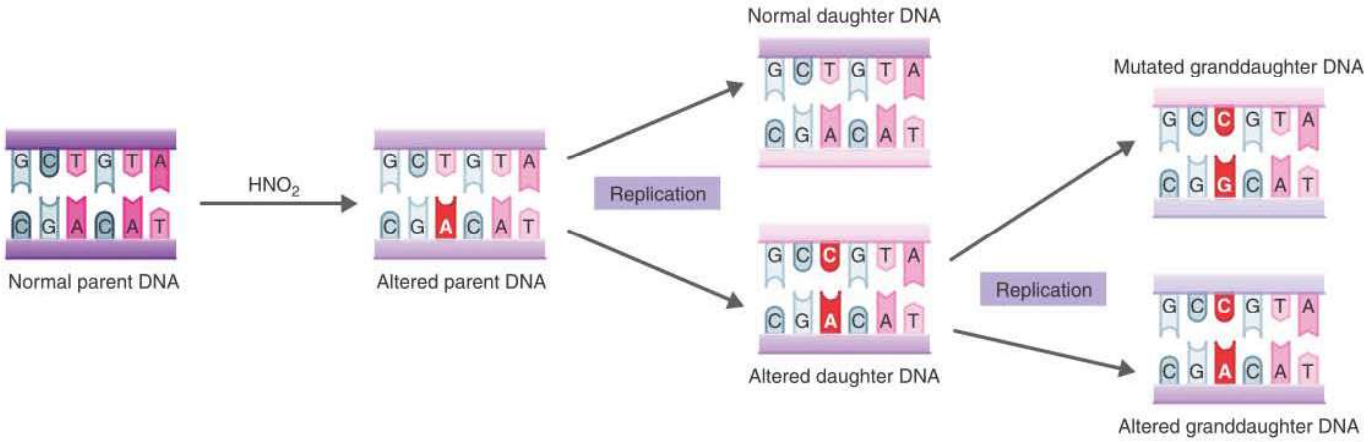


Figure 8.18 Nitrous acid (HNO_2) as a mutagen. The nitrous acid alters an adenine in such a way that it pairs with cytosine instead of thymine.

Q **What is a mutagen?**

Mutagens

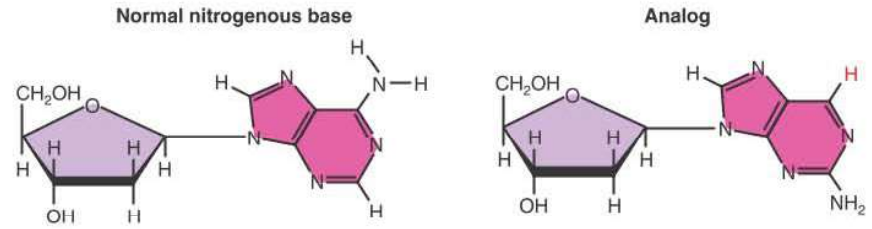
Chemical Mutagens

One of the many chemicals known to be a mutagen is nitrous acid. Figure 8.18 shows how exposure of DNA to nitrous acid can convert the base adenine (A) to a form that no longer pairs with thymine (T) but instead pairs with cytosine (C). When DNA containing such modified adenines replicates, one daughter DNA molecule will have a base-pair sequence different from that of the parent DNA. Eventually, some AT base pairs of the parent will have been changed to GC base pairs in a granddaughter cell. Nitrous acid makes a specific base-pair change in DNA. Like all mutagens, it alters DNA at random locations.

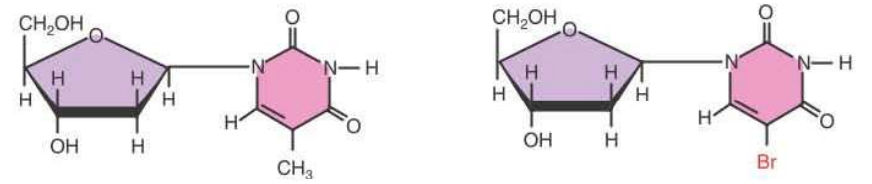
Another type of chemical mutagen is the **nucleoside analog.** These molecules are structurally similar to normal nitrogenous bases, but they have slightly altered base-pairing properties. Examples, 2-aminopurine and 5-bromouracil, are shown in Figure 8.19. When nucleoside analogs are given to growing cells, the analogs are randomly incorporated into cellular DNA in

Figure 8.19 Nucleoside analogs and the nitrogenous bases they replace. A nucleoside is phosphorylated and the resulting nucleotide used to synthesize DNA.

Q **Why do these drugs kill cells?**



(a) The 2-aminopurine is incorporated into DNA in place of adenine but can pair with cytosine, so an AT pair becomes a CG pair.



(b) The 5-bromouracil is used as an anticancer drug because it is mistaken for thymine by cellular enzymes but pairs with cytosine. In the next DNA replication, an AT pair becomes a GC pair.

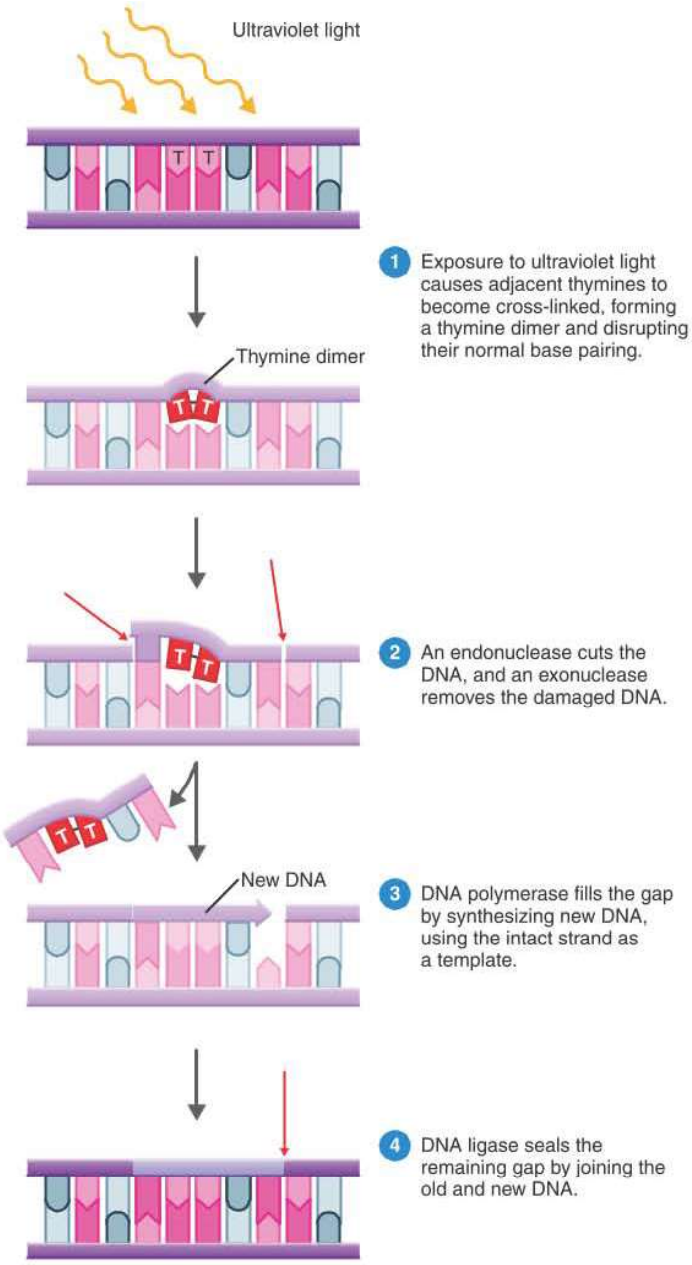


Figure 8.20 The creation and repair of a thymine dimer caused by ultraviolet light. After exposure to UV light, adjacent thymines can become cross-linked, forming a thymine dimer. In the absence of visible light, the nucleotide excision repair mechanism is used in a cell to repair the damage.

Q How do excision repair enzymes "know" which strand is incorrect?

place of the normal bases. Then, during DNA replication, the analogs cause mistakes in base pairing. The incorrectly paired bases will be copied during subsequent replication of the DNA, resulting in base-pair substitutions in the progeny cells. Some antiviral and antitumor drugs are nucleoside analogs, including AZT (azidothymidine), one of the primary drugs used to treat HIV infection.

Still other chemical mutagens cause small deletions or insertions, which can result in frameshifts. For instance, under certain conditions, benzopyrene, which is present in smoke and soot, is an effective *frameshift mutagen.* Aflatoxin—produced by *Aspergillus flavus* (a-spėr-jil′lus flā′vus), a mold that grows on peanuts and grain—is a frameshift mutagen, as are the acridine dyes used experimentally against herpesvirus infections. Frameshift mutagens usually have the right size and chemical properties to slip between the stacked base pairs of the DNA double helix. They may work by slightly offsetting the two strands of DNA, leaving a gap or bulge in one strand or the other. When the staggered DNA strands are copied during DNA synthesis, one or more base pairs can be inserted or deleted in the new double-stranded DNA. Interestingly, frameshift mutagens are often potent carcinogens.

Radiation

X rays and gamma rays are forms of radiation that are potent mutagens because of their ability to ionize atoms and molecules. The penetrating rays of ionizing radiation cause electrons to pop out of their usual shells (see Chapter 2). These electrons bombard other molecules and cause more damage, and many of the resulting ions and free radicals (molecular fragments with unpaired electrons) are very reactive. Some of these ions can combine with bases in DNA, resulting in errors in DNA replication and repair that produce mutations. An even more serious outcome is the breakage of covalent bonds in the sugar-phosphate backbone of DNA, which causes physical breaks in chromosomes.

Another form of mutagenic radiation is ultraviolet (UV) light, a nonionizing component of ordinary sunlight. However, the most mutagenic component of UV light (wavelength 260 nm) is screened out by the ozone layer of the atmosphere. The most important effect of direct UV light on DNA is the formation of harmful covalent bonds between certain bases. Adjacent thymines in a DNA strand can cross-link to form thymine dimers. Such dimers, unless repaired, may cause serious damage or death to the cell because it cannot properly transcribe or replicate such DNA.

Bacteria and other organisms have enzymes that can repair UV-induced damage. **Photolyases,** also known as *light-repair enzymes,* use visible light energy to separate the dimer back to the original two thymines. **Nucleotide excision repair,** shown in Figure 8.20, is not restricted to UV-induced damage; it can repair

mutations from other causes as well. Enzymes cut out the incorrect base and fill in the gap with newly synthesized DNA that is complementary to the correct strand. For many years biologists questioned how the incorrect base could be distinguished from the correct base if it was not physically distorted like a thymine dimer. In 1970, Hamilton Smith provided the answer with the discovery of **methylases.** These enzymes add a methyl group to selected bases soon after a DNA strand is made. A repair endonuclease then cuts the nonmethylated strand.

Exposure to UV light in humans, such as by excessive suntanning, causes a large number of thymine dimers in skin cells. Unrepaired dimers may result in skin cancers. Humans with xeroderma pigmentosum, an inherited condition that results in increased sensitivity to UV light, have a defect in nucleotide excision repair; consequently, they have an increased risk of skin cancer.

The Frequency of Mutation

The **mutation rate** is the probability that a gene will mutate when a cell divides. The rate is usually stated as a power of 10, and because mutations are very rare, the exponent is always a negative number. For example, if there is one chance in 10,000 that a gene will mutate when the cell divides, the mutation rate is 1/10,000, which is expressed as 10^{-4}. Spontaneous mistakes in DNA replication occur at a very low rate, perhaps only once in 10^9 replicated base pairs (a mutation rate of 10^{-9}). Because the average gene has about 10^3 base pairs, the spontaneous rate of mutation is about one in 10^6 (a million) replicated genes.

Mutations usually occur more or less randomly along a chromosome. The occurrence of random mutations at low frequency is an essential aspect of the adaptation of species to their environment, for evolution requires that genetic diversity be generated randomly and at a low rate. For example, in a bacterial population of significant size—say, greater than 10^7 cells—a few new mutant cells will always be produced in every generation. Most mutations either are harmful and likely to be removed from the gene pool when the individual cell dies or are neutral. However, a few mutations may be beneficial. For example, a mutation that confers antibiotic resistance is beneficial to a population of bacteria that is regularly exposed to antibiotics. Once such a trait has appeared through mutation, cells carrying the mutated gene are more likely than other cells to survive and reproduce as long as the environment stays the same. Soon most of the cells in the population will have the gene; an evolutionary change will have occurred, although on a small scale.

A mutagen usually increases the spontaneous rate of mutation, which is about one in 10^6 replicated genes, by a factor of 10 to 1000 times. In other words, in the presence of a mutagen, the normal rate of 10^{-6} mutations per replicated gene becomes a rate of 10^{-5} to 10^{-3} per replicated gene. Mutagens are used experimentally to enhance the production of mutant cells for research on the genetic properties of microorganisms and for commercial purposes. **Animations** Mutations: Types, Repair; Mutagens. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

- How are mutations caused by chemicals? By radiation? **8-9**
- How can mutations be repaired? **8-10**
- How do mutagens affect the mutation rate? **8-11**

Identifying Mutants

Mutants can be detected by selecting or testing for an altered phenotype. Whether or not a mutagen is used, mutant cells with specific mutations are always rare compared with other cells in the population. The problem is detecting such a rare event.

Experiments are usually performed with bacteria because they reproduce rapidly, so large numbers of organisms (more than 10^9 per milliliter of nutrient broth) can easily be used. Furthermore, because bacteria generally have only one copy of each gene per cell, the effects of a mutated gene are not masked by the presence of a normal version of the gene, as in many eukaryotic organisms.

Positive (direct) selection involves the detection of mutant cells by rejection of the unmutated parent cells. For example, suppose we were trying to find mutant bacteria that are resistant to penicillin. When the bacterial cells are plated on a medium containing penicillin, the mutant can be identified directly. The few cells in the population that are resistant (mutants) will grow and form colonies, whereas the normal, penicillin-sensitive parental cells cannot grow.

To identify mutations in other kinds of genes, **negative (indirect) selection** can be used. This process selects a cell that cannot perform a certain function, using the technique of **replica plating.** For example, suppose we wanted to use replica plating to identify a bacterial cell that has lost the ability to synthesize the amino acid histidine (**Figure 8.21**). First, about 100 bacterial cells are inoculated onto an agar plate. This plate, called the master plate, contains a medium with histidine on which all cells will grow. After 18 to 24 hours of incubation, each cell reproduces to form a colony. Then a pad of sterile material, such as latex, filter paper, or velvet, is pressed over the master plate, and some of the cells from each colony adhere to the velvet. Next, the velvet is pressed down onto two (or more) sterile plates. One plate contains a medium without histidine, and one contains a medium with histidine on which the original, nonmutant bacteria can grow. Any colony that grows on the medium with histidine on the master plate but that cannot synthesize its own histidine will not be able to grow on the medium without histidine. The mutant colony can then be identified on

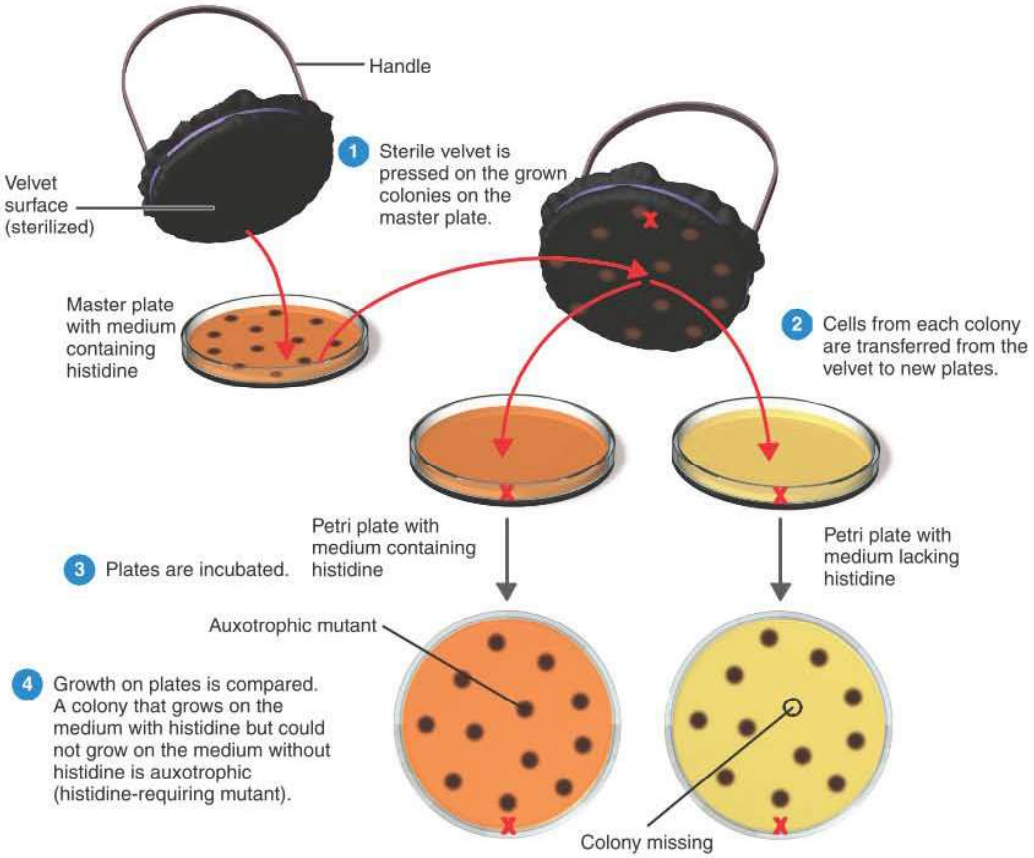


Figure 8.21 Replica plating. In this example, the auxotrophic mutant cannot synthesize histidine. The plates must be carefully marked (with an X here) to maintain orientation so that colony positions are known in relation to the original master plate.

Q What is an auxotroph?

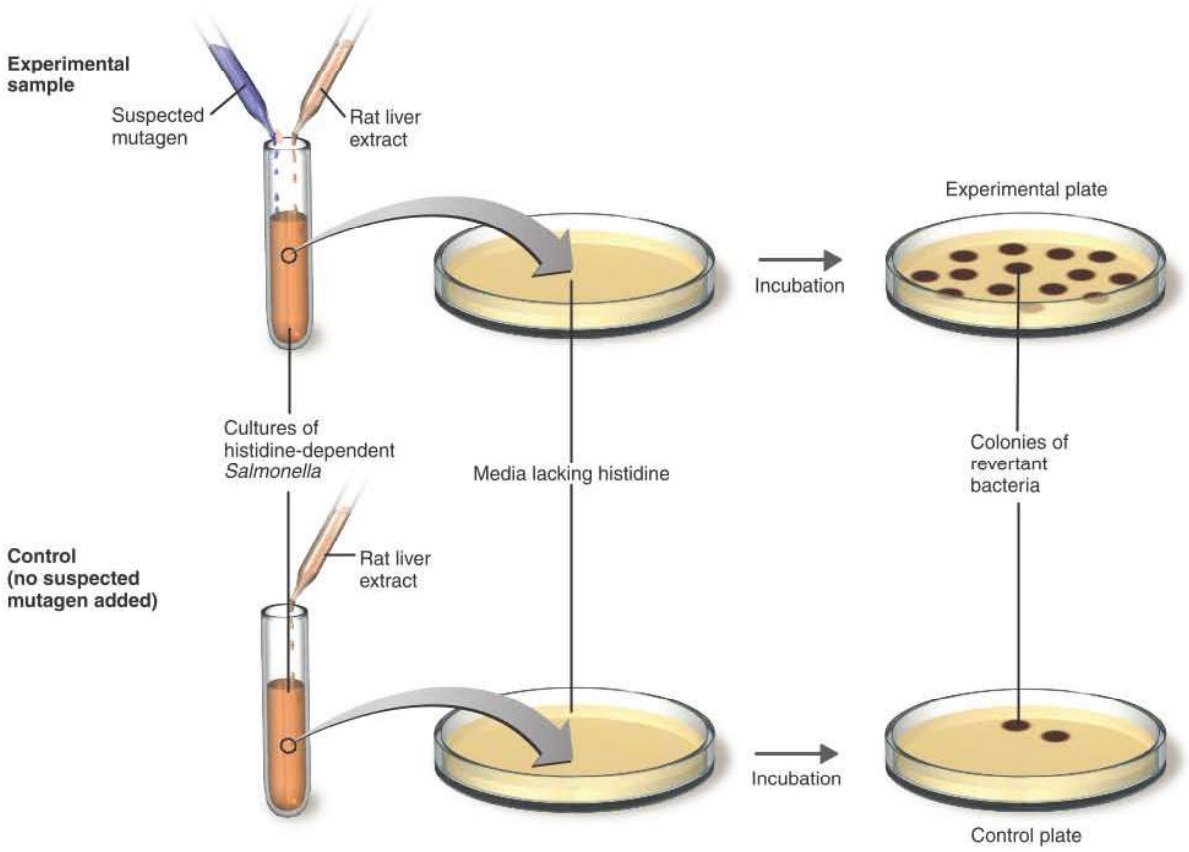
the master plate. Of course, because mutants are so rare (even those induced by mutagens), many plates must be screened with this technique to isolate a specific mutant.

Replica plating is a very effective means of isolating mutants that require one or more new growth factors. Any mutant microorganism having a nutritional requirement that is absent in the parent is known as an **auxotroph.** For example, an auxotroph may lack an enzyme needed to synthesize a particular amino acid and will therefore require that amino acid as a growth factor in its nutrient medium.

Identifying Chemical Carcinogens

Many known mutagens have been found to be **carcinogens,** substances that cause cancer in animals, including humans. In recent years, chemicals in the environment, the workplace, and the diet have been implicated as causes of cancer in humans. The usual subjects of tests to determine potential carcinogens are animals, and the testing procedures are time-consuming and expensive. Now there are faster and less expensive procedures for the preliminary screening of potential carcinogens. One of these, called the **Ames test,** uses bacteria as carcinogen indicators.

The Ames test is based on the observation that exposure of mutant bacteria to mutagenic substances may cause new mutations that reverse the effect (the change in phenotype) of the original mutation. These are called *reversions.* Specifically, the test measures the reversion of histidine auxotrophs of *Salmonella* (his^- cells, mutants that have lost the ability to synthesize histidine) to histidine-synthesizing cells (his^+) after treatment with a mutagen (Figure 8.22). Bacteria are incubated in both the presence and absence of the substance being tested. Because animal enzymes must activate many chemicals into forms that are chemically reactive for mutagenic or carcinogenic activity to appear, the chemical to be tested and the mutant bacteria are incubated together with rat liver extract, a rich source of activation enzymes. If the substance being tested is mutagenic, it will cause the reversion of his^- bacteria to his^+ bacteria at a rate higher than the spontaneous reversion rate. The number of



1. Two cultures are prepared of *Salmonella* bacteria that have lost the ability to synthesize histidine (histidine-dependent).
2. The suspected mutagen is added to the experimental sample only; rat liver extract (an activator) is added to both samples.
3. Each sample is poured onto a plate of medium lacking histidine. The plates are then incubated at 37°C for two days. Only bacteria whose histidine-dependent phenotype has mutated back (reverted) to histidine-synthesizing will grow into colonies.
4. The numbers of colonies on the experimental and control plates are compared. The control plate may show a few spontaneous histidine-synthesizing revertants. The test plates will show an increase in the number of histidine-synthesizing revertants if the test chemical is indeed a mutagen and potential carcinogen. The higher the concentration of mutagen used, the more revertant colonies will result.

Figure 8.22 The Ames reverse gene mutation test.

Q **Do all mutagens cause cancer?**

observed revertants indicates the degree to which a substance is mutagenic and therefore possibly carcinogenic.

The test can be used in many ways. Several potential mutagens can be qualitatively tested by spotting the individual chemicals on small paper disks on a single plate inoculated with bacteria. In addition, mixtures such as wine, blood, smoke condensates, and extracts of foods can also be tested to see whether they contain mutagenic substances.

About 90% of the substances found by the Ames test to be mutagenic have also been shown to be carcinogenic in animals. By the same token, the more mutagenic substances have generally been found to be more carcinogenic.

CHECK YOUR UNDERSTANDING

- How would you isolate an antibiotic-resistant bacterium? An antibiotic-sensitive bacterium? **8-12**
- What is the principle behind the Ames test? **8-13**

Genetic Transfer and Recombination

LEARNING OBJECTIVES

8-14 Differentiate horizontal and vertical gene transfer.

8-15 Compare the mechanisms of genetic recombination in bacteria.

8-16 Describe the functions of plasmids and transposons.

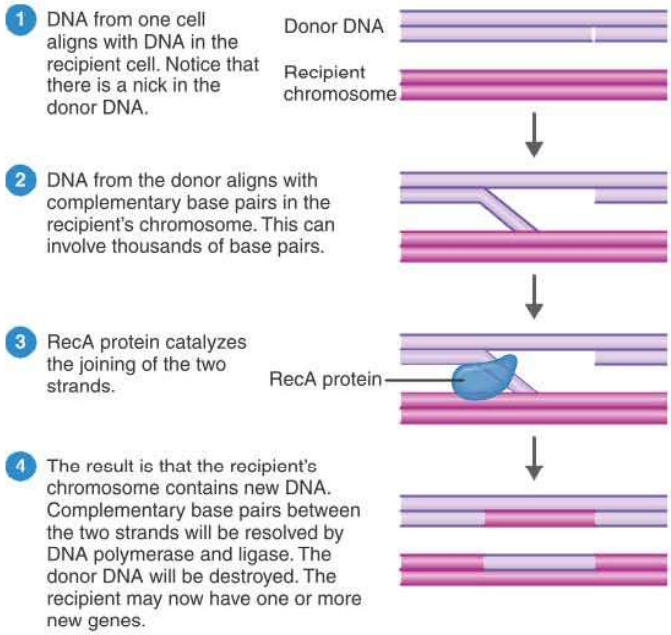


Figure 8.23 Genetic recombination by crossing over. Foreign DNA can be inserted into a chromosome by breaking and rejoining the chromosome. This can insert one or more new genes into the chromosome. A photograph of RecA protein is shown in Figure 3.11a.

Q What type of enzyme breaks the DNA? What enzyme rejoins the pieces of DNA?

Genetic recombination refers to the exchange of genes between two DNA molecules to form new combinations of genes on a chromosome. Figure 8.23 shows one mechanism for genetic recombination. If a cell picks up a foreign DNA (called donor DNA in the figure), some of it could insert into the cell's chromosome—a process called **crossing over**—and some of the genes carried by the chromosomes are shuffled. The DNA has recombined, so that the chromosome now carries a portion of the donor's DNA.

If A and B represent DNA from different individuals, how are they brought close enough together to recombine? In eukaryotes, genetic recombination is an ordered process that usually occurs as part of the sexual cycle of the organism. Crossing over generally takes place during the formation of reproductive cells, such that these cells contain recombinant DNA. In bacteria, genetic recombination can happen in a number of ways, which we will discuss in the following sections.

Like mutation, genetic recombination contributes to a population's genetic diversity, which is the source of variation in evolution. In highly evolved organisms such as present-day microbes, recombination is more likely than mutation to be beneficial because recombination will less likely destroy a gene's function and may bring together combinations of genes that enable the organism to carry out a valuable new function.

The major protein that constitutes the flagella of *Salmonella* is also one of the primary proteins that causes our immune systems to respond. However, these bacteria have the capability of producing two different flagellar proteins. As our immune system mounts a response against those cells containing one form of the flagellar protein, those organisms producing the second are not affected. Which flagellar protein is produced is determined by a recombination event that apparently occurs somewhat randomly within the chromosomal DNA. Thus, by altering the flagellar protein produced, *Salmonella* can better avoid the defenses of the host.

Vertical gene transfer occurs when genes are passed from an organism to its offspring. Plants and animals transmit their genes by vertical transmission. Bacteria can pass their genes not only to their offspring, but also laterally, to other microbes of the same generation. This is known as **horizontal gene transfer** (see Figure 8.2). Horizontal gene transfer between bacteria occurs in several ways. In all of the mechanisms, the transfer involves a **donor cell** that gives a portion of its total DNA to a **recipient cell.** Once transferred, part of the donor's DNA is usually incorporated into the recipient's DNA; the remainder is degraded by cellular enzymes. The recipient cell that incorporates donor DNA into its own DNA is called a *recombinant.* The transfer of genetic material between bacteria is by no means a frequent event; it may occur in only 1% or less of an entire population. Let's examine in detail the specific types of genetic transfer.

Transformation in Bacteria

During the process of **transformation,** genes are transferred from one bacterium to another as "naked" DNA in solution. This process was first demonstrated over 70 years ago, although it was not understood at the time. Not only did transformation show that genetic material could be transferred from one bacterial cell to another, but study of this phenomenon eventually led to the conclusion that DNA is the genetic material. The initial experiment on transformation was performed by Frederick Griffith in England in 1928 while he was working with two strains of *Streptococcus pneumoniae.* One, a virulent (pathogenic) strain, has a polysaccharide capsule that prevents phagocytosis. The bacteria grow and cause pneumonia. The other, an avirulent strain, lacks the capsule and does not cause disease.

Griffith was interested in determining whether injections of heat-killed bacteria of the encapsulated strain could be used to vaccinate mice against pneumonia. As he expected, injections of living encapsulated bacteria killed the mouse (Figure 8.24a); injections of live nonencapsulated bacteria (Figure 8.24b) or dead encapsulated bacteria (Figure 8.24c) did not kill the mouse. However, when the dead encapsulated bacteria were mixed with live nonencapsulated bacteria and injected into the mice, many

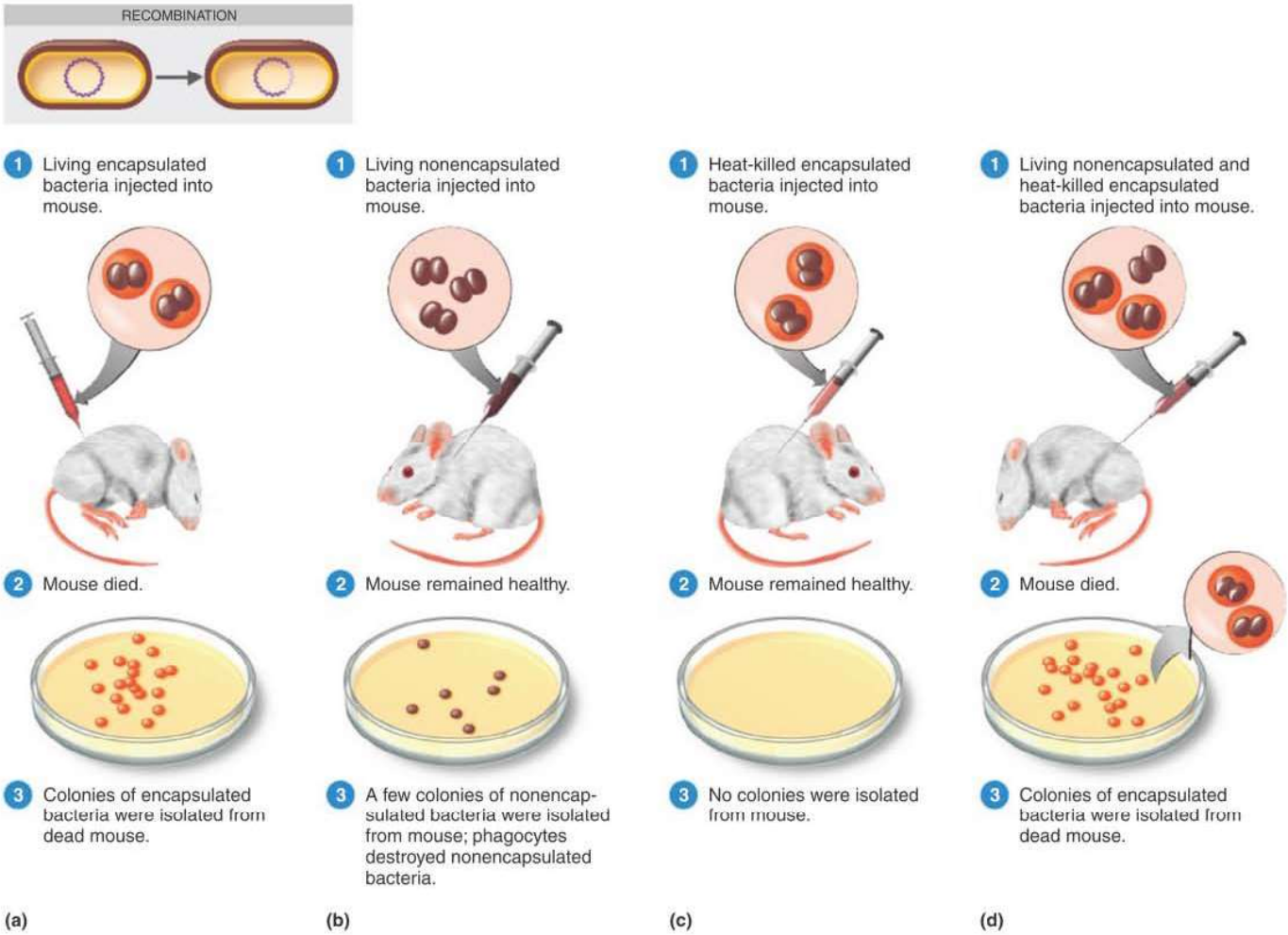


Figure 8.24 Griffith's experiment demonstrating genetic transformation. (**a**) Living encapsulated bacteria caused disease and death when injected into a mouse. (**b**) Living nonencapsulated bacteria are readily destroyed by the phagocytic defenses of the host, so the mouse remained healthy after injection. (**c**) After being killed by heat, encapsulated bacteria lost the ability to cause disease. (**d**) However, the combination of living nonencapsulated bacteria and heat-killed encapsulated bacteria (neither of which alone cause disease) did cause disease. Somehow, the live nonencapsulated bacteria were transformed by the dead encapsulated bacteria so that they acquired the ability to form a capsule and therefore cause disease. Subsequent experiments proved the transforming factor to be DNA.

Q Why did encapsulated bacteria kill the mouse while nonencapsulated bacteria did not? What killed the mouse in (d)?

of the mice died. In the blood of the dead mice, Griffith found living, encapsulated bacteria. Hereditary material (genes) from the dead bacteria had entered the live cells and changed them genetically so that their progeny were encapsulated and therefore virulent (Figure 8.24d).

Subsequent investigations based on Griffith's research revealed that bacterial transformation could be carried out without mice. A broth was inoculated with live nonencapsulated bacteria. Dead encapsulated bacteria were then added to the broth. After incubation, the culture was found to contain living bacteria that were encapsulated and virulent. The nonencapsulated bacteria had been transformed; they had acquired a new hereditary trait by incorporating genes from the killed encapsulated bacteria.

The next step was to extract various chemical components from the killed cells to determine which component caused the transformation. These crucial experiments were performed in the United States by Oswald T. Avery and his associates Colin M. MacLeod and Maclyn McCarty. After years of research, they announced in 1944 that the component responsible for transforming harmless *S. pneumoniae* into virulent strains was DNA. Their results provided one of the conclusive indications that DNA was indeed the carrier of genetic information.

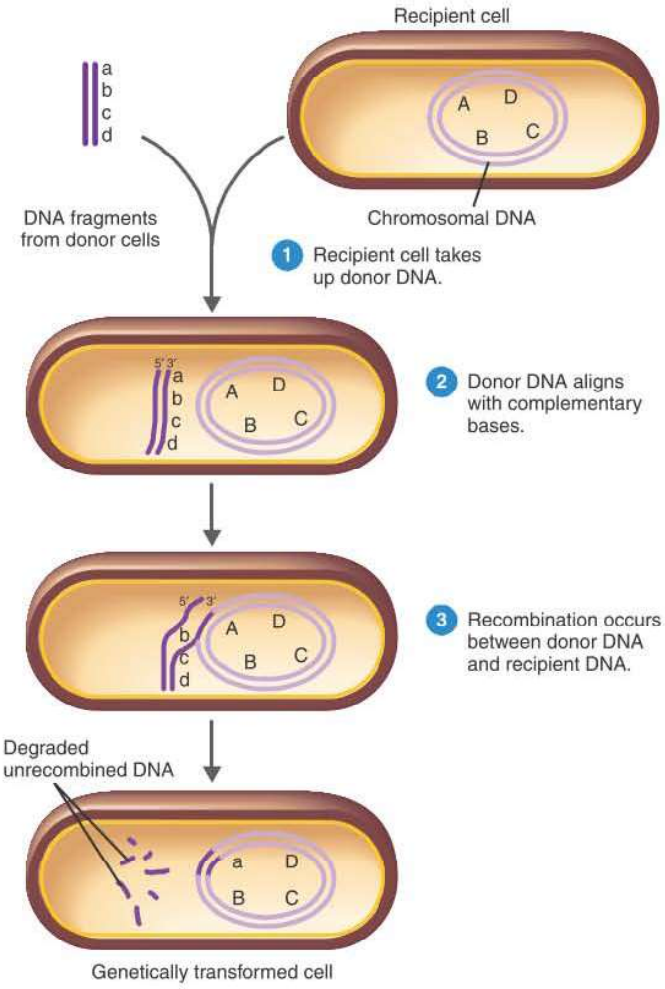


Figure 8.25 The mechanism of genetic transformation in bacteria. Some similarity is needed for the donor and recipient to align. Genes *a*, *b*, *c*, and *d* may be mutations of genes *A*, *B*, *C*, and *D*.

Q What type of enzyme cuts the donor DNA?

Since the time of Griffith's experiment, considerable information has been gathered about transformation. In nature, some bacteria, perhaps after death and cell lysis, release their DNA into the environment. Other bacteria can then encounter the DNA and, depending on the particular species and growth conditions, take up fragments of DNA and integrate them into their own chromosomes by recombination. A protein called RecA (see Figure 3.11a, page 65) binds to the cell's DNA and then to donor DNA causing the exchange of strands. A recipient cell with this new combination of genes is a kind of hybrid, or recombinant cell (**Figure 8.25**). All the descendants of such a recombinant cell will be identical to it. Transformation occurs naturally among very few genera of bacteria, including *Bacillus*, *Haemophilus* (hē-mä′fi-lus), *Neisseria*, *Acinetobacter* (a-si-ne′tō-bak-tėr), and certain strains of the genera *Streptococcus* and *Staphylococcus*.

Transformation works best when the donor and recipient cells are very closely related. Even though only a small portion of a cell's DNA is transferred to the recipient, the molecule that must pass through the recipient cell wall and membrane is still very large. When a recipient cell is in a physiological state in which it can take up the donor DNA, it is said to be competent. **Competence** results from alterations in the cell wall that make it permeable to large DNA molecules.

The well-understood and widely used bacterium *E. coli* is not naturally competent for transformation. However, a simple laboratory treatment enables *E. coli* to readily take up DNA. The discovery of this treatment has enabled researchers to use *E. coli* for genetic engineering, discussed in Chapter 9.

Conjugation in Bacteria

Another mechanism by which genetic material is transferred from one bacterium to another is known as **conjugation.** Conjugation is mediated by one kind of *plasmid*, a circular piece of DNA that replicates independently from the cell's chromosome (discussed on page 238). However, plasmids differ from bacterial chromosomes in that the genes they carry are usually not essential for the growth of the cell under normal conditions. The plasmids responsible for conjugation are transmissible between cells during conjugation.

Conjugation differs from transformation in two major ways. First, conjugation requires direct cell-to-cell contact. Second, the conjugating cells must generally be of opposite mating type; donor cells must carry the plasmid, and recipient cells usually do not. In gram-negative bacteria, the plasmid carries genes that code for the synthesis of *sex pili*, projections from the donor's cell surface that contact the recipient and help bring the two cells into direct contact (**Figure 8.26a**). Gram-positive bacterial cells produce sticky surface molecules that cause cells to come into direct contact with each other. In the process of conjugation, the plasmid is replicated during the transfer of a single-stranded copy of the plasmid DNA to the recipient, where the complementary strand is synthesized (**Figure 8.26b**).

Because most experimental work on conjugation has been done with *E. coli*, we will describe the process in this organism. In *E. coli*, the **F factor (fertility factor)** was the first plasmid observed to be transferred between cells during conjugation. Donors carrying F factors (F^+ cells) transfer the plasmid to recipients (F^- cells), which become F^+ cells as a result (**Figure 8.27a**). In some cells carrying F factors, the factor integrates into the chromosome, converting the F^+ cell to an **Hfr cell** (high frequency of recombination) (**Figure 8.27b**). When conjugation occurs between an Hfr cell and an F^- cell, the Hfr cell's chromosome (with its integrated F factor) replicates, and a parental strand of the chromosome is transferred to the recipient

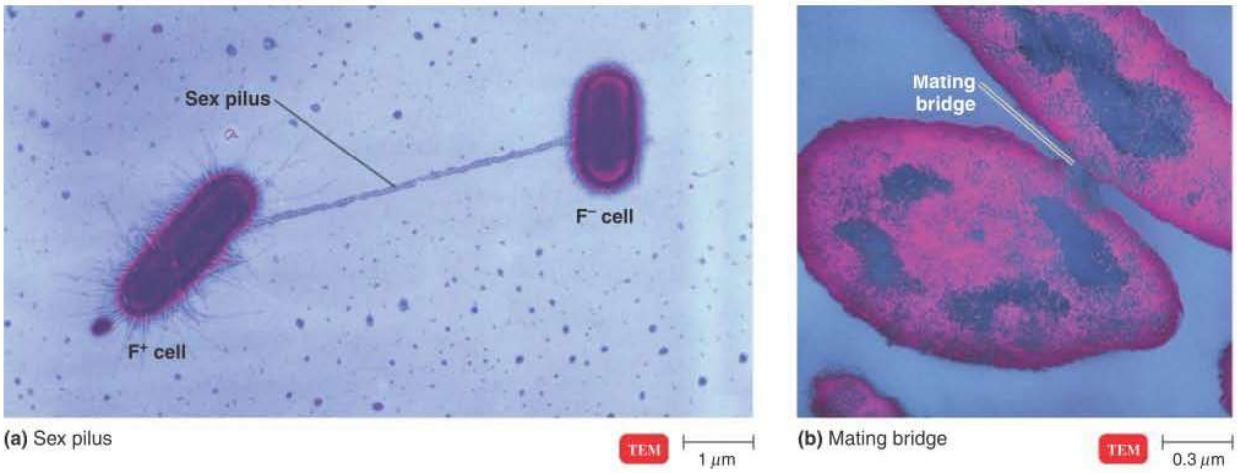


Figure 8.26 Bacterial conjugation.

Q What is an F^+ cell?

cell (Figure 8.27c). Replication of the Hfr chromosome begins in the middle of the integrated F factor, and a small piece of the F factor leads the chromosomal genes into the F^- cell. Usually, the chromosome breaks before it is completely transferred. Once within the recipient cell, donor DNA can recombine with the recipient's DNA. (Donor DNA that is not integrated is degraded.) Therefore, by conjugation with an Hfr cell, an F^- cell may acquire new versions of chromosomal genes (just as in transformation). However, it remains an F^- cell because it did not receive a complete F factor during conjugation.

Conjugation is used to map the location of genes on a bacterial chromosome (see Figure 8.1b). The genes for the synthesis of threonine *(thr)* and leucine *(leu)* are first, reading clockwise from 0. Their locations were determined by conjugation experiments. Assume that conjugation is allowed for only 1 minute between an Hfr strain that is his^+, pro^+, thr^+, and leu^+, and an F^- strain that is his^-, pro^-, thr^-, and leu^-. If the F^- acquired the ability to synthesize threonine, then the *thr* gene is located early in the chromosome, between 0 and 1 minute. If after 2 minutes the F^- cell now becomes thr^+ and leu^+, the order of these two genes on the chromosome must be *thr, leu.*

Transduction in Bacteria

A third mechanism of genetic transfer between bacteria is **transduction.** In this process, bacterial DNA is transferred from a donor cell to a recipient cell inside a virus that infects bacteria, called a **bacteriophage,** or **phage.** (Phages will be discussed further in Chapter 13.)

To understand how transduction works, we will consider the life cycle of one type of transducing phage of *E. coli;* this phage carries out **generalized transduction** (Figure 8.28).

During phage reproduction, phage DNA and proteins are synthesized by the host bacterial cell. The phage DNA should be packaged inside the phage protein coat. However, bacterial DNA, plasmid DNA, or even DNA of another virus may be packaged inside a phage protein coat.

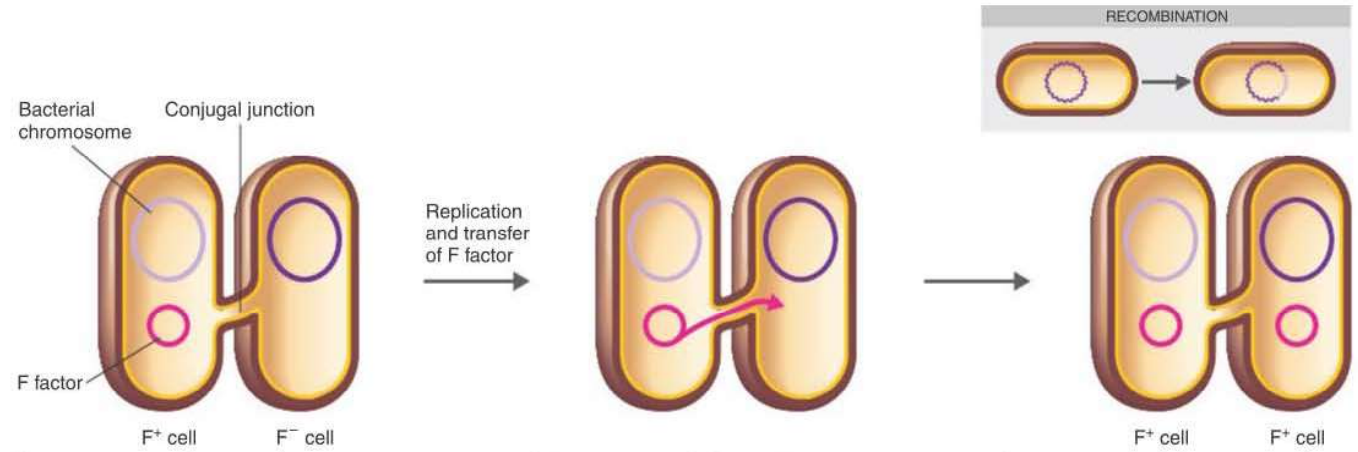
Q&A All genes contained within a bacterium infected by a generalized transducing phage are equally likely to be packaged in a phage coat and transferred. In another type of transduction, called **specialized transduction,** only certain bacterial genes are transferred. In one type of specialized transduction, the phage codes for certain toxins produced by their bacterial hosts, such as diphtheria toxin for *Corynebacterium diphtheriae* (kôr′-i-nē-bak-ti-rē-um dif-thi′-re-ī), erythrogenic toxin for *Streptococcus pyogenes*, and Shiga toxin for *E. coli* O157:H7. Specialized transduction will be discussed in Chapter 13 (page 382). In addition to mutation, transformation, and conjugation, transduction is another way bacteria acquire new genotypes. **Animations** Horizontal Gene Transfer: Overview; Transformation; Transduction: Generalized Transduction; Conjugation: Overview, F Factor, Hfr Conjugation, Chromosome Mapping. **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

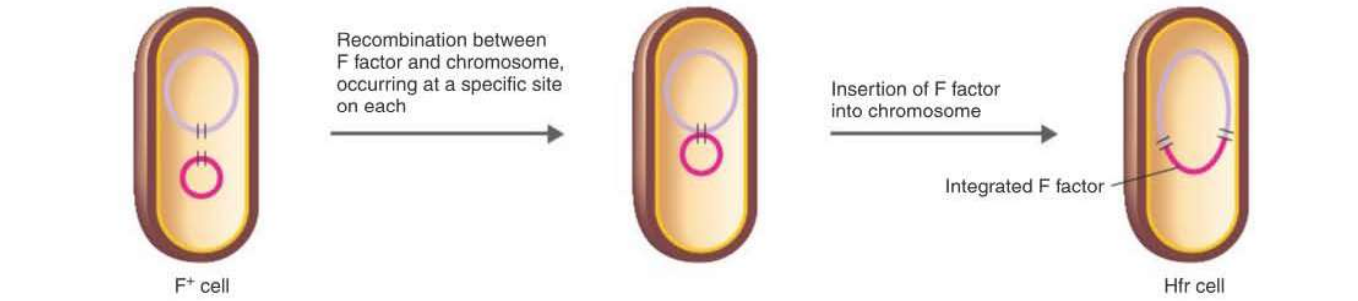
- Differentiate horizontal and vertical gene transfer. **8-14**
- Compare conjugation between the following pairs: $F^+ \times F^-$, Hfr $\times$ F^-. **8-15**

Plasmids and Transposons

Plasmids and transposons are genetic elements that provide additional mechanisms for genetic change. They occur in both



(a) When an F factor (a plasmid) is transferred from a donor (F^+) to a recipient (F^-), the F^- cell is converted to an F^+ cell.



(b) When an F factor becomes integrated into the chromosome of an F^+ cell, it makes the cell a high frequency of recombination (Hfr) cell.

(c) When an Hfr donor passes a portion of its chromosome into an F^- recipient, a recombinant F^- cell results.

Figure 8.27 Conjugation in *E. coli*.

Q How does conjugation differ from transformation?

prokaryotic and eukaryotic organisms, but this discussion focuses on their role in genetic change in prokaryotes.

Plasmids

Recall from Chapter 4 (page 85) that plasmids are self-replicating, gene-containing circular pieces of DNA about 1–5% the size of the bacterial chromosome (Figure 8.29a). They are found mainly in bacteria but also in some eukaryotic microorganisms, such as *Saccharomyces cerevisiae*. The F factor is a **conjugative plasmid** that carries genes for sex pili and for the transfer of the plasmid to another cell. Although plasmids are usually dispensable, under certain conditions genes carried by plasmids can be crucial to the survival and growth of the cell. For example, **dissimilation plasmids** code for enzymes

that trigger the catabolism of certain unusual sugars and hydrocarbons. Some species of *Pseudomonas* can actually use such exotic substances as toluene, camphor, and hydrocarbons of petroleum as primary carbon and energy sources because they have catabolic enzymes encoded by genes carried on plasmids. Such specialized capabilities permit the survival of those microorganisms in very diverse and challenging environments. Because of their ability to degrade and detoxify a variety of unusual compounds, many of them are being investigated for possible use in the cleanup of environmental wastes. (See the box in Chapter 2, page 33.)

Other plasmids code for proteins that enhance the pathogenicity of a bacterium. The strain of *E. coli* that causes infant diarrhea and traveler's diarrhea carries plasmids that code for toxin production and for bacterial attachment to intestinal cells. Without these plasmids, *E. coli* is a harmless resident of the large intestine; with them, it is pathogenic. Other plasmid-encoded toxins include the exfoliative toxin of *Staphylococcus aureus, Clostridium tetani* neurotoxin, and toxins of *Bacillus anthracis.* Still other plasmids contain genes for the synthesis of **bacteriocins,** toxic proteins that kill other bacteria. These plasmids have been found in many bacterial genera, and they are useful markers for the identification of certain bacteria in clinical laboratories.

Resistance factors (R factors) are plasmids that have significant medical importance. They were first discovered in Japan in the late 1950s after several dysentery epidemics. In some of these epidemics, the infectious agent was resistant to the usual antibiotic. Following isolation, the pathogen was also found to be resistant to a number of different antibiotics. In addition, other normal bacteria from the patients (such as *E. coli*) proved to be resistant as well. Researchers soon discovered that these bacteria acquired resistance through the spread of genes from one organism to another. The plasmids that mediated this transfer are R factors.

R factors carry genes that confer upon their host cell resistance to antibiotics, heavy metals, or cellular toxins. Many R factors contain two groups of genes. One group is called the **resistance transfer factor (RTF)** and includes genes for plasmid replication and conjugation. The other group, the **r-determinant,** has the resistance genes; it codes for the production of enzymes that inactivate certain drugs or toxic substances (**Figure 8.29b**). Different R factors, when present in the same cell, can recombine to produce R factors with new combinations of genes in their r-determinants.

In some cases, the accumulation of resistance genes within a single plasmid is quite remarkable. For example, Figure 8.29b shows a genetic map of resistance plasmid R100. Carried on this plasmid are resistance genes for sulfonamides, streptomycin, chloramphenicol, and tetracycline, as well as genes for resistance to mercury. This particular plasmid can be transferred between a number of enteric species, including *Escherichia, Klebsiella,* and *Salmonella.*

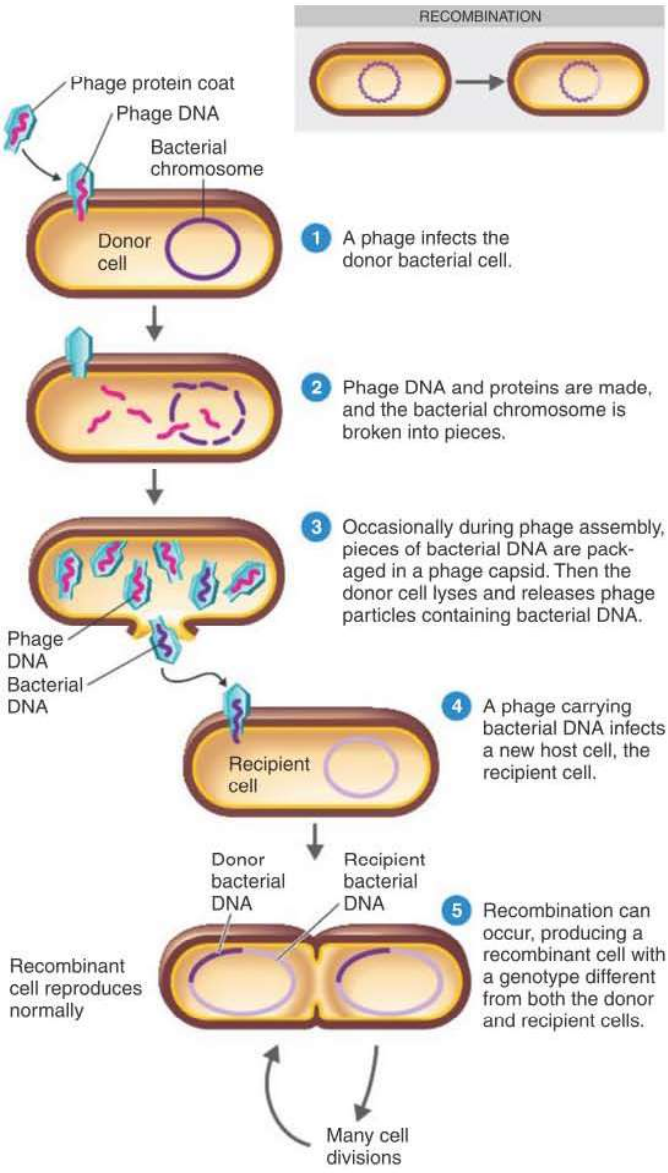
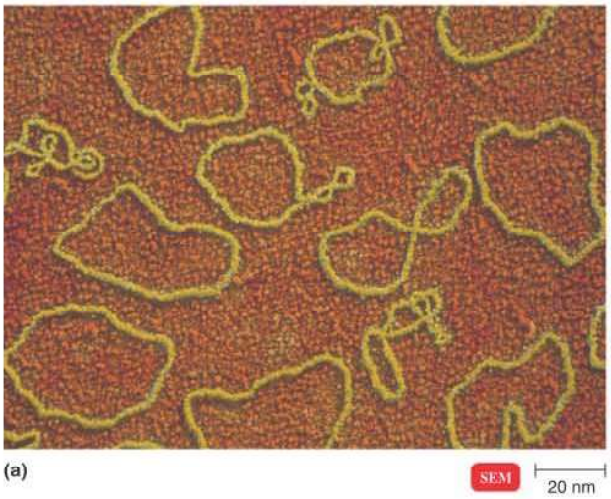


Figure 8.28 Transduction by a bacteriophage. Shown here is generalized transduction, in which any bacterial DNA can be transferred from one cell to another.

Q **What is transduction?**

R factors present very serious problems for treating infectious diseases with antibiotics. The widespread use of antibiotics in medicine and agriculture (see the box in Chapter 20 on page 577) has led to the preferential survival (selection) of bacteria that have R factors, so populations of resistant bacteria grow larger and larger. The transfer of resistance between bacterial cells of a population, and even between bacteria of different genera, also contributes to the problem. The ability to reproduce sexually with



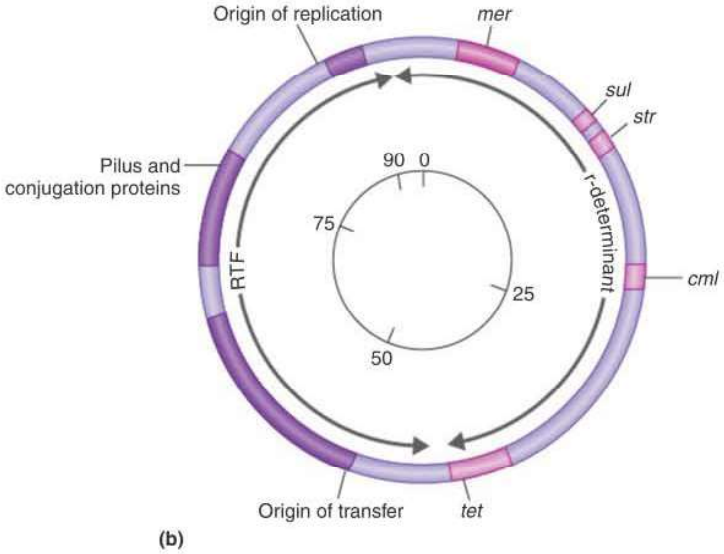


Figure 8.29 R factor, a type of plasmid. (**a**) Plasmids from *Bacteroides fragilis* bacteria that encode resistance to the antibiotic clindamycin. (**b**) A diagram of an R factor, which has two parts: the RTF contains genes needed for plasmid replication and transfer of the plasmid by conjugation, and the r-determinant carries genes for resistance to four different antibiotics and mercury (*sul* = sulfonamide resistance, *str* = streptomycin resistance, *cml* = chloramphenicol resistance, *tet* = tetracycline resistance, *mer* = mercury resistance); numbers are base pairs × 1000.

Q **Why are R factors important in the treatment of infectious diseases?**

members of its own species defines a eukaryotic species. However, a bacterial species can conjugate and transfer plasmids to other species. *Neisseria* may have acquired its penicillinase-producing plasmid from *Streptococcus,* and *Agrobacterium* can transfer plasmids to plant cells (see Figure 9.20, page 265). Nonconjugative plasmids may be transferred from one cell to another by inserting themselves into a conjugative plasmid or a chromosome or by transformation when released from a dead cell. Insertion is made possible by an insertion sequence, which will be discussed shortly.

Plasmids are an important tool for genetic engineering, discussed in Chapter 9 (page 250).

Transposons

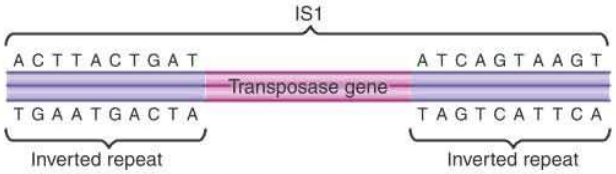
Transposons are small segments of DNA that can move (be "transposed") from one region of a DNA molecule to another. These pieces of DNA are 700 to 40,000 base pairs long.

In the 1950s, American geneticist Barbara McClintock discovered transposons in corn, but they occur in all organisms and have been studied most thoroughly in microorganisms. They may move from one site to another site on the same chromosome or to another chromosome or plasmid. As you might imagine, the frequent movement of transposons could wreak havoc inside a cell. For example, as transposons move about on chromosomes, they may insert themselves *within* genes, inactivating them. Fortunately, transposition occurs relatively rarely. The frequency of transposition is comparable to the spontaneous mutation rate that occurs in bacteria—that is, from 10^{-5} to 10^{-7} per generation.

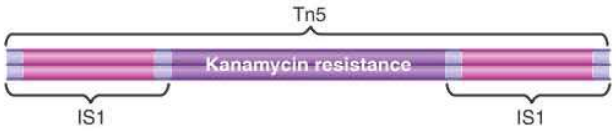
All transposons contain the information for their own transposition. As shown in **Figure 8.30a**, the simplest transposons, also called **insertion sequences (IS),** contain only a gene that codes for an enzyme (*transposase,* which catalyzes the cutting and resealing of DNA that occurs in transposition) and recognition sites. *Recognition sites* are short inverted repeat sequences of DNA that the enzyme recognizes as recombination sites between the transposon and the chromosome.

Complex transposons also carry other genes not connected with the transposition process. For example, bacterial transposons may contain genes for enterotoxin or for antibiotic resistance (**Figure 8.30b**). Plasmids such as R factors are frequently made up of a collection of transposons (**Figure 8.30c**).

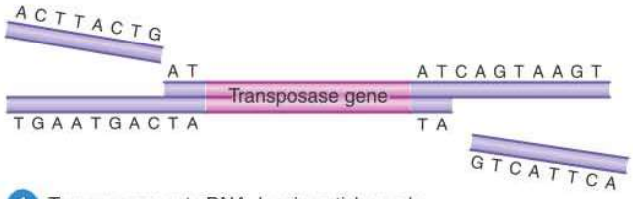
Transposons with antibiotic resistance genes are of practical interest, but there is no limitation on the kinds of genes that transposons can have. Thus, transposons provide a natural



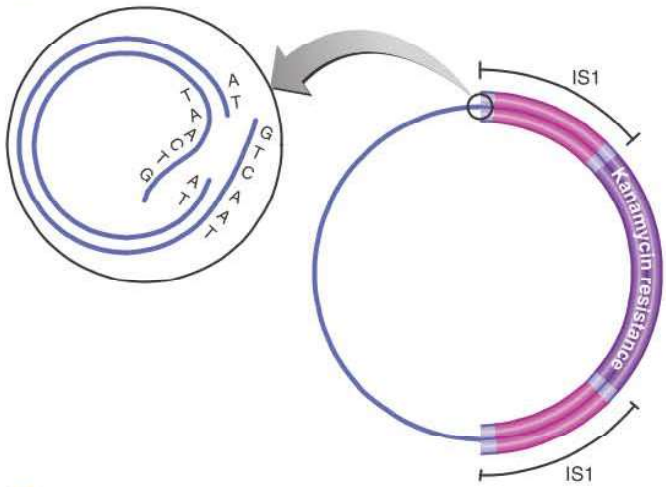
(a) An insertion sequence (IS), the simplest transposon, contains a gene for transposase, the enzyme that catalyzes transposition. The tranposase gene is bounded at each end by inverted repeat sequences that function as recognition sites for the transposon. IS1 is one example of an insertion sequence, shown here with simplified IR sequences.



(b) Complex transposons carry other genetic material in addition to transposase genes. The example shown here, Tn5, carries the gene for kanamycin resistance and has complete copies of the insertion sequence IS1 at each end.



1 Transposase cuts DNA, leaving sticky ends.



2 Sticky ends of transposon and target DNA anneal.

(c) Insertion of the transposon Tn5 into R100 plasmid.

Figure 8.30 Transposons and insertion.

Q Why are transposons sometimes referred to as "jumping genes"?

mechanism for the movement of genes from one chromosome to another. Furthermore, because they may be carried between cells on plasmids or viruses, they can also spread from one organism—or even species—to another. For example, vancomycin resistance was transferred from *Enterococcus faecalis* to *Staphylococcus aureus* via a transposon called Tn1546. Transposons are thus a potentially powerful mediator of evolution in organisms. **Animations** Transposons: Overview, Insertion Sequences, Complex Transposons **www.microbiologyplace.com**

CHECK YOUR UNDERSTANDING

✓ What types of genes do plasmids carry? **8-16**

Genes and Evolution

LEARNING OBJECTIVE

8-17 Discuss how genetic mutation and recombination provide material for natural selection to act upon.

We have now seen how gene activity can be controlled by the cell's internal regulatory mechanisms and how genes themselves can be altered or rearranged by mutation, transposition, and recombination. All these processes provide diversity in the descendants of cells. Diversity provides the raw material for evolution, and natural selection provides its driving force. Natural selection will act on diverse populations to ensure the survival of those fit for that particular environment. The different kinds of microorganisms that exist today are the result of a long history of evolution. Microorganisms have continually changed by alterations in their genetic properties and acquisition of adaptations to many different habitats. See the box on antibiotic resistance in Chapter 26, page 751, for an example of natural selection.

CHECK YOUR UNDERSTANDING

✓ Natural selection means that the environment favors survival of some genotypes. From where does diversity in genotypes come? **8-17**

STUDY OUTLINE

The **MyMicrobiologyPlace** website (**www.microbiologyplace.com**) will help you get ready for tests with its simple three-step approach: **1 take a pre-test** and obtain a personalized study plan, **2 learn and practice** with animations, tutorials, and MP3 tutor sessions, and **3 test yourself** with quizzes and a chapter post-test.

Structure and Function of the Genetic Material (pp. 211–221)

1. Genetics is the study of what genes are, how they carry information, how their information is expressed, and how they are replicated and passed to subsequent generations or other organisms.
2. DNA in cells exists as a double-stranded helix; the two strands are held together by hydrogen bonds between specific nitrogenous base pairs: AT and CG.
3. A gene is a segment of DNA, a sequence of nucleotides, that codes for a functional product, usually a protein.
4. The DNA in a cell is duplicated before the cell divides, so each daughter cell receives the same genetic information.

Genotype and Phenotype (p. 211)

5. Genotype is the genetic composition of an organism, its entire complement of DNA.
6. Phenotype is the expression of the genes: the proteins of the cell and the properties they confer on the organism.

DNA and Chromosomes (pp. 211–212)

7. The DNA in a chromosome exists as one long double helix associated with various proteins that regulate genetic activity.
8. Bacterial DNA is circular; the chromosome of *E. coli,* for example, contains about 4 million base pairs and is approximately 1000 times longer than the cell.
9. Genomics is the molecular characterization of genomes.

The Flow of Genetic Information (p. 212)

10. Information contained in the DNA is transcribed into RNA and translated into proteins.

DNA Replication (pp. 212–215)

11. During DNA replication, the two strands of the double helix separate at the replication fork, and each strand is used as a template by DNA polymerases to synthesize two new strands of DNA according to the rules of nitrogenous base pairing.
12. The result of DNA replication is two new strands of DNA, each having a base sequence complementary to one of the original strands.
13. Because each double-stranded DNA molecule contains one original and one new strand, the replication process is called semiconservative.
14. DNA is synthesized in one direction designated 5′ → 3′. At the replication fork, the leading strand is synthesized continuously and the lagging strand discontinuously.
15. DNA polymerase proofreads new molecules of DNA and removes mismatched bases before continuing DNA synthesis.
16. Each daughter bacterium receives a chromosome that is virtually identical to the parent's.

RNA and Protein Synthesis (pp. 216–221)

17. During transcription, the enzyme RNA polymerase synthesizes a strand of RNA from one strand of double-stranded DNA, which serves as a template.
18. RNA is synthesized from nucleotides containing the bases A, C, G, and U, which pair with the bases of the DNA strand being transcribed.
19. RNA polymerase binds the promoter; transcription begins at AUG; the region of DNA that is the end point of transcription is the terminator; RNA is synthesized in the 5′ → 3′ direction.
20. Translation is the process in which the information in the nucleotide base sequence of mRNA is used to dictate the amino acid sequence of a protein.
21. The mRNA associates with ribosomes, which consist of rRNA and protein.
22. Three-base segments of mRNA that specify amino acids are called codons.
23. The genetic code refers to the relationship among the nucleotide base sequence of DNA, the corresponding codons of mRNA, and the amino acids for which the codons code.
24. The genetic code is degenerate; that is, most amino acids are coded for by more than one codon.
25. Of the 64 codons, 61 are sense codons (which code for amino acids), and 3 are nonsense codons (which do not code for amino acids and are stop signals for translation).
26. The start codon, AUG, codes for methionine.
27. Specific amino acids are attached to molecules of tRNA. Another portion of the tRNA has a base triplet called an anticodon.
28. The base pairing of codon and anticodon at the ribosome results in specific amino acids being brought to the site of protein synthesis.
29. The ribosome moves along the mRNA strand as amino acids are joined to form a growing polypeptide; mRNA is read in the 5′ → 3′ direction.
30. Translation ends when the ribosome reaches a stop codon on the mRNA.

The Regulation of Bacterial Gene Expression (pp. 221–226)

1. Regulating protein synthesis at the gene level is energy-efficient because proteins are synthesized only as they are needed.
2. Constitutive enzymes produce products at a fixed rate. Examples are genes for the enzymes in glycolysis.
3. For these gene regulatory mechanisms, the control is aimed at mRNA synthesis.

Repression and Induction (p. 224)

4. Repression controls the synthesis of one or several (repressible) enzymes.
5. When cells are exposed to a particular end-product, the synthesis of enzymes related to that product decreases.

CHAPTER
30

Genetics & Gene Therapy

CHAPTER CONTENTS

INTRODUCTION

The study of viral genetics falls into two general areas: (1) mutations and their effect on replication and pathogenesis; and (2) the interaction of two genetically distinct viruses that infect the same cell. In addition, viruses serve as **vectors** in gene therapy and in recombinant vaccines, two areas that hold great promise for the treatment of genetic diseases and the prevention of infectious diseases.

MUTATIONS

Mutations in viral DNA and RNA occur by the same processes of base substitution, deletion, and frameshift as those described for bacteria in Chapter 4. Probably the most important practical use of mutations is in the production of vaccines containing live, attenuated virus. These attenuated mutants have lost their pathogenicity but have retained their antigenicity; therefore, they induce immunity without causing disease.

There are two other kinds of mutants of interest. The first are **antigenic variants** such as those that occur frequently with influenza viruses, which have an altered

surface protein and are therefore no longer inhibited by a person's preexisting antibody. The variant can thus cause disease, whereas the original strain cannot. Human immunodeficiency virus and hepatitis C virus also produce many antigenic variants. These viruses have an "**error-prone**" **polymerase** that causes the mutations. The second are **drug-resistant mutants**, which are insensitive to an antiviral drug because the target of the drug, usually a viral enzyme, has been modified.

Conditional lethal mutations are extremely valuable in determining the function of viral genes. These mutations function normally under permissive conditions but fail to replicate or to express the mutant gene under restrictive conditions. For example, **temperature-sensitive** conditional lethal mutants express their phenotype normally at a low (permissive) temperature, but at a higher (restrictive) temperature, the mutant gene product is inactive. To give a specific example, temperature-sensitive mutants of Rous sarcoma virus can transform cells to malignancy at the permissive temperature of 37°C. When the transformed cells are grown at the restrictive temperature of 41°C, their phenotype reverts to normal appearance and behavior. The malignant phenotype is regained when the permissive temperature is restored.

Note that temperature-sensitive mutants have now entered clinical practice. Temperature-sensitive mutants of influenza virus are now being used to make a vaccine, because this virus will grow in the cooler, upper airways where it causes few symptoms and induces antibodies, but it will not grow in the warmer, lower airways where it can cause pneumonia.

Some deletion mutants have the unusual property of being **defective interfering particles**. They are defective because they cannot replicate unless the deleted function is supplied by a "helper" virus. They also interfere with the growth of normal virus if they infect first and preempt the required cellular functions. Defective interfering particles may play a role in recovery from viral infection; they interfere with the production of progeny virus, thereby limiting the spread of the virus to other cells.

INTERACTIONS BETWEEN VIRUSES

When two genetically distinct viruses infect a cell, three different phenomena can ensue.

(1) **Recombination** is the exchange of genes between two chromosomes that is based on crossing over within regions of significant base sequence homology.

Recombination can be readily demonstrated for viruses with double-stranded DNA as the genetic material and has been used to determine their genetic map. However, recombination by RNA viruses occurs at a very low frequency, if at all.

Reassortment is the term used when viruses with segmented genomes, such as influenza virus, exchange segments. This usually results in a much higher frequency of gene exchange than does recombination. Reassortment of influenza virus RNA segments is involved in the major antigenic changes in the virus that are the basis for recurrent influenza epidemics.

(2) **Complementation** can occur when either one or both of the two viruses that infect the cell have a mutation that results in a nonfunctional protein (Figure 30–1). The nonmutated virus "complements" the mutated one by making a functional protein that serves for both viruses. Complementation is an important method by which a helper virus permits replication of a defective virus. One clinically important example of complementation is hepatitis B virus providing its surface antigen to hepatitis delta virus, which is defective in its ability to produce its own outer protein.

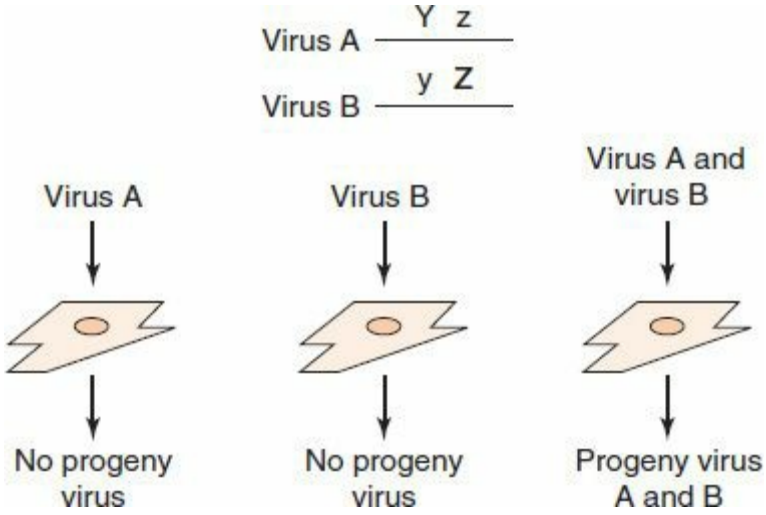


FIGURE 30–1 Complementation. If *either* virus A *or* virus B infects a cell, no virus is produced because each has a mutated gene. If *both* virus A and virus B infect a cell, the protein product of gene Y of virus A will complement virus B, the protein product of gene Z of virus B will complement virus A, and progeny of both virus A and virus B will be produced. Note that no recombination has occurred and that the virus A progeny will contain the mutated z gene and the virus B progeny will contain the mutant y gene. Y, Z, functional genes; y, z, mutated, nonfunctional genes.

This phenomenon is the basis for the complementation test, which can be used to determine how many genes exist in a viral genome. It is performed by determining

whether mutant virus A can complement mutant virus B. If it can, the two mutations are in separate genes because they make different, complementary proteins. If it cannot, the two mutations are in the same gene, and both proteins are nonfunctional. By performing many of these paired tests with different mutants, it is possible to determine functional domains of complementation groups that correspond to genes. Appropriate controls are needed to obviate the effects of recombination.

(3) In **phenotypic mixing,** the genome of virus type A can be coated with the surface proteins of virus type B (Figure 30–2). This phenotypically mixed virus can infect cells as determined by its type B protein coat. However, the progeny virus from this infection has a type A coat; it is encoded solely by its type A genetic material. An interesting example of phenotypic mixing is that of **pseudotypes,** which consist of the nucleocapsid of one virus and the envelope of another. Pseudotypes composed of the nucleocapsid of vesicular stomatitis virus (a rhabdovirus) and the envelope of human immunodeficiency virus (HIV; a retrovirus) are currently being used to study the immune response to HIV.

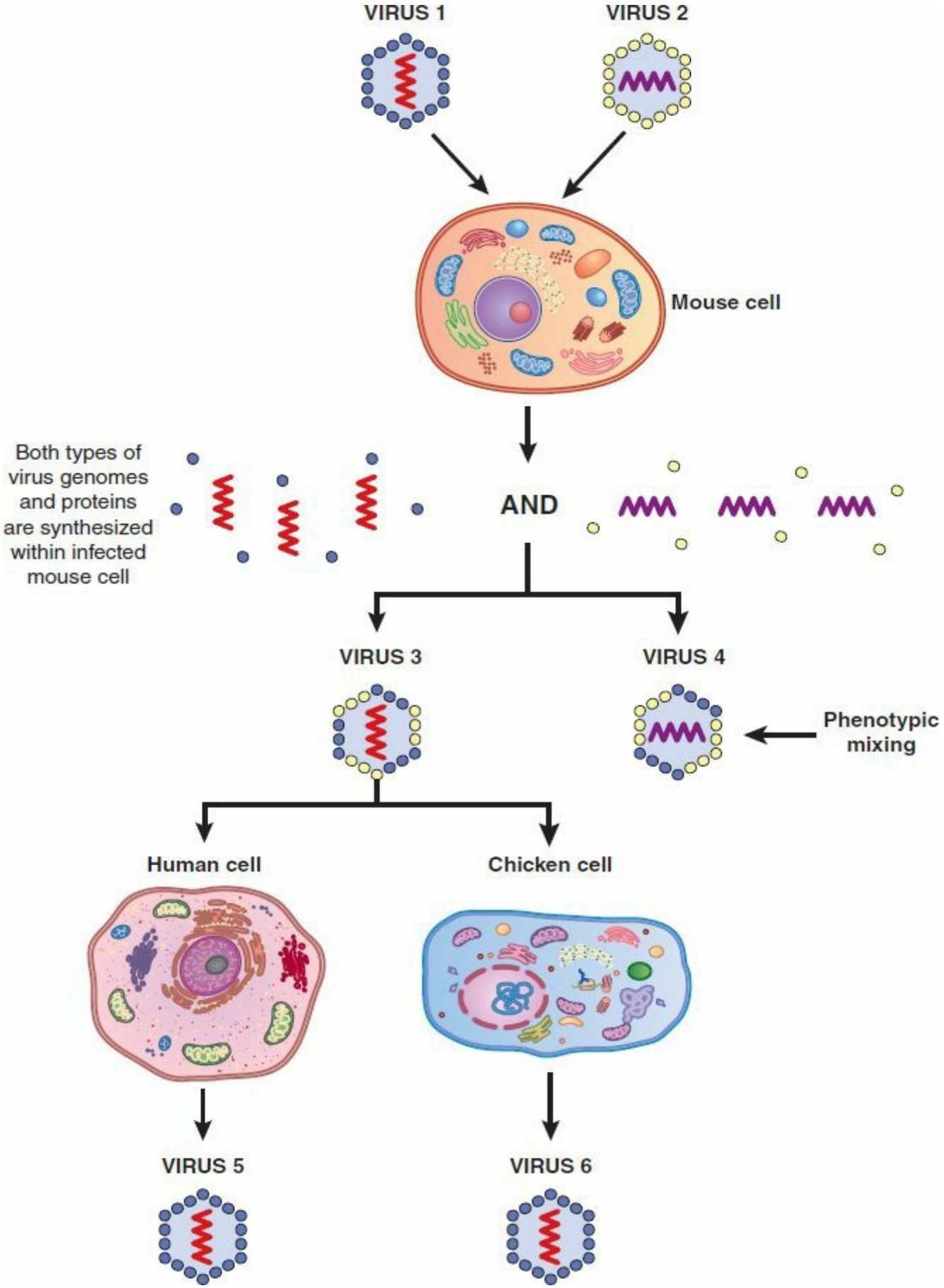

VIRUS 1
VIRUS 2
Mouse cell
Both types of virus genomes and proteins are synthesized within infected mouse cell
AND
VIRUS 3
VIRUS 4
Phenotypic mixing
Human cell
Chicken cell
VIRUS 5
VIRUS 6

FIGURE 30–2 Phenotypic mixing. Initially, Virus 1 (Blue capsid proteins and vertical genome) and Virus 2 (Yellow capsid proteins and horizontal genome) infect the same mouse cell. Assume that Virus 1 can infect human cells but not chicken cells (a property determined by the blue surface proteins) and that Virus 2 can infect chicken cells but not human cells (a property determined by the yellow surface proteins). However, both Virus 1 and Virus 2 can infect a mouse cell. Within the mouse cell, both genomes are replicated and both blue and yellow capsid proteins are synthesized.

As shown, some of the progeny virus (Viruses 3 and 4) exhibit *phenotypic mixing* because they have both the blue and the yellow surface proteins and therefore can infect both chicken cells and human cells. Note that in the next round of infection, when progeny Virus 3 infects either human cells or chicken cells, the progeny of that infection (Viruses 5 and 6) is determined by the vertical genome and will be identical to Virus 1 with only blue capsid proteins and a vertical genome. Similarly (but not shown), when progeny Virus 4 infects either human cells or chicken cells, the progeny of that infection is determined by the horizontal genome and will be identical to Virus 2. (Modified and reproduced with permission from Joklik W et al. *Zinsser Microbiology*, 20th ed. Appleton & Lange, Norwalk, CT, 1992.)

GENE THERAPY & RECOMBINANT VACCINES

Viruses are being used as genetic vectors in two novel ways: (1) to deliver new, functional genes to patients with genetic diseases (gene therapy); and (2) to produce new viral vaccines that contain recombinant viruses carrying the genes of several different viruses, thereby inducing immunity to several diseases with one immunization.

Gene Therapy

Retroviruses are currently being used as vectors of the gene encoding adenine deaminase (ADA) in patients with immunodeficiencies resulting from a defective ADA gene. Retroviruses are excellent vectors because a DNA copy of their RNA genome is stably integrated into the host cell DNA and the integrated genes are expressed efficiently. Retroviral vectors are constructed by removing the genes encoding several viral proteins from the virus and replacing them with the human gene of interest (e.g., the ADA gene). Virus particles containing the human gene are produced within "helper cells" that contain the deleted viral genes and therefore can supply, by complementation, the missing viral proteins necessary for the virus to replicate. The retroviruses produced by the helper cells can infect the patient's cells

and introduce the human gene into the cells, but the viruses cannot replicate because they lack several viral genes. This inability of these viruses to replicate is an important advantage in human gene therapy.

Recombinant Vaccines

Recombinant viral vaccines contain viruses that have been genetically engineered to carry the genes of other viruses. Viruses with large genomes (e.g., vaccinia virus) are excellent candidates for this purpose. To construct the recombinant virus, any vaccinia virus gene that is not essential for viral replication is deleted, and the gene from the other virus that encodes the antigen that elicits neutralizing antibody is introduced. For example, the gene for the surface antigen of hepatitis B virus has been introduced into vaccinia virus and is expressed in infected cells. Recombinant vaccines are not yet clinically available, but vaccines of this type promise to greatly improve the efficiency of our immunization programs.

PEARLS

- Mutations in the viral genome can produce antigenic variants and drug-resistant variants. Mutations can also produce **attenuated** (weakened) variants that cannot cause disease but retain their antigenicity and are useful in vaccines.
- Temperature-sensitive mutants can replicate at a low (permissive) temperature but not at a high (restrictive) temperature. Temperature-sensitive mutants of influenza virus are used in one of the vaccines against this disease.
- **Reassortment** (exchange) of segments of the genome RNA of influenza virus is important in the pathogenesis of the worldwide epidemics caused by this virus.
- **Complementation** occurs when one virus produces a protein that can be used by another virus. A medically important example is hepatitis D virus, which uses the surface antigen of hepatitis B virus as its outer coat protein.
- **Phenotypic mixing** occurs when two different viruses infect the same cell and progeny viruses contain proteins of both parental viruses. This can endow the progeny viruses with the ability to

infect cells of species that ordinarily parental virus could not.

SELF-ASSESSMENT QUESTIONS

1. In the lab, a virologist was studying the properties of HIV. She infected the same cell with both HIV and rabies virus. (HIV can infect only human CD4-positive cells, whereas rabies virus can infect both human cells and dog cells.) Some of the progeny virions were able to infect dog cells, within which she found HIV-specific RNA. Which one of the following is the term used to describe these results?

   (A) Complementation

   (B) Phenotypic mixing

   (C) Reassortment

   (D) Recombination

2. You have isolated two mutants of poliovirus, one mutated at gene X and the other mutated at gene Y. If you infect cells with each one alone, no virus is produced. If you infect a single cell with both mutants, which one of the following statements is most accurate?

   (A) If complementation between the mutant gene products occurs, both X and Y progeny viruses will be made.

   (B) If phenotypic mixing occurs, then both X and Y progeny viruses will be made.

   (C) If the genome is transcribed into DNA, then both X and Y viruses will be made.

   (D) Because reassortment of the genome segments occurs at high frequency, both X and Y progeny viruses will be made.

ANSWERS

1. (**B**)
2. (**A**)

PRACTICE QUESTIONS: USMLE & COURSE

EXAMINATIONS

Questions on the topics discussed in this chapter can be found in the Basic Virology section of Part XIII: USMLE (National Board) Practice Questions starting on page 700. Also see Part XIV: USMLE (National Board) Practice Examination starting on page 731.

طفيليات دكتوراه مجهري

CHAPTER
52

Blood & Tissue Protozoa

CHAPTER CONTENTS

INTRODUCTION

The medically important organisms in this category of protozoa consist of the sporozoans *Plasmodium* and *Toxoplasma* and the flagellates *Trypanosoma* and *Leishmania*. *Pneumocystis* is discussed in this book as a protozoan because it is considered as such from a medical point of view. However, molecular data indicate that it is related to yeasts such as *Saccharomyces cerevisiae*. Table 51–2 summarizes several important features of these blood and tissue protozoa.

The medically important stages in the life cycle of the blood and tissue protozoa are described in Table 52–1.

TABLE 52–1 **Medically Important Stages in Life Cycle of Blood and Tissue Protozoa**

Organism	Insect Vector	Stage That Infects Humans	Stage(s) in Humans Most Associated with Disease	Important Stage(s) Outside of Humans
Plasmodium	Female mosquito (*Anopheles*)	Sporozoite in mosquito saliva	Trophozoites and merozoites in red blood cells	Mosquito ingests gametocytes → fuse to form zygote → ookinete → sporozoites
Toxoplasma	None	Tissue cyst (pseudocysts) in undercooked meat or oocyst in cat feces	Rapidly multiplying trophozoites (tachyzoites) within various cell types; tachyzoites can pass placenta and infect fetus; slowly multiplying trophozoites (bradyzoites) in tissue cysts	Cat ingests tissue cysts containing bradyzoites → gametes → ookinete → oocysts in feces
Pneumocystis	None	Uncertain; probably cyst	Cysts	None known
Trypanosoma cruzi	Reduviid bug (*Triatoma*)	Trypomastigote in bug feces	Amastigotes in cardiac muscle and neurons	Bug ingests trypomastigote in human blood → epimastigote → trypomastigote
Trypanosoma gambiense and *Trypanosoma rhodesiense*	Tsetse fly (*Glossina*)	Trypomastigote in fly saliva	Trypomastigotes in blood and brain	Fly ingests trypomastigote in human blood → epimastigote → trypomastigote
Leishmania donovani	Sandfly (*Phlebotomus* and *Lutzomyia*)	Promastigotes in fly saliva	Amastigotes in macrophages in spleen, liver, and bone marrow	Fly ingests macrophages containing amastigotes → promastigotes
Leishmania tropica and others	Sandfly (*Phlebotomus* and *Lutzomyia*)	Promastigotes in fly saliva	Amastigotes in macrophages in skin	Fly ingests macrophages containing amastigotes → promastigotes

PLASMODIUM

Disease

Malaria is caused by four plasmodia: *Plasmodium vivax, Plasmodium ovale, Plasmodium malariae*, and *Plasmodium falciparum. P. vivax* and *P. falciparum* are more common causes of malaria than are *P. ovale* and *P. malariae*. Worldwide, malaria is one of the most common infectious diseases and a leading cause of death.

Important Properties

The life cycle of *Plasmodium* species is shown in Figure 52–1. The vector and definitive host for plasmodia is the **female *Anopheles* mosquito** (only the female takes a blood meal). There are two phases in the life cycle: the sexual cycle, which occurs primarily in mosquitoes, and the asexual cycle, which occurs in humans, the

intermediate hosts.[1]

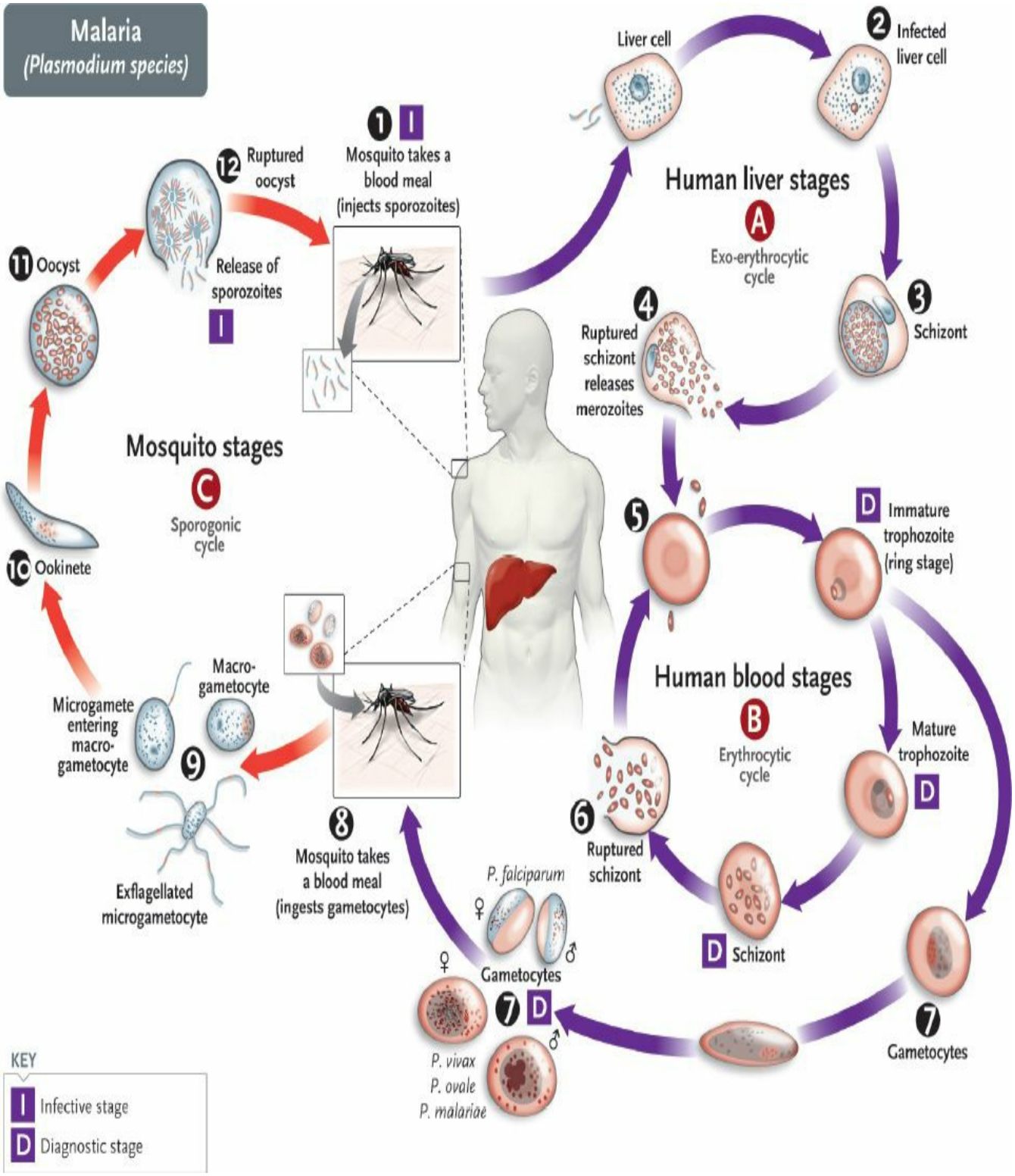


FIGURE 52–1 *Plasmodium* species. Life cycle. Right side of figure describes the stages within the human (blue arrows). Cycle A (top right) is the exo-erythrocyte

stage that occurs in the liver. Cycle B (bottom right) is the erythrocyte stage that occurs in the red blood cell. Note that at step 6 in the cycle, merozoites released from the ruptured schizonts then infect other red blood cells. The synchronized release of merozoites causes the periodic fever and chills characteristic of malaria. Left side of figure describes the stages within the mosquito (red arrows). Humans are infected at step 1 when mosquito injects sporozoites. Mosquito is infected at step 8 when mosquito ingests gametocytes in human blood. (Provider: Centers for Disease Control and Prevention/Dr. Alexander J. da Silva and Melanie Moser.)

The sexual cycle is called **sporogony** because sporozoites are produced (sporogonic cycle is labeled C in Figure 52–1), and the asexual cycle is called **schizogony** because schizonts are made.

The life cycle in humans begins with the introduction of sporozoites into the blood from the saliva of the biting mosquito. The sporozoites are taken up by hepatocytes within 30 minutes. This "exoerythrocytic" phase (labeled A in Figure 52–1) consists of cell multiplication and differentiation into **merozoites**. *P. vivax* and *P. ovale* produce a latent form (**hypnozoite**) in the liver; this form is the cause of relapses seen with vivax and ovale malaria.

Merozoites are released from the liver cells and infect red blood cells. During the erythrocytic phase (labeled B in Figure 52–1), the organism differentiates into a ring-shaped trophozoite (Figures 52–2A and B and 52–3). The ring form grows into an ameboid form and then differentiates into a schizont filled with merozoites (Figure 52–2C). After release, the merozoites infect other erythrocytes (step 6 in Figure 52–1). This cycle in the red blood cell repeats at regular intervals typical for each species. The periodic release of merozoites causes the typical recurrent symptoms of chills, fever, and sweats seen in malaria patients.

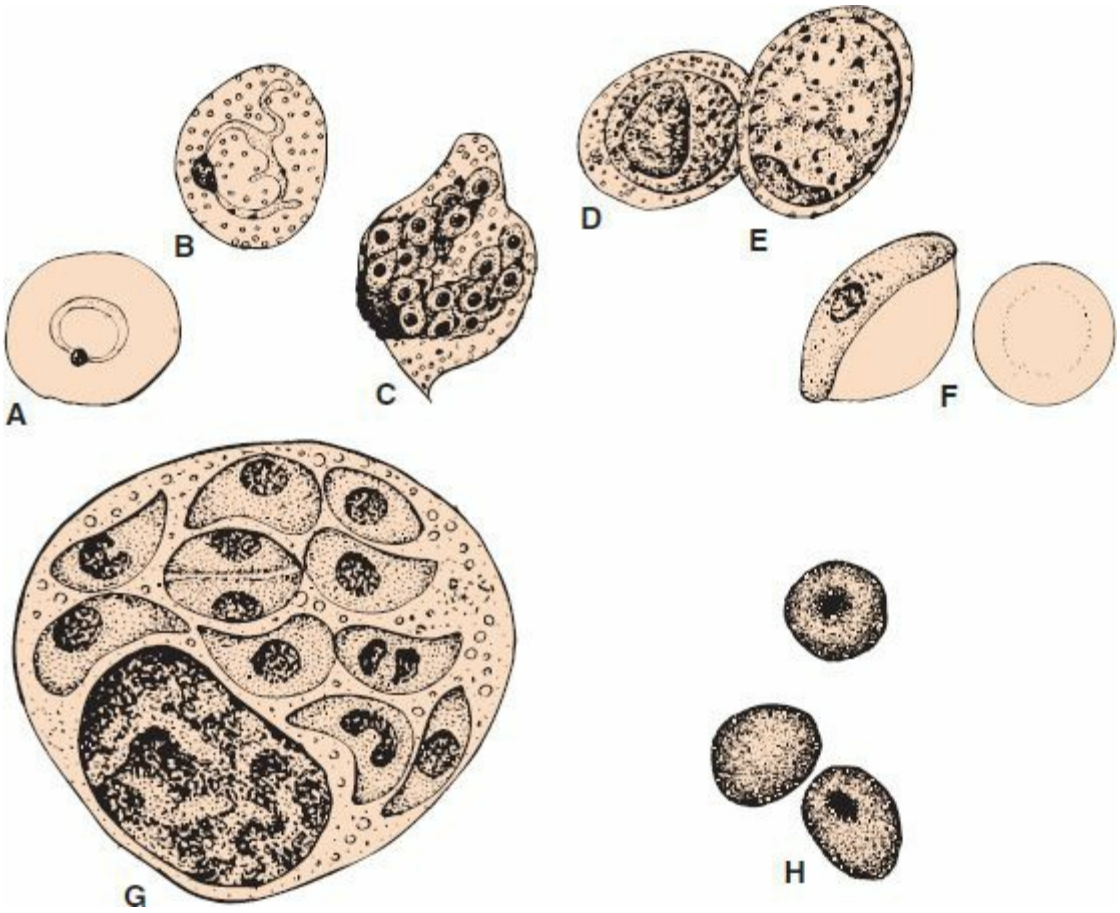


FIGURE 52–2 **A:** *Plasmodium vivax* signet-ring trophozoite within a red blood cell. **B:** *Plasmodium vivax* ameboid trophozoite within a red blood cell, showing Schüffner's dots. **C:** *Plasmodium vivax* mature schizont with merozoites inside. **D:** *Plasmodium vivax* microgametocyte. **E:** *Plasmodium vivax* macrogametocyte. **F:** *Plasmodium falciparum* "banana-shaped" gametocyte with attached red cell ghost. **G:** *Toxoplasma gondii* trophozoites within macrophage. **H:** *Pneumocystis jiroveci* cysts. (A–G, 1200×; H, 800×.)

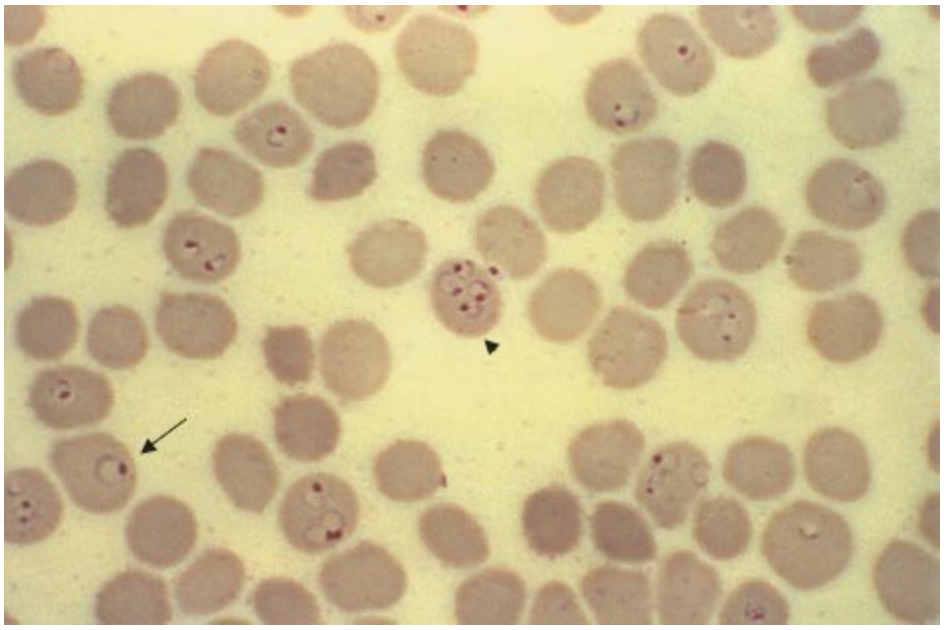


FIGURE 52–3 *Plasmodium falciparum*—ring-shaped trophozoite. Long arrow points to a red blood cell containing a ring-shaped trophozoite. Arrowhead points to a red blood cell containing four ring-shaped trophozoites. Note the very high percentage of red cells containing ring forms. This high-level parasitemia is more often seen in *Plasmodium falciparum* infection than in infection by the other plasmodia. (Figure courtesy of Dr. S. Glenn, Public Health Image Library, Centers for Disease Control and Prevention.)

The sexual cycle begins in the human red blood cells when some merozoites develop into male and others into female gametocytes (Figures 52–2D to F and 52–4, and step 7 in Figure 52–1). The gametocyte-containing red blood cells are ingested by the female *Anopheles* mosquito and, within her gut, produce a female macrogamete and eight spermlike male microgametes. After fertilization, the diploid zygote differentiates into a motile ookinete that burrows into the gut wall, where it grows into an oocyst within which many haploid sporozoites are produced. The sporozoites are released and migrate to the salivary glands, ready to complete the cycle when the mosquito takes her next blood meal.

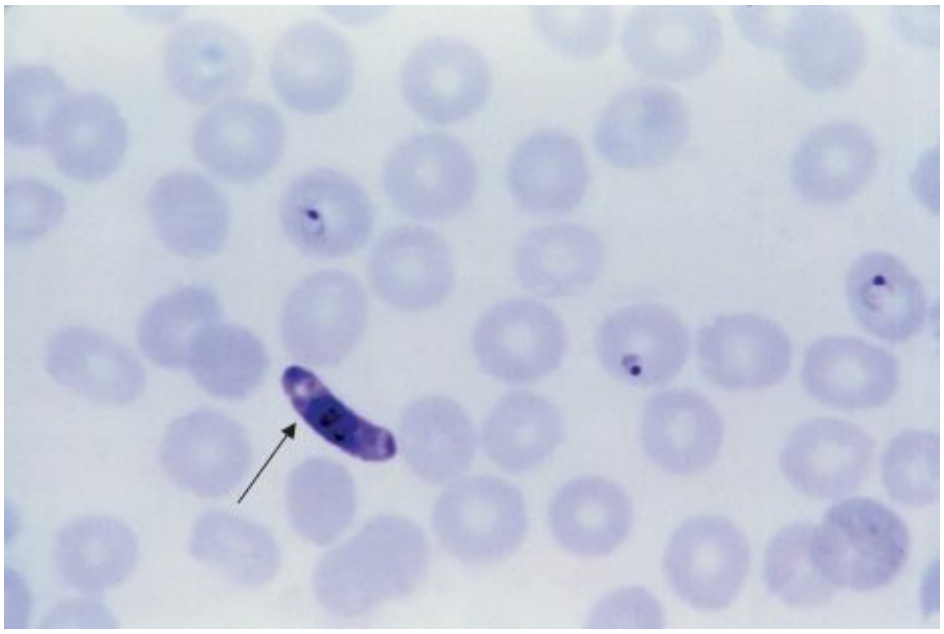


FIGURE 52–4 *Plasmodium falciparum*—gametocyte. Arrow points to a "banana-shaped" gametocyte of *Plasmodium falciparum*. (Figure courtesy of Dr. S. Glenn, Public Health Image Library, Centers for Disease Control and Prevention.)

A very important feature of *P. falciparum* is **chloroquine resistance**. Chloroquine-resistant strains now predominate in most areas of the world where malaria is endemic. Chloroquine resistance is mediated by a mutation in the gene encoding the chloroquine transporter in the cell membrane of the organism.

Pathogenesis & Epidemiology

Most of the pathologic findings of malaria result from the **destruction of red blood cells**. Red cells are destroyed both by the release of the merozoites and by the action of the spleen to first sequester the infected red cells and then to lyse them. The enlarged spleen characteristic of malaria is due to congestion of sinusoids with erythrocytes, coupled with hyperplasia of lymphocytes and macrophages.

Malaria caused by *P. falciparum* is **more severe** than that caused by other plasmodia. It is characterized by infection of far more red cells than the other malarial species and by occlusion of the capillaries with aggregates of parasitized red cells. This leads to life-threatening hemorrhage and necrosis, particularly in the brain (cerebral malaria). Furthermore, extensive hemolysis and kidney damage occur, with resulting hemoglobinuria. The dark color of the patient's urine has given rise to the term "blackwater fever." The hemoglobinuria can lead to acute renal failure.

The timing of the fever cycle is 72 hours for *P. malariae* and 48 hours for the other plasmodia. Disease caused by *P. malariae* is called quartan malaria because it recurs every fourth day, whereas malaria caused by the other plasmodia is called tertian malaria because it recurs every third day. Tertian malaria is subdivided into malignant malaria, caused by *P. falciparum*, and benign malaria, caused by *P. vivax* and *P. ovale*.

P. falciparum causes a high level of parasitemia because it can infect red cells of all ages. In contrast, *P. vivax* infects only reticulocytes and *P. malariae* infects only mature red cells; therefore, they produce much lower levels of parasites in the blood. Individuals with sickle cell trait (heterozygotes) are protected against malaria because their red cells have too little ATPase activity and cannot produce sufficient energy to support the growth of the parasite. People with homozygous sickle cell anemia are also protected but rarely live long enough to obtain much benefit.

The receptor for *P. vivax* is the Duffy blood group antigen. People who are homozygous recessive for the genes that encode this protein are resistant to infection by *P. vivax*. More than 90% of black West Africans and many of their American descendants do not produce the Duffy antigen and are thereby resistant to vivax malaria.

People with glucose-6-phosphate dehydrogenase (G6PD) deficiency are also protected against the severe effects of falciparum malaria. G6PD deficiency is an X-linked hemoglobinopathy found in high frequency in tropical areas where malaria is endemic. Both male and female carriers of the mutated gene are protected against malaria.

Malaria is transmitted primarily by mosquito bites, but transmission across the placenta, in blood transfusions, and by intravenous drug use also occurs.

Partial immunity based on humoral antibodies that block merozoites from invading the red cells occurs in infected individuals. A low level of parasitemia and low-grade symptoms result; this condition is known as **premunition**. In contrast, a nonimmune person, such as a first-time traveler to an area where falciparum malaria is endemic, is at risk of severe, life-threatening disease.

More than 200 million people worldwide have malaria, and more than 1 million die of it each year, making it the most common lethal infectious disease. It occurs primarily in tropical and subtropical areas, especially in Asia, Africa, and Central and South America. Malaria in the United States is seen in Americans who travel to areas of endemic infection without adequate chemoprophylaxis and in immigrants from areas of endemic infection. It is not endemic in the United States. Certain regions in Southeast Asia, South America, and east Africa are particularly affected by chloroquine-resistant strains of *P. falciparum*. People who have lived or traveled in areas where malaria occurs should seek medical attention for febrile illnesses up

to 3 years after leaving the malarious area.

Clinical Findings

Malaria presents with abrupt onset of fever and chills, accompanied by headache, myalgias, and arthralgias, about 2 weeks after the mosquito bite. Fever may be continuous early in the disease; the typical periodic cycle does not develop for several days after onset. The fever spike, which can reach 41°C, is frequently accompanied by shaking chills, nausea, vomiting, and abdominal pain. The fever is followed by drenching sweats. Patients usually feel well between the febrile episodes. Splenomegaly is seen in most patients, and hepatomegaly occurs in roughly one-third. Anemia is prominent.

Untreated malaria caused by *P. falciparum* is potentially life-threatening as a result of extensive brain (cerebral malaria) and kidney (blackwater fever) damage. Malaria caused by the other three plasmodia is usually self-limited, with a low mortality rate. However, relapses of *P. vivax* and *P. ovale* malaria can occur up to several years after the initial illness as a result of hypnozoites latent in the liver.

Laboratory Diagnosis

Diagnosis rests on microscopic examination of blood, using both **thick** and **thin** Giemsa-stained smears. The thick smear is used to screen for the presence of organisms, and the thin smear is used for species identification. It is important to identify the species because the treatment of different species can differ. Ring-shaped trophozoites can be seen within infected red blood cells (Figure 52–3). The gametocytes of *P. falciparum* are **crescent-shaped** ("banana-shaped"), whereas those of the other plasmodia are spherical (Figure 52–2F). If more than 5% of red blood cells are parasitized, the diagnosis is usually *P. falciparum* malaria.

If blood smears do not reveal the diagnosis, then a polymerase chain reaction (PCR)-based test for *Plasmodium* nucleic acids or an enzyme-linked immunosorbent assay (ELISA) test for a protein specific for *P. falciparum* can be useful.

Treatment

The treatment of malaria is complicated, and the details are beyond the scope of this book. Table 52–2 presents the drugs commonly used in the United States. The main criteria used for choosing specific drugs are the severity of the disease and whether the organism is resistant to chloroquine. Chloroquine resistance is determined by the geographical location where the infection was acquired rather than by laboratory testing.

TABLE 52–2 Drugs Commonly Used for the Treatment of Malaria in the United States

TABLE 32-2 Drugs Commonly Used for the Treatment of Malaria in the United States

Species	Drug(s)	Comments
Chloroquine-sensitive *Plasmodium falciparum* and *Plasmodium malariae*	Chloroquine	Oral
Chloroquine-sensitive *Plasmodium vivax* and *Plasmodium ovale*	Chloroquine plus primaquine	Oral Do not use primaquine if G6PD deficient
Chloroquine-resistant *P. falciparum*; uncomplicated infection	Coartem (artemether and lumefantrine) or Malarone (atovaquone and proguanil)	Oral
Chloroquine-resistant *P. falciparum*; severe complicated infection	Artesunate[1] or quinidine[2]	Intravenous

G6PD = glucose-6-phosphate dehydrogenase.

[1]Available in the United States through the Centers for Disease Control and Prevention.

[2]If intravenous quinidine is used, cardiac monitoring should be in place.

Chloroquine is the drug of choice for treatment of uncomplicated malaria caused by non-falciparum species in areas without chloroquine resistance. Chloroquine kills the merozoites, thereby reducing the parasitemia, but does not affect the hypnozoites of *P. vivax* and *P. ovale* in the liver. These are killed by primaquine, which must be used to prevent relapses. Primaquine may induce severe hemolysis in those with G6PD deficiency, so testing for this enzyme should be done before the drug is given. Primaquine should not be given if the patient is severely G6PD deficient. If primaquine is not given, one approach is to wait to see whether symptoms recur and then treat with chloroquine.

Uncomplicated, chloroquine-resistant *P. falciparum* infection is treated with either Coartem (artemether plus lumefantrine) or Malarone (atovaquone and proguanil). In severe complicated cases of chloroquine-resistant falciparum malaria, intravenous administration of either artesunate or quinidine is used.

Outside the United States, the artemisinins, such as artesunate or artemether, are widely used in combination with other antimalarial drugs. The artemisinins are inexpensive and have few side effects, and most plasmodia have not developed resistance to these drugs. However, resistance to artesunate has emerged in some strains of *P. falciparum* in Southeast Asia (e.g., Cambodia, Myanmar, and Thailand).

Prevention

Chemoprophylaxis of malaria for travelers to areas where chloroquine-resistant *P. falciparum* is endemic consists of mefloquine or doxycycline. A combination of atovaquone and proguanil (Malarone), in a fixed dose, can also be used.

Chloroquine should be used in areas where *P. falciparum* is sensitive to that drug. Travelers to areas where the other three plasmodia are found should take chloroquine starting 2 weeks before arrival in the endemic area and continuing for 6 weeks after departure. This should be followed by a 2-week course of primaquine if exposure was high. Primaquine will kill the hypnozoites of *P. vivax* and *P. ovale*.

Other preventive measures include the use of mosquito netting, window screens, protective clothing, and insect repellents. The mosquitoes feed from dusk to dawn, so protection is particularly important during the night. Communal preventive measures are directed against reducing the mosquito population. Many insecticide sprays, such as DDT, are no longer effective because the mosquitoes have developed resistance. Drainage of stagnant water in swamps and ditches reduces the breeding areas. There is no vaccine.

TOXOPLASMA

Disease

Toxoplasma gondii causes toxoplasmosis, including congenital toxoplasmosis.

Important Properties

The life cycle of *T. gondii* is shown in Figure 52–5. The definitive host is the **domestic cat** and other felines; humans and other mammals are intermediate hosts. Infection of humans begins with the **ingestion of cysts** in undercooked meat or from accidental contact with cysts in cat feces. In the small intestine, the cysts rupture and release forms that invade the gut wall, where they are ingested by macrophages and differentiate into rapidly multiplying trophozoites (**tachyzoites**), which kill the cells and infect other cells (Figures 52–2G and 52–6). Cell-mediated immunity usually limits the spread of tachyzoites, and the parasites enter host cells in the brain, muscle, and other tissues, where they develop into cysts in which the parasites multiply slowly. These forms are called **bradyzoites**. These tissue cysts are both an important diagnostic feature and a source of organisms when the tissue cyst breaks in an immunocompromised patient.

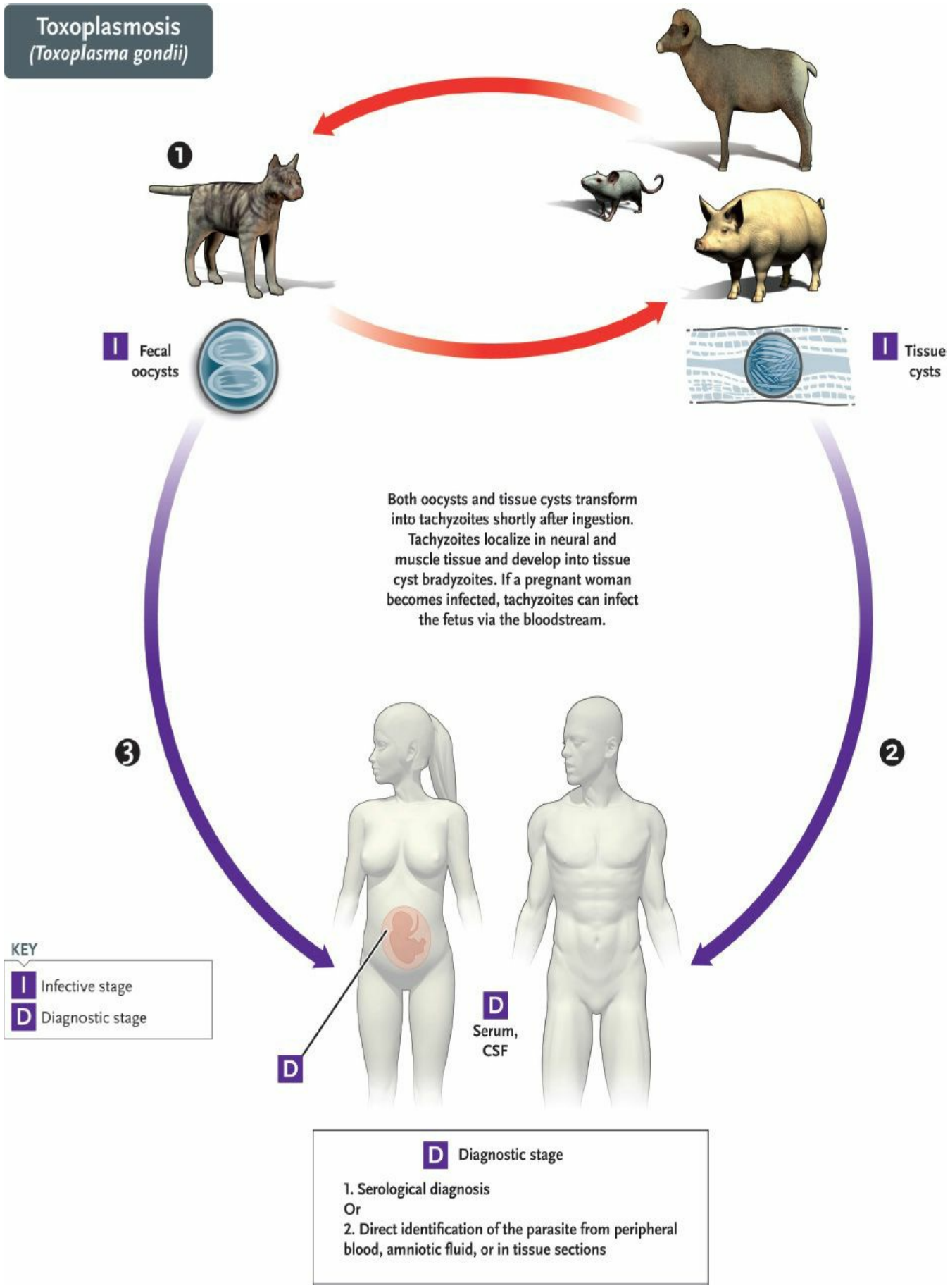

Toxoplasmosis
(Toxoplasma gondii)
1
I Fecal oocysts
I Tissue cysts
Both oocysts and tissue cysts transform into tachyzoites shortly after ingestion. Tachyzoites localize in neural and muscle tissue and develop into tissue cyst bradyzoites. If a pregnant woman becomes infected, tachyzoites can infect the fetus via the bloodstream.
2
3
KEY
I Infective stage
D Diagnostic stage
D Serum, CSF
D
D Diagnostic stage
1. Serological diagnosis
Or
2. Direct identification of the parasite from peripheral blood, amniotic fluid, or in tissue sections

FIGURE 52–5 *Toxoplasma gondii*. Life cycle. Top red arrows show the natural life cycle as *T. gondii* circulates between cats (#1), which excrete oocysts in the feces that are eaten by mice, but also by domestic animals such as pigs and sheep. Cysts form in tissue such as muscle and brain. The natural cycle is completed when cats eat mice. Humans are accidental hosts. They can be infected by the ingestion of under-cooked pork and lamb (blue arrow #2) containing tissue cysts in muscle or by ingestion of food contaminated with cat feces containing oocysts (blue arrow #3). (Provider: Centers for Disease Control and Prevention/Dr. Alexander J. da Silva and Melanie Moser.)

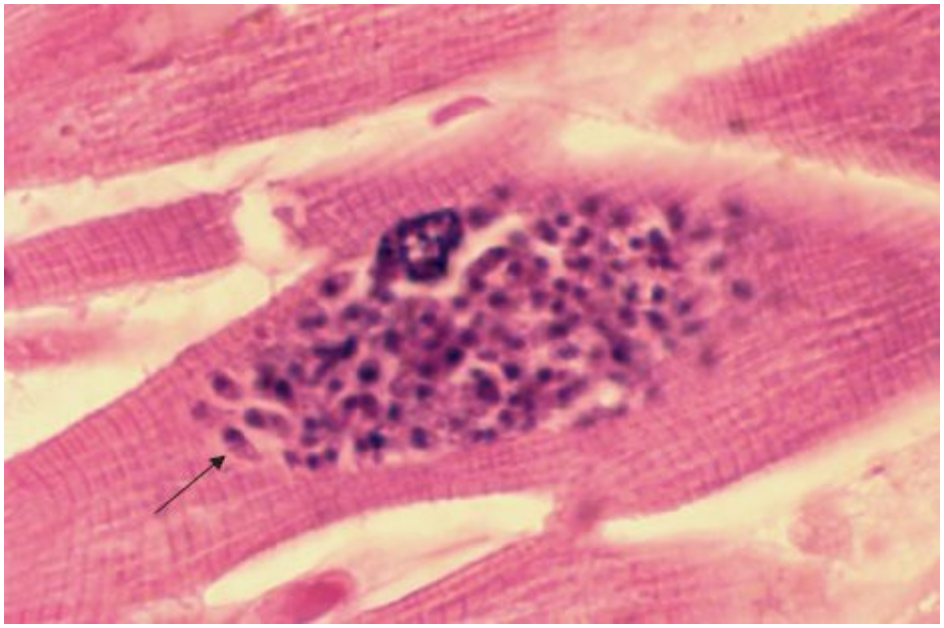


FIGURE 52–6 *Toxoplasma gondii*—tachyzoite. Arrow points to a tachyzoite of *T. gondii* in cardiac muscle. (Figure courtesy of Dr. E. Ewing, Jr., Public Health Image Library, Centers for Disease Control and Prevention.)

The cycle within the cat begins with the ingestion of cysts in raw meat (e.g., mice). Bradyzoites are released from the cysts in the small intestine, infect the mucosal cells, and differentiate into male and female gametocytes, whose gametes fuse to form oocysts that are excreted in cat feces. The cycle is completed when soil contaminated with cat feces is accidentally ingested. Human infection usually occurs from eating undercooked meat (e.g., lamb and pork) from animals that grazed in soil contaminated with infected cat feces.

Pathogenesis & Epidemiology

T. gondii is usually acquired by **ingestion** of cysts in uncooked meat or cat feces.

Transplacental transmission from an infected mother to the fetus occurs also. Human-to-human transmission, other than transplacental transmission, does not occur. After infection of the intestinal epithelium, the organisms spread to other organs, especially the brain, lungs, liver, and eyes. Progression of the infection is usually limited by a competent immune system. **Cell-mediated immunity** plays the major role, but circulating antibody enhances killing of the organism. Most initial infections are asymptomatic. When contained, the organisms persist as cysts within tissues. There is no inflammation, and the individual remains well unless immunosuppression allows activation of organisms in the cysts.

Congenital infection of the fetus occurs *only* when the mother is infected during pregnancy. If she is infected before the pregnancy, the organism will be in the cyst form and there will be no trophozoites to pass through the placenta. The mother who is reinfected during pregnancy but who has immunity from a previous infection will not transmit the organism to her child. Roughly one-third of mothers infected during pregnancy give birth to infected infants, but only 10% of these infants are symptomatic.

Infection by *T. gondii* occurs worldwide. Serologic surveys reveal that in the United States antibodies are found in 5% to 50% of people in various regions. Infection is usually sporadic, but outbreaks associated with ingestion of raw meat or contaminated water occur. Approximately 1% of domestic cats in the United States shed *Toxoplasma* cysts.

Clinical Findings

Most primary infections in immunocompetent adults are asymptomatic, but some resemble infectious mononucleosis, except that the heterophil antibody test is negative. Congenital infection can result in abortion, stillbirth, or neonatal disease with encephalitis, **chorioretinitis,** and hepatosplenomegaly. Fever, jaundice, and **intracranial calcifications** are also seen. Most infected newborns are asymptomatic, but chorioretinitis or mental retardation will develop in some children months or years later. Congenital infection with *Toxoplasma* is one of the leading causes of blindness in children. In patients with reduced cell-mediated immunity (e.g., patients with acquired immunodeficiency syndrome [AIDS]), life-threatening disseminated disease, primarily encephalitis, occurs.

Laboratory Diagnosis

For the diagnosis of acute and congenital infections, an immunofluorescence assay for **IgM antibody** is used. IgM is used to diagnose congenital infection, because IgG can be maternal in origin. Tests of IgG antibody can be used to diagnose acute

infections if a significant rise in antibody titer in paired sera is observed.

Microscopic examination of Giemsa-stained preparations shows crescent-shaped trophozoites during acute infections. Cysts may be seen in the tissue. The organism can be grown in cell culture. Inoculation into mice can confirm the diagnosis.

Treatment

Congenital toxoplasmosis, whether symptomatic or asymptomatic, should be treated with a combination of sulfadiazine and pyrimethamine. These drugs also constitute the treatment of choice for disseminated disease in immunocompromised patients. Acute toxoplasmosis in an immunocompetent individual is usually self-limited, but any patient with chorioretinitis should be treated.

Prevention

The most effective means of preventing toxoplasmosis is to cook meat thoroughly to kill the cysts. Pregnant women should be especially careful to avoid undercooked meat and contact with cat feces. They should refrain from emptying cat litter boxes. Cats should not be fed raw meat. Trimethoprim-sulfamethoxazole is used to prevent *Toxoplasma* encephalitis in patients infected with human immunodeficiency virus (HIV).

PNEUMOCYSTIS

Disease

Pneumocystis jiroveci is an important cause of pneumonia in immunocompromised individuals. In 2002, taxonomists renamed the human species of *Pneumocystis* as *P. jiroveci* and recommended that *Pneumocystis carinii* be used only to describe the rat species of *Pneumocystis*.

Important Properties

The classification and life cycle of *Pneumocystis* are unclear. Many aspects of its biochemistry indicate that it is a yeast, but it also has several attributes of a protozoan. An analysis of rRNA sequences published in 1988 indicates that *Pneumocystis* should be classified as a **fungus** related to yeasts such as *Saccharomyces cerevisiae*. Subsequent analysis of mitochondrial DNA and of various enzymes supports the idea that it is a fungus. However, it does not have ergosterol in its membranes as do the fungi. It has cholesterol.

Medically, it is still thought of as a protozoan. In tissue, it appears as a cyst that resembles the cysts of protozoa (Figures 52–2H and 52–7). The findings that it does

not grow on fungal media and that antifungal drugs are ineffective have delayed acceptance of its classification as a fungus.

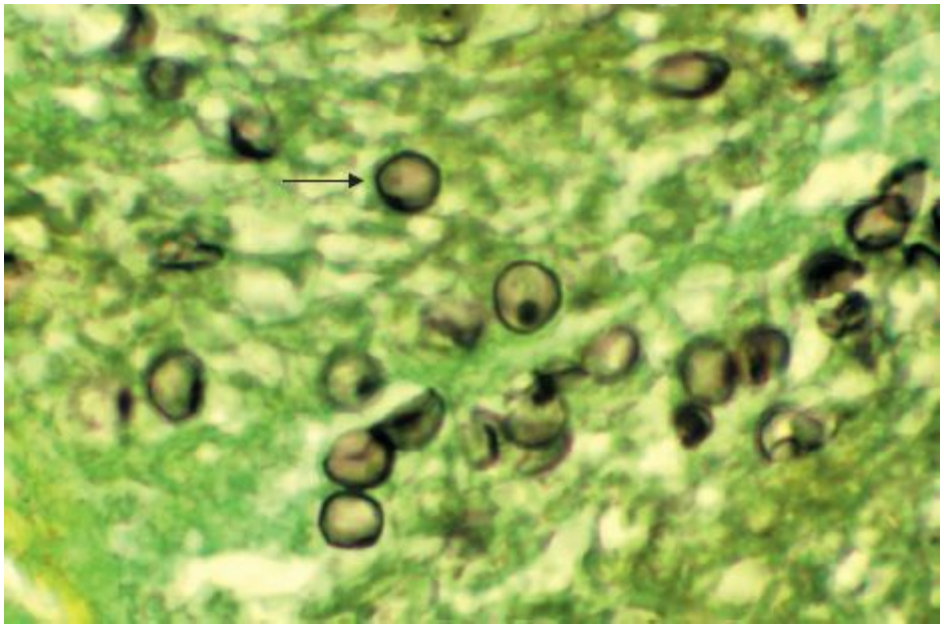


FIGURE 52–7 *Pneumocystis jiroveci*—arrow points to a cyst of P. jiroveci in lung tissue. (Figure courtesy of Dr. E. Ewing, Jr., Public Health Image Library, Centers for Disease Control and Prevention.)

Pneumocystis species are found in domestic animals such as horses and sheep and in a variety of rodents, but it is thought that these animals are not a reservoir for human infection. Each mammalian species is thought to have its own species of *Pneumocystis*.

Pneumocystis species have a major surface glycoprotein that exhibits significant antigenic variation in a manner similar to that of *Trypanosoma brucei*. *Pneumocystis* species have multiple genes encoding these surface proteins, but only one is expressed at a time. This process of programmed rearrangements was first observed in *T. brucei*.

Pathogenesis & Epidemiology

Transmission occurs by **inhalation,** and infection is predominantly in the lungs. The presence of cysts in the alveoli induces an inflammatory response consisting primarily of plasma cells, resulting in a frothy exudate that blocks oxygen exchange. (The presence of plasma cells has led to the name "plasma cell pneumonia.") The organism does not invade the lung tissue.

Pneumonia occurs when host defenses (e.g., the number of CD4-positive [helper]

T cells) are reduced. This accounts for the prominence of *Pneumocystis* pneumonia in patients with AIDS and in premature or debilitated infants. Hospital outbreaks do not occur, and patients with *Pneumocystis* pneumonia are not isolated.

P. jiroveci is distributed worldwide. It is estimated that 70% of people have been infected. Most 5-year-old children in the United States have antibodies to this organism. Asymptomatic infection is therefore quite common. Prior to the advent of immunosuppressive therapy, *Pneumocystis* pneumonia was rarely seen in the United States. Its incidence has paralleled the increase in immunosuppression and the rise in the number of AIDS cases.

Most *Pneumocystis* infections in AIDS patients are new rather than a reactivation of a prior latent infection. This conclusion is based on the finding that *Pneumocystis* recovered from AIDS patients shows resistance to drugs that the patients have not taken.

Clinical Findings

The sudden onset of fever, nonproductive cough, dyspnea, and tachypnea is typical of *Pneumocystis* pneumonia. Bilateral rales and rhonchi are heard, and the chest X-ray shows a diffuse interstitial pneumonia with "ground glass" infiltrates bilaterally. In infants, the disease usually has a more gradual onset. Extrapulmonary *Pneumocystis* infections occur in the late stages of AIDS and affect primarily the liver, spleen, lymph nodes, and bone marrow. The mortality rate of untreated *Pneumocystis* pneumonia approaches 100%.

Laboratory Diagnosis

Diagnosis is made by finding the typical cysts by microscopic examination of lung tissue or fluids obtained by bronchoscopy, bronchial lavage, or open lung biopsy (Figure 52–7). Sputum is usually less suitable. The cysts can be visualized with methenamine silver, Giemsa, or other tissue stains. Fluorescent-antibody staining is also commonly used for diagnosis. PCR-based tests using respiratory tract specimens are also useful. The organism stains poorly with Gram stain. There is no serologic test, and the organism has not been grown in culture.

Treatment

The treatment of choice is a combination of trimethoprim and sulfamethoxazole (Bactrim, Septra). Pentamidine and atovaquone are alternative drugs.

Prevention

Trimethoprim-sulfamethoxazole or aerosolized pentamidine should be used as

chemoprophylaxis in patients whose CD4 counts are below 200.

TRYPANOSOMA

The genus *Trypanosoma* includes three major pathogens: *Trypanosoma cruzi, Trypanosoma gambiense*, and *Trypanosoma rhodesiense*.[2]

1. Trypanosoma cruzi

Disease

T. cruzi is the cause of Chagas' disease (American trypanosomiasis).

Important Properties

The life cycle of *T. cruzi* is shown in Figure 52–8. The life cycle involves the **reduviid bug** (*Triatoma*, cone-nose or kissing bug) as the vector, and both humans and animals as reservoir hosts. The animal reservoirs include domestic cats and dogs and wild species such as the armadillo, raccoon, and rat. The cycle in the reduviid bug begins with ingestion of trypomastigotes in the blood of the reservoir host. In the insect gut, they multiply and differentiate first into epimastigotes and then into trypomastigotes. When the bug bites again, the site is contaminated with feces containing trypomastigotes, which enter the blood of the person (or other reservoir) and form nonflagellated amastigotes within host cells. Many cells can be affected, but myocardial, glial, and reticuloendothelial cells are the most frequent sites. To complete the cycle, amastigotes differentiate into trypomastigotes, which enter the blood and are taken up by the reduviid bug (Figures 52–9A to C and 52–10).

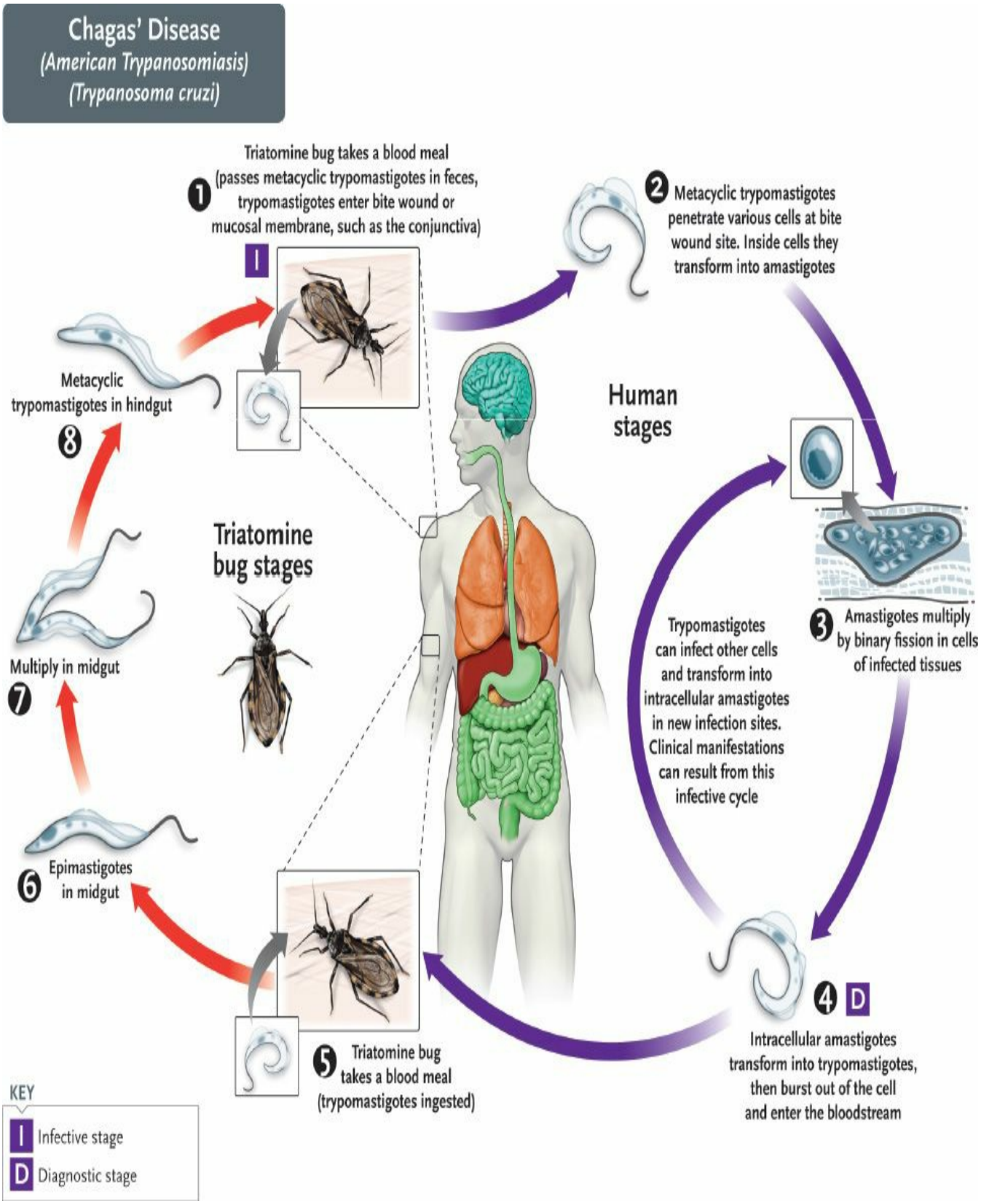


FIGURE 52–8 *Trypanosoma cruzi*. Life cycle. Right side of figure describes the stages within the human (blue arrows). Humans are infected at step 1 when

triatomine (reduviid) bug bites human and defecates at bite site. Trypomastigotes in feces enter bite wound. Amastigotes form within cells, especially heart muscle and neural tissue. Reduviid bug is infected at step 5 when it ingests trypomastigotes in human blood. Left side of figure describes the stages within the reduviid bug (red arrows). (Provider: Centers for Disease Control and Prevention/Dr. Alexander J. da Silva and Melanie Moser.)

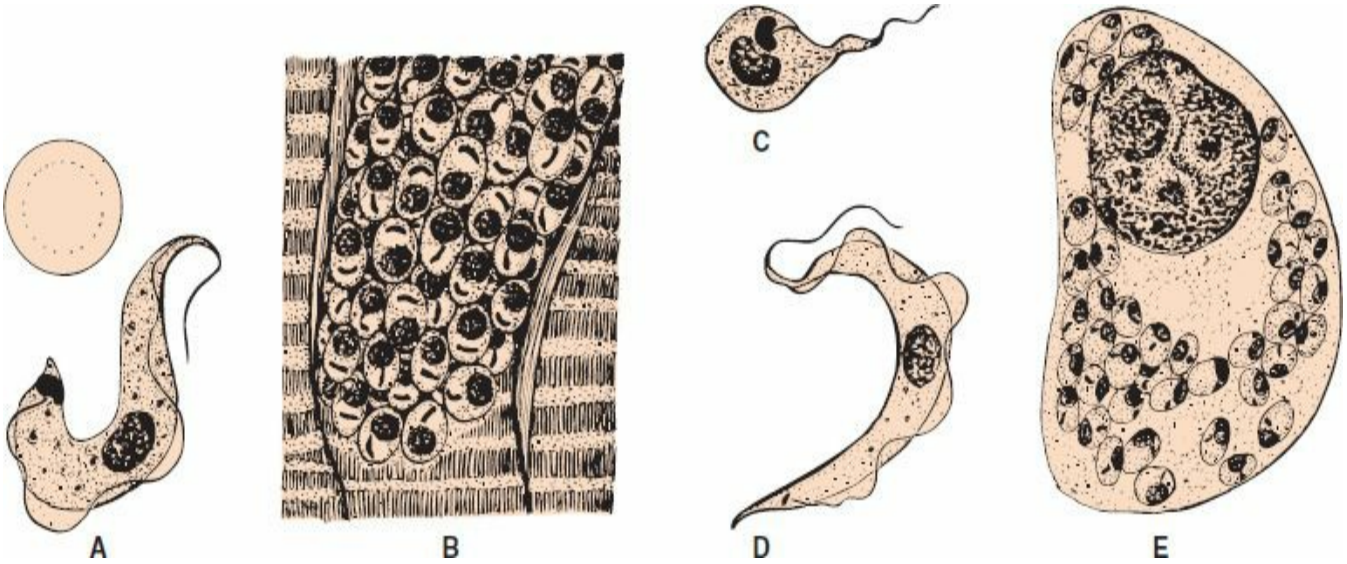


FIGURE 52–9 **A:** *Trypanosoma cruzi* trypomastigote found in human blood (1200×). **B:** *T. cruzi* amastigotes found in cardiac muscle (850×). **C:** *T. cruzi* epimastigote found in reduviid bug (1200×). **D:** *Trypanosoma brucei gambiense* or *rhodesiense* trypomastigote found in human blood (1200×). **E:** *Leishmania donovani* amastigotes within splenic macrophages (1000×). (Circle with inner dotted line represents a red blood cell.)

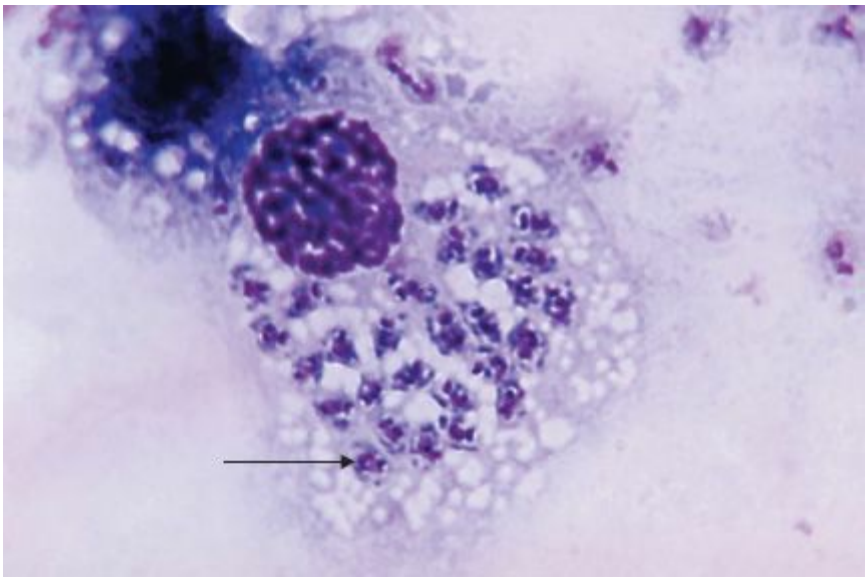


FIGURE 52–10 *Trypanosoma cruzi*—amastigotes. Arrow points to an amastigote (nonflagellated form) in cytoplasm. (Figure courtesy of Dr. A. J. Sulzer, Public Health Image Library, Centers for Disease Control and Prevention.)

Pathogenesis & Epidemiology

Chagas' disease occurs primarily in rural Central and South America. Acute Chagas' disease occurs rarely in the United States, but the chronic form causing myocarditis and congestive heart failure is seen with increasing frequency in immigrants from Latin America. The disease is seen primarily in rural areas because the reduviid bug lives in the walls of rural huts and feeds at night. It bites preferentially around the mouth or eyes, hence the name "kissing bug."

The amastigotes can kill cells and cause inflammation, consisting mainly of mononuclear cells. **Cardiac muscle** is the most frequently and severely affected tissue. In addition, neuronal damage leads to cardiac arrhythmias and loss of tone in the colon (**megacolon**) and esophagus (**megaesophagus**). During the acute phase, there are both trypomastigotes in the blood and amastigotes intracellularly in the tissues. In the chronic phase, the organism persists in the amastigote form.

Chagas' disease has occurred in the United States in recipients of either blood transfusions or organ transplants from infected donors. The organism can also be transmitted congenitally from an infected mother to the fetus across the placenta.

Clinical Findings

The acute phase of Chagas' disease consists of facial edema and a nodule (chagoma) near the bite, coupled with fever, lymphadenopathy, and hepatosplenomegaly. A bite around the eye can result in unilateral palpebral swelling called Romaña's sign. The acute phase resolves in about 2 months. Most individuals then remain asymptomatic, but some progress to the chronic form with myocarditis and megacolon. Death from chronic Chagas' disease is usually due to cardiac arrhythmias or congestive heart failure.

Laboratory Diagnosis

Acute disease is diagnosed by demonstrating the presence of trypomastigotes in thick or thin films of the patient's blood. Both stained and wet preparations should be examined, the latter for motile organisms. Because the trypomastigotes are not numerous in the blood, other diagnostic methods may be required, namely, (1) a stained preparation of a bone marrow aspirate or muscle biopsy specimen (which may reveal amastigotes); (2) culture of the organism on special medium; and (3) **xenodiagnosis,** which consists of allowing an uninfected, laboratory-raised reduviid

bug to feed on the patient and, after several weeks, examining the intestinal contents of the bug for the organism.

Serologic tests can be helpful also. The indirect fluorescent antibody test is the earliest to become positive. Indirect hemagglutination and complement fixation tests are also available. Diagnosis of chronic disease is difficult because there are few trypomastigotes in the blood. Xenodiagnosis and serologic tests are used.

Treatment

The drug of choice for the acute phase is nifurtimox, which kills trypomastigotes in the blood but is much less effective against amastigotes in tissue. Benznidazole is an alternative drug. There is no effective drug against the chronic form.

Prevention

Prevention involves protection from the reduviid bite, improved housing, and insect control. No prophylactic drug or vaccine is available. Blood for transfusion is tested for the presence of antibodies to *T. cruzi*. Blood containing antibodies should not be used.

2. Trypanosoma gambiense & Trypanosoma rhodesiense

Disease

These organisms cause sleeping sickness (African trypanosomiasis). They are also known as *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense*.

Important Properties

The life cycle of *Trypanosoma brucei* is shown in Figure 52–11. The morphology and life cycle of the two species are similar. The vector for both is the **tsetse fly,** *Glossina*, but different species of fly are involved for each. Humans are the reservoir for *T. gambiense*, whereas *T. rhodesiense* has reservoirs in both domestic animals (especially cattle) and wild animals (e.g., antelopes).

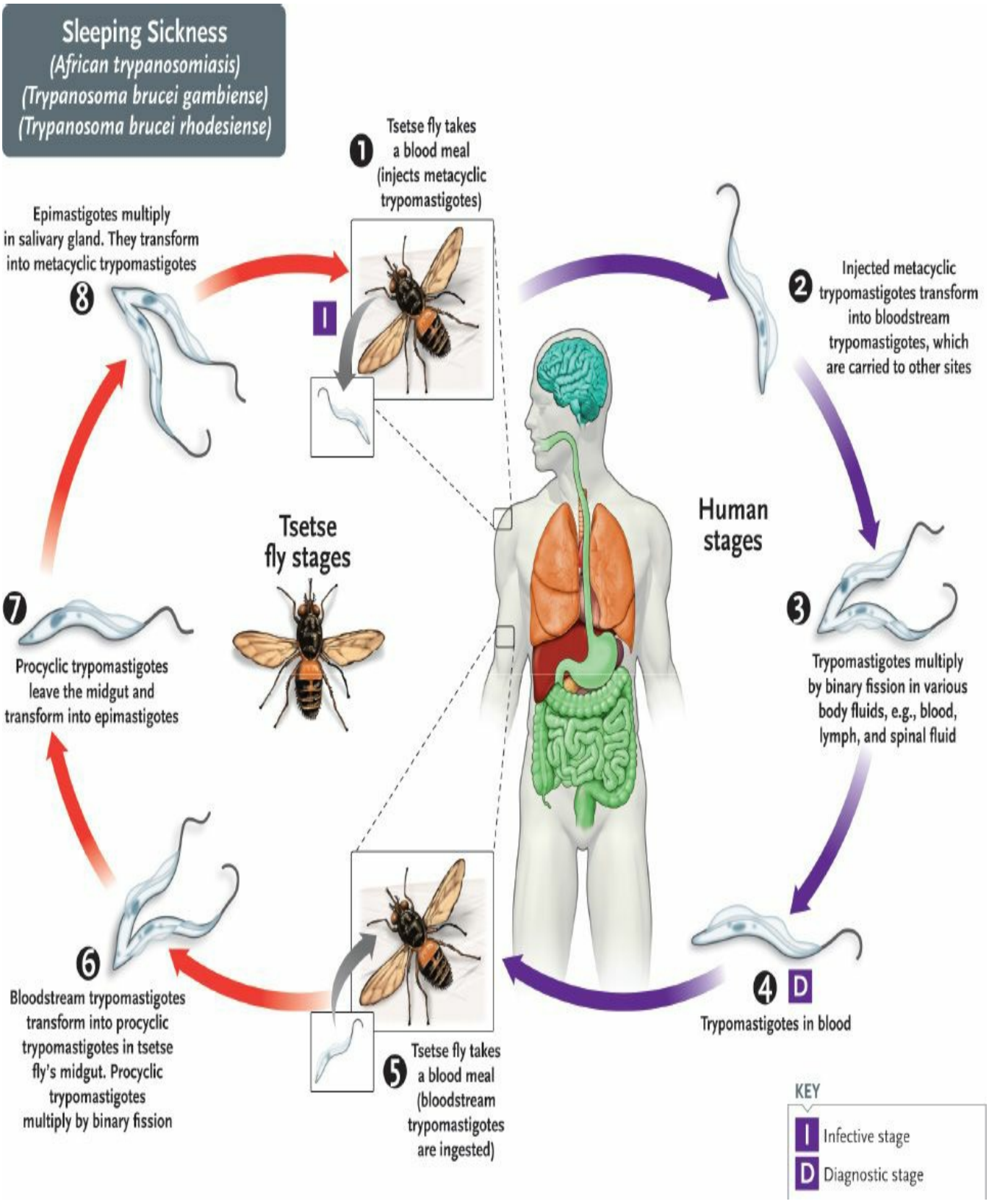


FIGURE 52–11 *Trypanosoma brucei*. Life cycle. Right side of figure describes the stages within the human (blue arrows). Humans are infected at step 1

when the tsetse fly bites human and injects trypomastigotes into bloodstream. Tsetse fly is infected at step 5 when it ingests trypomastigotes in human blood. Left side of figure describes the stages within the tsetse fly (red arrows). (Provider: Centers for Disease Control and Prevention/Dr. Alexander J. da Silva and Melanie Moser.)

The 3-week life cycle in the tsetse fly begins with ingestion of trypomastigotes in a blood meal from the reservoir host. They multiply in the insect gut and then migrate to the salivary glands, where they transform into epimastigotes, multiply further, and then form metacyclic trypomastigotes, which are transmitted by the tsetse fly bite. The organisms in the saliva are injected into the skin, where they enter the bloodstream, differentiate into blood-form trypomastigotes, and multiply, thereby completing the cycle (Figures 52–9D and 52–12). Note that these species are rarely found as amastigotes in tissue, in contrast to *T. cruzi* and *Leishmania* species, in which amastigotes are commonly found.

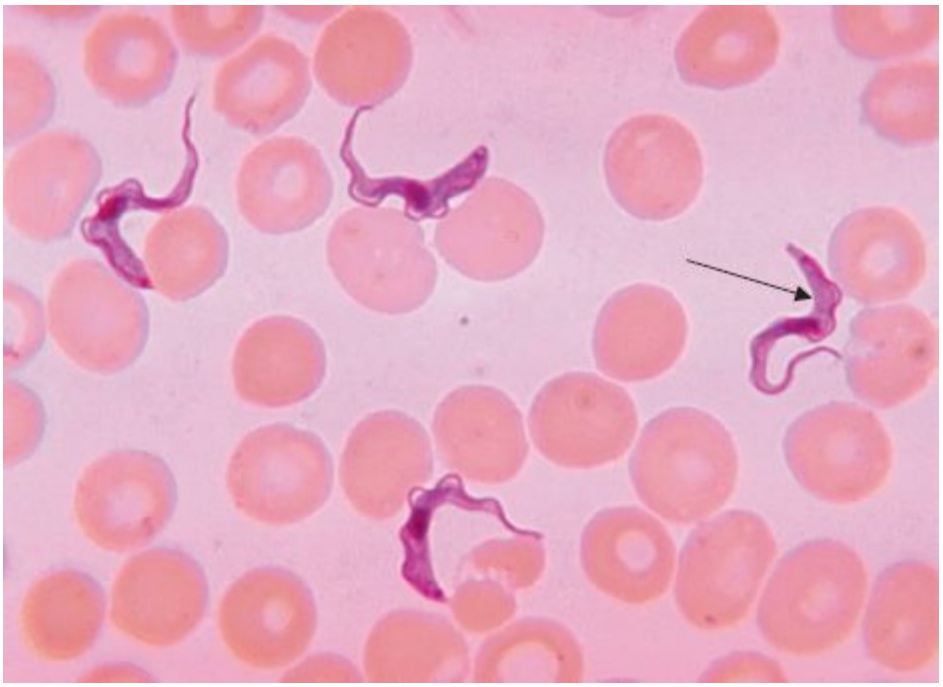


FIGURE 52–12 *Trypanosoma brucei*—trypomastigotes. Arrow points to a trypomastigote (the flagellated form) in the blood. (Figure courtesy of Dr. M. Schultz, Public Health Image Library, Centers for Disease Control and Prevention.)

These trypanosomes exhibit remarkable **antigenic variation** of their surface glycoproteins, with hundreds of antigenic types found. One antigenic type will coat the surface of the parasites for approximately 10 days, followed by other types in sequence in the new progeny. This variation is due to sequential movement of the glycoprotein genes to a preferential location on the chromosome, where only that

specific gene is transcribed into mRNA. These antigenic variations allow the organism to continually evade the host immune response.

Pathogenesis & Epidemiology

The trypomastigotes spread from the skin through the blood to the lymph nodes and the brain. The typical somnolence (**sleeping sickness**) progresses to coma as a result of a demyelinating encephalitis.

In the acute form, a cyclical fever spike (approximately every 2 weeks) occurs that is related to antigenic variation. As antibody-mediated agglutination and lysis of the trypomastigotes occur, the fever subsides. However, a few antigenic variants survive, multiply, and cause a new fever spike. This cycle repeats itself over a long period. The lytic antibody is directed against the surface glycoprotein.

The disease is endemic in sub-Saharan Africa, the natural habitat of the tsetse fly. Both sexes of fly take blood meals and can transmit the disease. The fly is infectious throughout its 2- to 3-month lifetime. *T. gambiense* is the species that causes the disease along water courses in west Africa, whereas *T. rhodesiense* is found in the arid regions of east Africa. Both species are found in central Africa.

Clinical Findings

Although both species cause sleeping sickness, the progress of the disease differs. *T. gambiense*–induced disease runs a low-grade chronic course over a few years, whereas *T. rhodesiense* causes a more acute, rapidly progressive disease that, if untreated, is usually fatal within several months.

The initial lesion is an indurated skin ulcer ("trypanosomal chancre") at the site of the fly bite. After the organisms enter the blood, intermittent weekly fever and lymphadenopathy develop. Enlargement of the posterior cervical lymph nodes (Winterbottom's sign) is commonly seen. The encephalitis is characterized initially by headache, insomnia, and mood changes, followed by muscle tremors, slurred speech, and apathy that progress to somnolence and coma. Untreated disease is usually fatal as a result of pneumonia.

Laboratory Diagnosis

During the early stages, microscopic examination of the blood (either wet films or thick or thin smears) reveals trypomastigotes (Figure 52–12). An aspirate of the chancre or enlarged lymph node can also demonstrate the parasites. The presence of trypanosomes in the spinal fluid, coupled with an elevated protein level and pleocytosis, indicates that the patient has entered the late, encephalitic stage. Serologic tests, especially the ELISA for IgM antibody, can be helpful.

Treatment

Treatment must be initiated before the development of encephalitis, because suramin, the most effective drug, does not pass the blood–brain barrier well. Suramin will effect a cure if given early. Pentamidine is an alternative drug. If central nervous system symptoms are present, suramin (to clear the parasitemia) followed by melarsoprol should be given.

Prevention

The most important preventive measure is protection against the fly bite, using netting and protective clothing. Clearing the forest around villages and using insecticides are helpful measures. No vaccine is available.

LEISHMANIA

The genus *Leishmania* includes four major pathogens: *Leishmania donovani, Leishmania tropica, Leishmania mexicana*, and *Leishmania braziliensis*.

1. Leishmania donovani

Disease

L. donovani is the cause of kala-azar (visceral leishmaniasis).

Important Properties

The life cycle of *L. donovani* is shown in Figure 52–13. The life cycle involves the **sandfly**[3] as the vector and a variety of mammals such as dogs, foxes, and rodents as reservoirs.

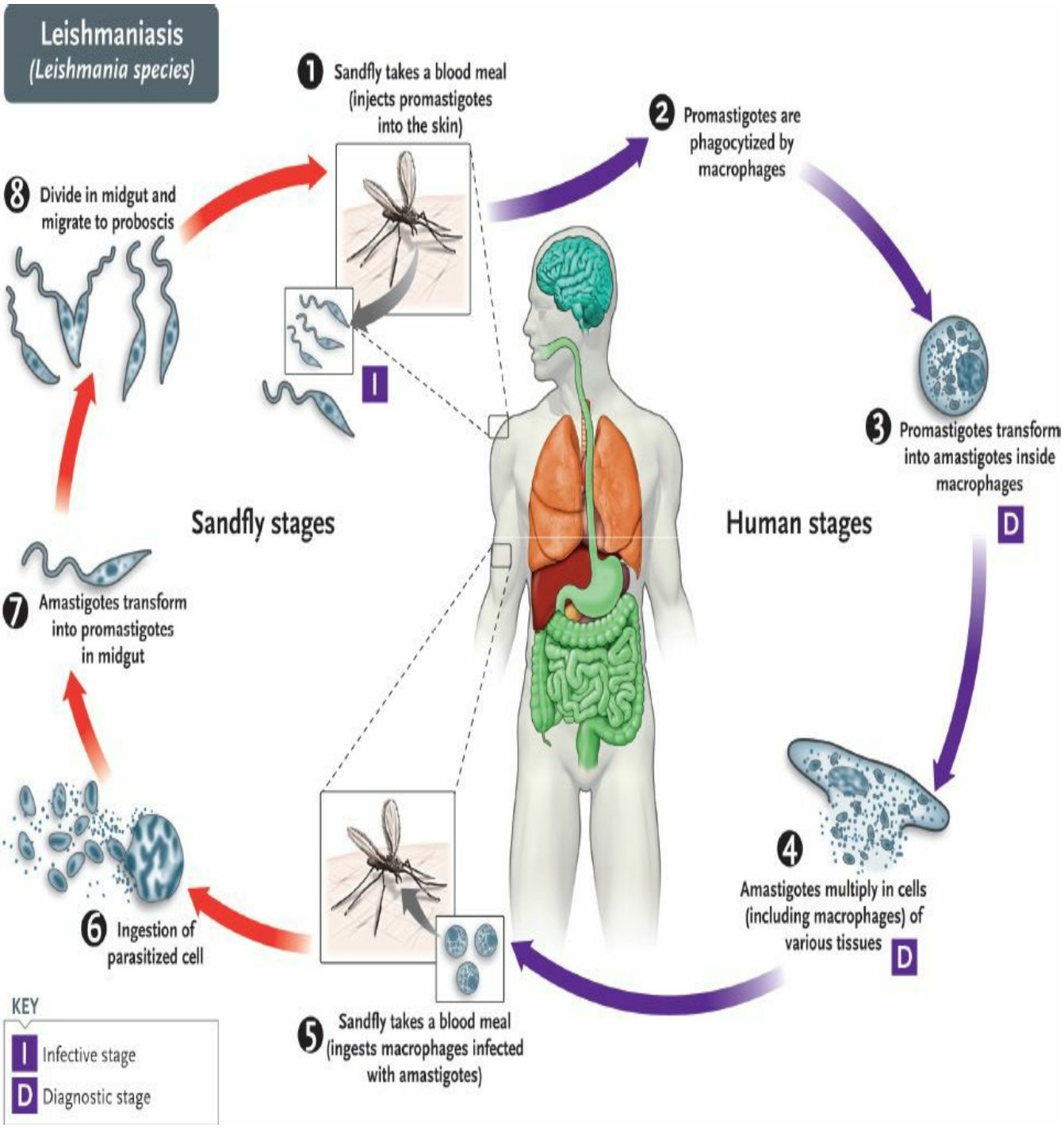


FIGURE 52–13 *Leishmania donovani*. Life cycle. Right side of figure describes the stages within the human (blue arrows). Humans are infected at step 1 when the sandfly bites human and injects promastigotes. Sandfly is infected at step 5 when it ingests macrophages containing amastigotes in human blood. Left side of figure describes the stages within the sandfly (red arrows). (Provider: Centers for Disease Control and Prevention/Dr. Alexander J. da Silva and Blaine Mathison.)

Only female flies are vectors because only they take blood meals (a requirement for egg maturation). When the sandfly sucks blood from an infected host, it ingests **macrophages-containing amastigotes** (Figures 52–9E and 52–14).[4]

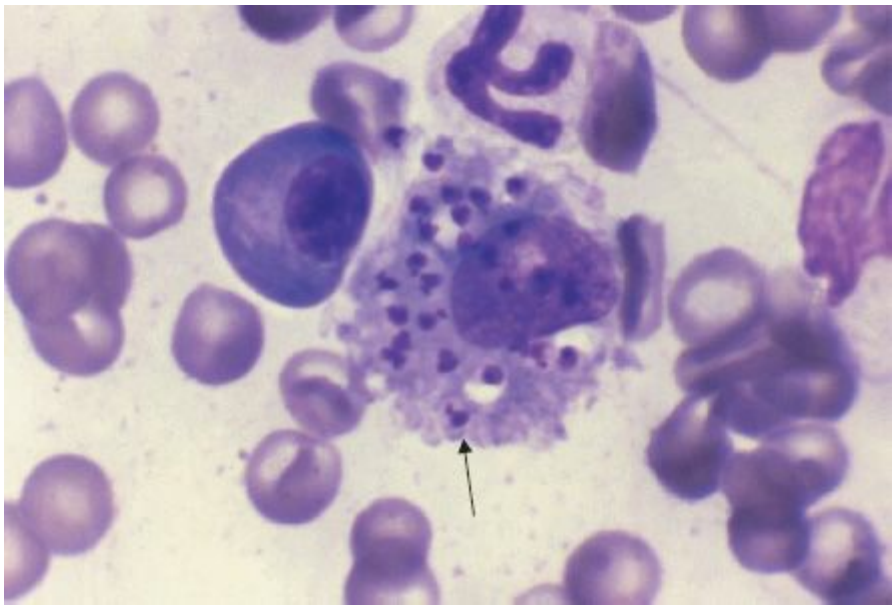


FIGURE 52–14 *Leishmania donovani*—amastigotes. Arrow points to an amastigote (nonflagellated form) in cytoplasm of bone marrow cell. (Figure courtesy of Dr. Francis Chandler, Public Health Image Library, Centers for Disease Control and Prevention.)

After dissolution of the macrophages, the freed amastigotes differentiate into promastigotes in the gut. They multiply and then migrate to the pharynx and proboscis, where they can be transmitted during the next bite. The cycle in the sandfly takes approximately 10 days.

Shortly after an infected sandfly bites a human, the promastigotes are engulfed by macrophages, where they transform into amastigotes (Figure 52–9E). Amastigotes can remain in the cytoplasm of macrophages because they can prevent fusion of the vacuole with lysosomes.

The infected cells die and release progeny amastigotes that infect other macrophages and reticuloendothelial cells. The cycle is completed when the fly ingests macrophages containing the amastigotes.

Pathogenesis & Epidemiology

In visceral leishmaniasis, the organs of the **reticuloendothelial** system (liver, spleen, and bone marrow) are the most severely affected. Reduced bone marrow activity, coupled with cellular destruction in the spleen, results in anemia,

leukopenia, and thrombocytopenia. This leads to secondary infections and a tendency to bleed. The striking **enlargement of the spleen** is due to a combination of proliferating macrophages and sequestered blood cells. The marked increase in IgG is neither specific nor protective.

Kala-azar occurs in three distinct epidemiologic patterns. In one area, which includes the Mediterranean basin, the Middle East, southern Russia, and parts of China, the reservoir hosts are primarily dogs and foxes. In sub-Saharan Africa, rats and small carnivores (e.g., civets) are the main reservoirs. A third pattern is seen in India and neighboring countries (and Kenya), in which humans appear to be the only reservoir.

Clinical Findings

Symptoms begin with intermittent fever, weakness, and weight loss. Massive enlargement of the spleen is characteristic. Hyperpigmentation of the skin is seen in light-skinned patients (kala-azar means **black sickness**). The course of the disease runs for months to years. Initially, patients feel reasonably well despite persistent fever. As anemia, leukopenia, and thrombocytopenia become more profound, weakness, infection, and gastrointestinal bleeding occur. Untreated severe disease is nearly always fatal as a result of secondary infection.

Laboratory Diagnosis

Diagnosis is usually made by detecting amastigotes in a bone marrow, spleen, or lymph node biopsy or "touch" preparation (Figure 52–14). The organisms can also be cultured. Serologic (indirect immunofluorescence) tests are positive in most patients. Although not diagnostic, a very high concentration of IgG is indicative of infection. A skin test using a crude homogenate of promastigotes (leishmanin) as the antigen is available. The skin test is negative during active disease but positive in patients who have recovered.

Treatment

The drug of choice is either liposomal amphotericin B or sodium stibogluconate. With proper therapy, the mortality rate is reduced to almost 5%. Recovery results in permanent immunity.

Prevention

Prevention involves protection from sandfly bites (use of netting, protective clothing, and insect repellents) and insecticide spraying.

2. Leishmania tropica, Leishmania mexicana, & Leishmania braziliensis

Disease

L. tropica and *L. mexicana* both cause cutaneous leishmaniasis; the former organism is found in the Old World, whereas the latter is found only in the Americas. *L. braziliensis* causes mucocutaneous leishmaniasis, which occurs only in Central and South America.

Important Properties

Sandflies are the vectors for these three organisms, as they are for *L. donovani*, and forest rodents are their main reservoirs. The life cycle of these parasites is essentially the same as that of *L. donovani*.

Pathogenesis & Epidemiology

The lesions are confined to the skin in cutaneous leishmaniasis and to the mucous membranes, cartilage, and skin in mucocutaneous leishmaniasis. A granulomatous response occurs, and a necrotic ulcer forms at the bite site. The lesions tend to become superinfected with bacteria.

Old World cutaneous leishmaniasis (Oriental sore, Delhi boil), caused by *L. tropica*, is endemic in the Middle East, Africa, and India. New World cutaneous leishmaniasis (chicle ulcer, bay sore), caused by *L. mexicana*, is found in Central and South America. Mucocutaneous leishmaniasis (espundia), caused by *L. braziliensis*, occurs mostly in Brazil and Central America, primarily in forestry and construction workers.

Clinical Findings

The initial lesion of cutaneous leishmaniasis is a red papule at the bite site, usually on an exposed extremity. This enlarges slowly to form multiple satellite nodules that coalesce and ulcerate. There is usually a single lesion that heals spontaneously in patients with a competent immune system. However, in certain individuals, if cell-mediated immunity does not develop, the lesions can spread to involve large areas of skin and contain enormous numbers of organisms.

Mucocutaneous leishmaniasis begins with a papule at the bite site, but then metastatic lesions form, usually at the mucocutaneous junction of the nose and mouth. Disfiguring granulomatous, ulcerating lesions destroy nasal cartilage but not adjacent bone. These lesions heal slowly, if at all. Death can occur from secondary infection.

CHAPTER
53

Minor Protozoan Pathogens

CHAPTER CONTENTS

The medically important stages in the life cycle of certain minor protozoa are described in Table 53–1.

TABLE 53–1 **Medically Important Stages in Life Cycle of Certain Minor Protozoa**

Organism	Insect Vector	Stage That Infects Humans	Stage(s) in Humans Most Associated with Disease	Important Stage(s) Outside of Humans
Acanthamoeba and *Naegleria*	None	Trophozoite	Trophozoites in meninges	Cyst
Babesia	Tick (*Ixodes*)	Sporozoite in tick saliva	Trophozoites and merozoites in red blood cells	None

ACANTHAMOEBA & NAEGLERIA

Acanthamoeba castellanii and *Naegleria fowleri* are free-living **amebas** that cause **meningoencephalitis**. The organisms are found in warm freshwater lakes and in soil. Their life cycle involves trophozoite and cyst stages. Cysts are quite resistant and are not killed by chlorination.

Naegleria trophozoites usually enter the body through mucous membranes while an individual is **swimming**. They can penetrate the nasal mucosa and cribriform plate to produce a purulent meningitis and encephalitis that are usually rapidly fatal (Figure 53–1). *Acanthamoeba* is carried into the skin or eyes during trauma. *Acanthamoeba* infections occur primarily in immunocompromised individuals, whereas *Naegleria* infections occur in otherwise healthy persons, usually children. In the United States, these rare infections occur mainly in the southern states and California.

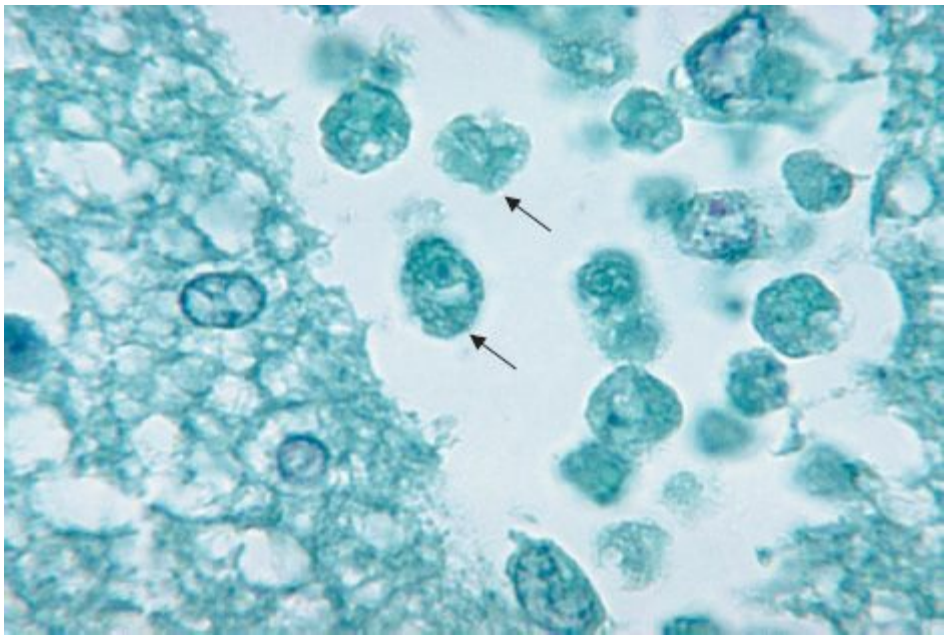


FIGURE 53–1 *Naegleria fowleri*—trophozoite. Arrows point to two ameba-shaped trophozoites in brain tissue. (Figure courtesy of Public Health Image Library, Centers for Disease Control and Prevention.)

Diagnosis is made by finding amebas in the spinal fluid. The prognosis is poor even in treated cases. Amphotericin B may be effective in *Naegleria* infections. Pentamidine, ketoconazole, or flucytosine may be effective in *Acanthamoeba* infections.

Acanthamoeba also causes **keratitis**—an inflammation of the cornea that occurs primarily in those who wear contact lenses. With increasing use of contact lenses, keratitis has become the most common disease associated with *Acanthamoeba* infection. The amebas have been recovered from contact lenses, lens cases, and lens disinfectant solutions. Tap water contaminated with amebas is the source of infection for lens users.

BABESIA

Babesia microti causes babesiosis—a zoonosis acquired chiefly in the coastal areas and islands off the northeastern coast of the United States (e.g., Nantucket Island). The sporozoan organism is endemic in rodents and is transmitted by the bite of the **tick** *Ixodes dammini* (renamed *I. scapularis*), the same species of tick that transmits *Borrelia burgdorferi*, the agent of Lyme disease. *Babesia* infects red blood cells, causing them to lyse, but unlike plasmodia, it has no exoerythrocytic phase. Asplenic patients are affected more severely.

The influenzalike symptoms begin gradually and may last for several weeks. Hepatosplenomegaly and anemia occur. Diagnosis is made by seeing intraerythrocytic ring-shaped parasites on Giemsa-stained blood smears. The intraerythrocytic ring-shaped trophozoites are often in tetrads in the form of a **Maltese cross** (Figure 53–2). Unlike the case with plasmodia, there is no pigment in the erythrocytes. The treatment of choice for mild to moderate disease is a combination of atovaquone and azithromycin. Patients with severe disease should receive a combination of quinidine and clindamycin. Exchange transfusion should also be considered in patients with severe disease. Prevention involves protection from tick bites and, if a person is bitten, prompt removal of the tick.

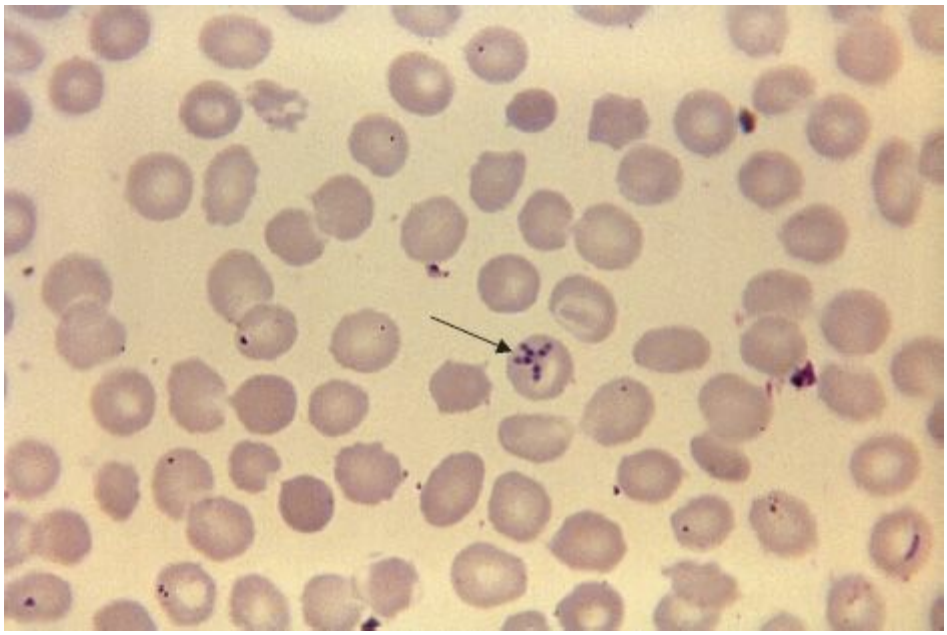


FIGURE 53–2 *Babesia microti*—trophozoites in tetrads. Arrow points to a red blood cell containing four trophozoites in a tetrad resembling a "Maltese cross." (Figure courtesy of Dr. S. Glenn, Public Health Image Library, Centers for Disease Control and Prevention.)

BALANTIDIUM

Balantidium coli is the **only ciliated protozoan** that causes human disease (i.e., **diarrhea**). It is found worldwide but only infrequently in the United States. Domestic animals, especially pigs, are the main reservoir for the organism, and humans are infected after ingesting the cysts in food or water contaminated with animal or human feces. The trophozoites excyst in the small intestine, travel to the colon, and, by burrowing into the wall, cause an ulcer similar to that of *Entamoeba histolytica*. However, unlike the case with *E. histolytica*, extraintestinal lesions do not occur.

Most infected individuals are asymptomatic; diarrhea rarely occurs. Diagnosis is made by finding large ciliated trophozoites or large cysts with a characteristic V-shaped nucleus in the stool. There are no serologic tests. The treatment of choice is tetracycline. Prevention consists of avoiding contamination of food and water by domestic animal feces.

CYCLOSPORA

Cyclospora cayetanensis is an intestinal protozoan that causes watery diarrhea in both immunocompetent and immunocompromised individuals. It is classified as a member of the Coccidia.[1]

The organism is acquired by fecal–oral transmission, especially via contaminated water supplies. One outbreak in the United States was attributed to the ingestion of contaminated raspberries. There is no evidence for an animal reservoir.

The diarrhea can be prolonged and relapsing, especially in immunocompromised patients. Infection occurs worldwide. The diagnosis is made microscopically by observing the spherical oocysts in a modified acid-fast stain of a stool sample. There are no serologic tests. The treatment of choice is trimethoprim-sulfamethoxazole.

ISOSPORA

Isospora belli is an intestinal protozoan that causes **diarrhea,** especially in **immunocompromised patients** (e.g., those with acquired immunodeficiency syndrome [AIDS]). Its life cycle parallels that of other members of the Coccidia. The organism is acquired by fecal–oral transmission of oocysts from either human or animal sources. The oocysts excyst in the upper small intestine and invade the mucosa, causing destruction of the brush border.

The disease in immunocompromised patients presents as a chronic, profuse,

watery diarrhea. The pathogenesis of the diarrhea is unknown. Diagnosis is made by finding the typical oocysts in fecal specimens. Serologic tests are not available. The treatment of choice is trimethoprim-sulfamethoxazole.

MICROSPORIDIA

Microsporidia are a group of protozoa characterized by obligate intracellular replication and spore formation. As the name implies, the spores are quite small, approximately 1 to 3 μm, about the size of *Escherichia coli*. One unique feature of these spores is a "polar tube," which is coiled within the spore and extrudes to attach to the human cells upon infection. The protoplasm of the spore then enters the human cell via the polar tube.

Enterocytozoon bieneusi and *Encephalitozoon intestinalis* are two important microsporidial species that cause severe, persistent, watery diarrhea in AIDS patients. The organisms are transmitted from human to human by the fecal–oral route. Microsporidia are also implicated in infections of the central nervous system, the genitourinary tract, and the eye. It is uncertain whether an animal reservoir exists. Diagnosis is made by visualization of spores in stool samples or intestinal biopsy samples. The treatment of choice is albendazole.

SELF-ASSESSMENT QUESTIONS

1. Regarding *Acanthamoeba* and *Naegleria* species, which one of the following is most accurate?

   (A) They are free-living amebas that live in warm fresh water.

   (B) *Naegleria* is a well-recognized cause of otitis media, primarily in children.

   (C) The drug of choice for infections caused by these organisms is chloroquine.

   (D) Their main clinical presentation is pneumonia acquired when water is aspirated into the lung.

2. Regarding *B. microti*, which one of the following is most accurate?

   (A) It infects macrophages, causing them to lyse.

   (B) Doxycycline is the drug of choice for babesiosis.

   (C) It is transmitted by the bite of *Culex* mosquitoes.

   (D) Seeing sporozoites within red cells supports the diagnosis of babesiosis.

   (E) *B. microti* causes disease primarily in the northeastern region of the United

CHAPTER

10

Antibacterial Drugs: Mechanism of Action

CHAPTER CONTENTS

ANTIMICROBIAL DRUG STEWARDSHIP

The discovery of antimicrobials is one of the great advances in medicine, and their use has substantially reduced morbidity and mortality worldwide. Unfortunately, with widespread antibiotic use, we have witnessed the emergence of multidrug-resistant pathogens and reduced efficacy of many of our most powerful antimicrobials. In addition, we have also recognized many adverse effects of antimicrobials, most notably the rising rates of *Clostridium difficile* colitis. Further, the cost of medical care is greatly increased due to overuse of antibiotics and treating infections caused by resistant organisms. It is critical that health profession learners understand the key concepts behind antimicrobial stewardship at the time they learn about microbial pathogens and antimicrobials.

The worldwide problem of antibiotic resistance makes the need for antimicrobial stewardship evident. The Centers for Disease Control and Prevention estimates that in the United States over 2 million infections with multidrug-resistant pathogens occur each year, leading to approximately 20,000 deaths. These pathogens include methicillin-resistant *Staphylococcus aureus* and extended-spectrum β-lactamase–producing gram-negative rods (e.g., *Escherichia coli* and *Klebsiella pneumoniae*). Hospital-associated infections, many of which are caused by antibiotic-resistant bacteria, are estimated to cost billions of dollars each year.

The basic principles of good stewardship are threefold: (1) reduce inappropriate use of antibiotics, (2) encourage targeted treatment with narrow-spectrum drugs, and (3) limit adverse effects (Table 10–1). Inappropriate antibiotic use can occur for many reasons, including the providers' desire to adhere to patient's wishes, even when it is not medically appropriate. The most common example of inappropriate antibiotic use is the prescribing of antibiotics for a viral respiratory tract infection. It is estimated that half of all prescriptions given for upper respiratory infections (pharyngitis, sinusitis) are inappropriate.

The concept of targeted treatment refers to making a microbiologic diagnosis promptly and using the most specific antibiotic that has the best safety profile for the patient. If multiple broad-spectrum antibiotics are used as empiric therapy early in infection, then switching to a narrow-spectrum antibiotic as soon as possible should be done. Cultures should be sent prior to starting antibiotics so that the drugs will not reduce the likelihood of isolating the causative organism. Further, switching from intravenous antibiotics to oral dosing reduces the risk of catheter-associated infections. The net result of targeted treatment is to reduce the ability of antibiotics to select for resistant mutants present in the bacterial population.

Limiting the occurrence of adverse effects caused by antibiotics is another major goal of antimicrobial stewardship. Minimizing the duration of antibiotic use to only as long as clinically indicated is a key intervention since duration of exposure correlates closely with the risk of many types of adverse effects. Patients with reduced renal function should have the dose of some antibiotics adjusted based on their estimated glomerular filtration rate.

Antibiotic allergies should be identified and explored in detail. Although reaction to antibiotics may be commonly

TABLE 10–1 Basic Principles of Antimicrobial Drug Stewardship

Current Problems in the Use of Antibiotics	Role of Antimicrobial Drug Stewardship in Mitigating These Problems
Inappropriate use of antibiotics	1. Use antibiotics only when a microbiologic diagnosis indicates effectiveness 2. Empiric therapy should be tailored to the most likely pathogen(s) 3. Send appropriate cultures before starting antibiotics
Overuse of broad-spectrum antibiotics	1. Use narrow-spectrum antibiotics whenever possible 2. Require approval for the use of advanced-generation broad-spectrum antibiotics
High rate of adverse effects	1. Stop antibiotics as soon as appropriate to reduce adverse effects, such as antibiotic-associated colitis caused by *Clostridium difficile* 2. Be aware of the effect of the patient's renal function on the dose of antibiotic prescribed 3. Be aware of the patient's hypersensitivity to specific antibiotics 4. Determine whether the patient's declared hypersensitivity is correct and clinically significant 5. Warn patients regarding certain idiosyncratic drug reactions, such as photosensitization

reported, they may not always be of significance such as some alleged hypersensitivity reactions. If optimal treatment requires a drug such as penicillin to which the patient says he or she is allergic, then skin testing can be employed to determine the accuracy of that claim. In addition, patients should be warned regarding the possibility that certain drugs may cause adverse effects. For example, certain photosensitizing antibiotics may cause a rash when the patient is exposed to sunlight.

The reasons that inappropriate use of antibiotics occurs are varied. Probably, the most important is the lack of knowledge or awareness of the physician. Risk avoidance on the part of the physician is also common. Inadequate microbiologic information plays a role as well. Patient expectation and direct demands for antibiotics contribute to the problem.

In summary, antimicrobial stewardship refers to the effort to improve the treatment of infectious diseases by the appropriate use of antibiotics. This is critical in this era of rising rates of multidrug-resistant pathogens. Targeted therapy with the most appropriate single antibiotic will hopefully improve clinical outcomes and reduce the cost of care.

PRINCIPLES OF ANTIMICROBIAL THERAPY

The most important concept underlying antimicrobial therapy is **selective toxicity** (i.e., selective inhibition of the growth of the microorganism without damage to the host). Selective toxicity is achieved by exploiting the differences between the metabolism and structure of the microorganism and the corresponding features of human cells. For example, penicillins and cephalosporins are effective antibacterial agents because they prevent the synthesis of peptidoglycan, thereby inhibiting the growth of bacterial but not human cells.

There are four major sites in the bacterial cell that are sufficiently different from the human cell that they serve as the basis for the action of clinically effective drugs: cell wall, ribosomes, nucleic acids, and cell membrane (Table 10–2 and Figure 10–1).

There are far more antibacterial drugs than antiviral drugs. This is a consequence of the difficulty of designing a drug that will selectively inhibit viral replication. Because viruses use many of the normal cellular functions of the host in their growth, it is not easy to develop a drug that specifically inhibits viral functions and does not damage the host cell.

Broad-spectrum antibiotics are active against several types of microorganisms (e.g., tetracyclines are active against many gram-negative rods, chlamydiae, mycoplasmas, and rickettsiae). **Narrow-spectrum** antibiotics are active against one or very few types (e.g., vancomycin is primarily used against certain gram-positive cocci, namely, staphylococci and enterococci).

BACTERICIDAL & BACTERIOSTATIC ACTIVITY

In some clinical situations, it is essential to use a bactericidal drug rather than a bacteriostatic one. A **bactericidal drug kills bacteria,** whereas a **bacteriostatic drug inhibits their growth but does not kill them** (Figure 10–2). The salient features of the behavior of bacteriostatic drugs are that (1) the bacteria can grow again when the drug is withdrawn, and (2) host defense mechanisms, such as phagocytosis, are required to kill the bacteria. Bactericidal drugs are particularly useful in certain infections (e.g., those that are immediately life-threatening; those in patients whose polymorphonuclear leukocyte count is below 500/μL; and endocarditis, in which phagocytosis is limited by the fibrinous network of the vegetations and bacteriostatic drugs do not effect a cure).

TABLE 10–2 Mechanism of Action of Important Antibacterial Drugs

Mechanism of Action	Drugs
Inhibition of cell wall synthesis	
Inhibits cross-linking (transpeptidation) of peptidoglycan	Penicillins, cephalosporins, imipenem, aztreonam, vancomycin
Inhibits other steps in peptidoglycan synthesis	Cycloserine, bacitracin
Inhibition of protein synthesis	
Acts on 50S ribosomal subunit	Chloramphenicol, erythromycin, clindamycin, linezolid
Acts on 30S ribosomal subunit	Tetracyclines and aminoglycosides
Inhibition of nucleic acid synthesis	
Inhibits nucleotide synthesis	Sulfonamides, trimethoprim
Inhibits DNA synthesis	Quinolones (e.g., ciprofloxacin)
Inhibits mRNA synthesis	Rifampin
Alteration of cell membrane function	
Disrupts membranes	Polymyxin, daptomycin
Other mechanisms of action	
Inhibits mycolic acid synthesis	Isoniazid
Acts as electron sink and damages DNA	Metronidazole
Inhibits arabinogalactan synthesis	Ethambutol
May inhibit fatty acid synthesis	Pyrazinamide

MECHANISMS OF ACTION

INHIBITION OF CELL WALL SYNTHESIS

1. Inhibition of Bacterial Cell Wall Synthesis

Penicillins

Penicillins (and cephalosporins) act by inhibiting **transpeptidases**, the enzymes that catalyze the final cross-linking step in the synthesis of peptidoglycan (see Figure 2–5). For example, in *S. aureus,* transpeptidation occurs between the amino group on the end of the pentaglycine cross-link and the terminal carboxyl group of the D-alanine on the tetrapeptide side chain. Because the stereochemistry of penicillin is similar to that of a dipeptide, D-alanyl-D-alanine, penicillin can bind to the active site of the transpeptidase and inhibit its activity.

Two additional factors are involved in the action of penicillin:

(1) The first is that penicillin binds to a variety of proteins in the bacterial cell membrane and cell wall, called **penicillin-binding proteins (PBPs).** Some PBPs are transpeptidases; the others function in the synthesis of peptidoglycan. Changes in PBPs are in part responsible for an organism's becoming resistant to penicillin.

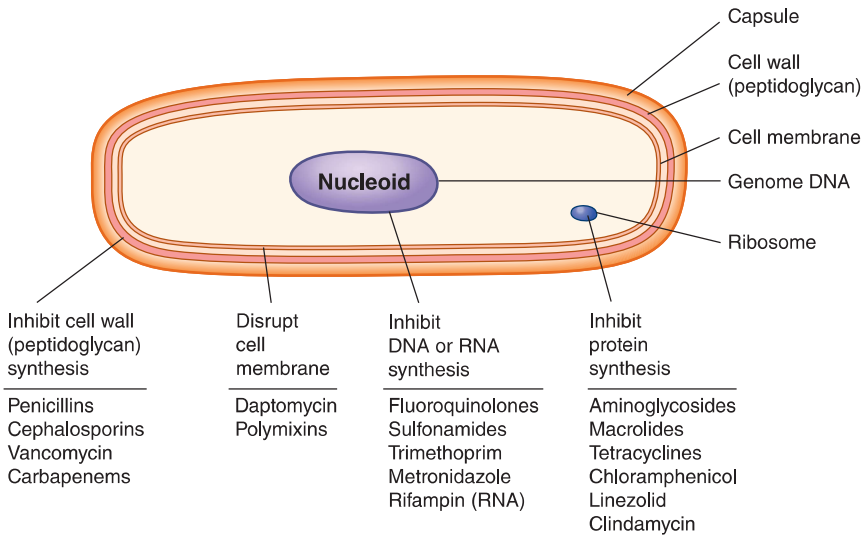


FIGURE 10–1 Model of typical bacterial cell showing sites of action of important antibacterial drugs.

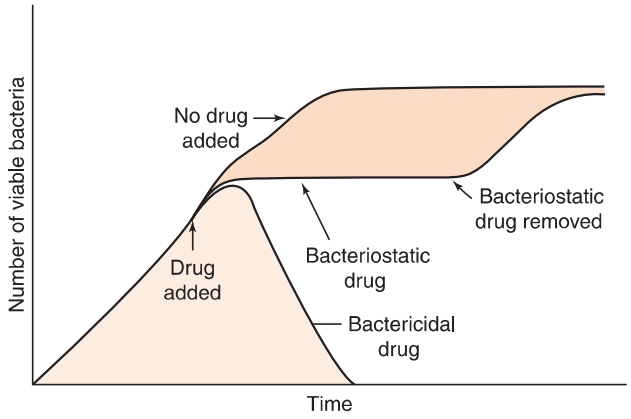


FIGURE 10–2 Bactericidal and bacteriostatic activity of antimicrobial drugs. Either a bactericidal or a bacteriostatic drug is added to the growing bacterial culture at the time indicated by the arrow. After a brief lag time during which the drug enters the bacteria, the bactericidal drug kills the bacteria, and a decrease in the number of viable bacteria occurs. The bacteriostatic drug causes the bacteria to stop growing, but if the bacteriostatic drug is removed from the culture, the bacteria resume growing.

(2) The second factor is that **autolytic enzymes** called murein hydrolases (murein is a synonym for peptidoglycan) are activated in penicillin-treated cells and degrade the peptidoglycan. Some bacteria (e.g., strains of *S. aureus*) are **tolerant** to the action of penicillin, because these autolytic enzymes are not activated. A tolerant organism is one that is inhibited but not killed by a drug that is usually bactericidal, such as penicillin. Penicillin-treated cells die by rupture as a result of the influx of water into the high-osmotic-pressure interior of the bacterial cell.

Penicillin is bactericidal, but it **kills cells only when they are growing.** When cells are growing, new peptidoglycan is being synthesized, and transpeptidation occurs. However, in non-growing cells, no new cross-linkages are required, and penicillin is inactive. Penicillins are therefore **more active during the log phase** of bacterial cell growth than during the stationary phase (see Chapter 3 for the bacterial cell growth cycle).

Penicillins (and cephalosporins) are called β-lactam drugs because of the importance of the β-lactam ring (Figure 10–3). An intact ring structure is essential for antibacterial activity; cleavage of the ring by penicillinases (**β-lactamases**) inactivates the drug. The most important naturally occurring compound is benzylpenicillin (penicillin G), which is composed of the 6-aminopenicillanic acid nucleus that all penicillins have, plus a benzyl side chain (see Figure 10–3). Penicillin G is available in three main forms:

(1) Aqueous penicillin G, which is metabolized most rapidly.

(2) Procaine penicillin G, in which penicillin G is conjugated to procaine. This form is metabolized more slowly and is less painful when injected intramuscularly because the procaine acts as an anesthetic.

(3) Benzathine penicillin G, in which penicillin G is conjugated to benzathine. This form is metabolized very slowly and is often called a "depot" preparation.

Benzylpenicillin is one of the most widely used and effective antibiotics. However, it has four disadvantages, three of which have been successfully overcome by chemical modification of

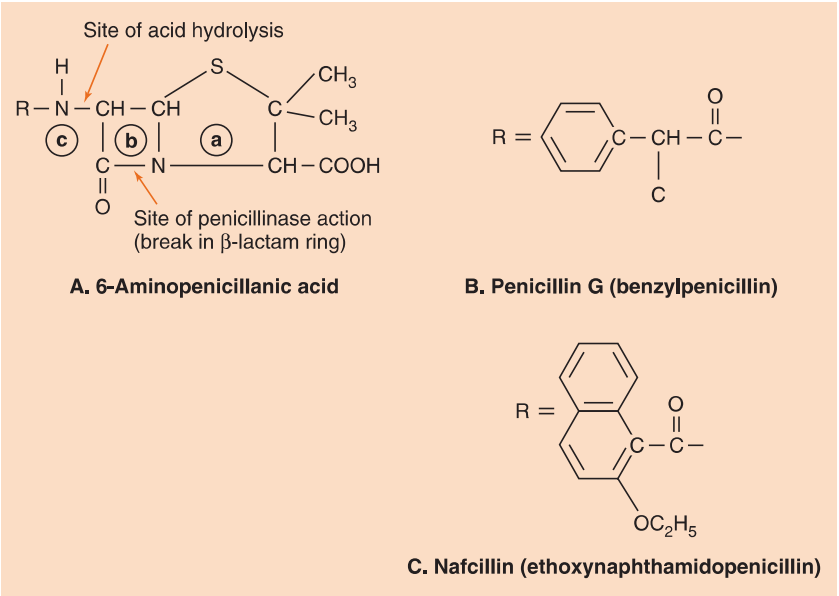


FIGURE 10–3 Penicillins. **A:** The 6-aminopenicillanic acid nucleus is composed of a thiazolidine ring (a), a β-lactam ring (b), and an amino group (c). The sites of inactivation by stomach acid and by penicillinase are indicated. **B:** The benzyl group, which forms benzylpenicillin (penicillin G) when attached at R. **C:** The large aromatic ring substituent that forms nafcillin, a β-lactamase–resistant penicillin, when attached at R. The large ring blocks the access of β-lactamase to the β-lactam ring.

the side chain. The three disadvantages are (1) limited effectiveness against many gram-negative rods, (2) hydrolysis by gastric acids, so that it cannot be taken orally, and (3) inactivation by β-lactamases. The limited effectiveness of penicillin G against gram-negative rods is due to the inability of the drug to penetrate the outer membrane of the organism. The fourth disadvantage common to all penicillins that has *not* been overcome is hypersensitivity, especially anaphylaxis, in some recipients of the drug.

The effectiveness of penicillins against gram-negative rods has been increased by a series of chemical changes in the side chain (Table 10–3). It can be seen that ampicillin and amoxicillin have activity against several gram-negative rods that the earlier penicillins do not have. However, these drugs are not useful against *Pseudomonas aeruginosa* and *K. pneumoniae.* Hence, other penicillins were introduced. Generally speaking, as the activity against gram-negative bacteria increases, the activity against gram-positive bacteria decreases.

The second important disadvantage—acid hydrolysis in the stomach—also has been addressed by modification of the side chain. The site of acid hydrolysis is the amide bond between the side chain and penicillanic acid nucleus (see Figure 10–3). Minor modifications of the side chain in that region, such as addition of an oxygen (to produce penicillin V) or an amino group (to produce ampicillin), prevent hydrolysis and allow the drug to be taken orally.

The inactivation of penicillin G by β-lactamases is another important disadvantage, especially in the treatment of *S. aureus* infections. Access of the enzyme to the β-lactam ring is blocked by modification of the side chain with the addition of large aromatic rings containing bulky methyl or ethyl groups (methicillin, oxacillin, nafcillin, etc.; see Figure 10–3). Another defense against β-lactamases is inhibitors such as clavulanic acid, tazobactam, sulbactam, and avibactam. These are structural analogues of penicillin that have little antibacterial activity but bind strongly to β-lactamases and thus protect the penicillin. Combinations, such as amoxicillin and clavulanic acid (Augmentin), are in clinical use. Some bacteria resistant to these combinations have been isolated from patient specimens.

Penicillins are usually nontoxic at clinically effective levels. The major disadvantage of these compounds is hypersensitivity, with a reported prevalence of 1% to 10% of patients. The immunoglobulin (Ig) E–mediated hypersensitivity reactions include anaphylactic shock, bronchospasm, and urticarial rash (see Chapter 65). IgG and cell-mediated hypersensitivity reactions include nonurticarial skin rashes, hemolytic anemia, nephritis, and drug fever. A maculopapular drug-induced rash is quite common. Anaphylactic shock, the most serious complication, occurs in 0.5% of patients. Death as a result of anaphylaxis occurs in 0.002% of patients (1 in 50,000 patients).

Patients who say they are allergic to penicillin can be treated with another equally effective antibiotic, if available. To determine whether the patient's allergy is clinically significant, a skin test using penicilloyl-polylysine as the test reagent can be performed. A wheal and flare reaction occurs at the site of injection in allergic individuals. If the patient's disease requires treatment with penicillin, the patient can be desensitized under the supervision of a trained allergist.

TABLE 10–3 Activity of Selected Penicillins

Drug	Clinically Useful Activity[1]
Penicillin G	Gram-positive cocci, gram-positive rods, *Neisseria,* spirochetes such as *Treponema pallidum,* and many anaerobes (except *Bacteroides fragilis*) but none of the gram-negative rods listed below
Ampicillin or amoxicillin	Certain gram-negative rods, such as *Haemophilus influenzae, Escherichia coli, Proteus, Salmonella,* and *Shigella* but not *Pseudomonas aeruginosa* or *Klebsiella pneumoniae*
Ticarcillin	*P. aeruginosa,* especially when used in synergistic combination with an aminoglycoside
Piperacillin	Similar to ticarcillin but with greater activity against *P. aeruginosa* and *Klebsiella pneumoniae*
Nafcillin or dicloxacillin	Penicillinase-producing *Staphylococcus aureus*

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

Cephalosporins

Cephalosporins are β-lactam drugs that, like penicillins, also inhibit the cross-linking of peptidoglycan. The structures, however, are different: Cephalosporins have a six-membered ring adjacent to the β-lactam ring and are substituted in two places on the 7-aminocephalosporanic acid nucleus (Figure 10–4), whereas penicillins have a five-membered ring and are substituted in only one place.

The first-generation cephalosporins are active primarily against gram-positive cocci (Table 10–4). Similar to the penicillins, new cephalosporins were synthesized with expansion of activity against gram-negative rods as the goal. These new cephalosporins have been categorized into second, third, and fourth generations, with each generation having expanded

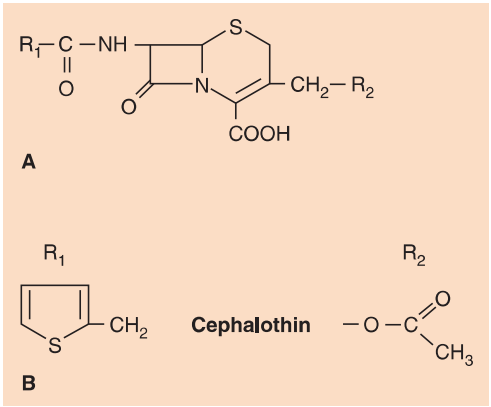


FIGURE 10–4 Cephalosporins. **A:** The 7-aminocephalosporanic acid nucleus. **B:** The two R groups in the drug cephalothin.

TABLE 10–4 Activity of Selected Cephalosporins[1]

Generation of Cephalosporin	Drug	Clinically Useful Activity
First	Cefazolin, cephalexin	Gram-positive cocci such as staphylococci and streptococci except enterococci and MRSA
Second	Cefuroxime	*Haemophilus influenzae*
	Cefoxitin	*Bacteroides fragilis*
Third	Ceftriaxone	Enteric gram-negative rods such as *Escherichia coli, Klebsiella* and *Proteus*. Also *Neisseria gonorrhoeae*
	Ceftazidime	*Pseudomonas aeruginosa* and other enteric gram-negative rods
Fourth	Cefepime	Enteric gram-negative rods that produce extended-spectrum β-lactamases; *Staphylococcus aureus* (not MRSA) and penicillin-resistant *Streptococcus pneumoniae*
Fifth	Ceftaroline	Gram-positive cocci and gram-negative rods that cause bacterial pneumonia and gram-positive cocci that cause skin infections including MRSA
	Ceftolozane	Enteric gram-negative rods that produce extended-spectrum β-lactamases; *Pseudomonas aeruginosa;* used in combination with tazobactam

MRSA = methicillin-resistant *Staphylococcus aureus*.

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

coverage against certain gram-negative rods. The fourth- and fifth-generation cephalosporins have activity against many gram-positive cocci as well.

Cephalosporins are effective against a broad range of organisms, are generally well tolerated, and produce fewer hypersensitivity reactions than do the penicillins. Despite the structural similarity, a patient allergic to penicillin has only about a 10% chance of being hypersensitive to cephalosporins also. Most cephalosporins are the products of molds of the genus *Cephalosporium;* a few, such as cefoxitin, are made by the actinomycete *Streptomyces.*

The inactivation of cephalosporins by β-lactamases (cephalosporinases) is an important clinical problem. β-Lactamase inhibitors such as tazobactam and avibactam are combined with certain cephalosporins to prevent inactivation of the cephalosporin. For example, the US Food and Drug Administration (FDA) has approved the combination of ceftazidime/avibactam (Avycaz) and ceftolozane/tazobactam (Zerbaxa) for the treatment of intra-abdominal infections and complicated urinary tract infections caused by antibiotic-resistant gram-negative rods.

Carbapenems

Carbapenems are β-lactam drugs that are structurally different from penicillins and cephalosporins. For example, imipenem (*N*-formimidoylthienamycin), a commonly used carbapenem, has a methylene group in the ring in place of the sulfur (Figure 10–5). Imipenem has one of the widest spectrums of activity of the β-lactam drugs. It has excellent bactericidal activity against many gram-positive, gram-negative, and anaerobic bacteria. It is effective against most gram-positive cocci (e.g., streptococci and staphylococci), most gram-negative cocci (e.g., *Neisseria*), many gram-negative rods (e.g., *Pseudomonas, Haemophilus,* and members of the family Enterobacteriaceae such as *E. coli*), and various anaerobes (e.g., *Bacteroides* and *Clostridium*). Imipenem is especially useful in treating infections caused by gram-negative rods that produce extended-spectrum β-lactamases that make them resistant to all penicillins and cephalosporins. Carbapenems are often the "drugs of last resort" against bacteria resistant to multiple antibiotics and are thus reserved for hospital settings.

Imipenem is prescribed in combination with cilastatin, which is an inhibitor of dehydropeptidase, a kidney enzyme that inactivates imipenem. Imipenem is not inactivated by most β-lactamases; however, carbapenemases produced by *K. pneumoniae* that degrade imipenem and other carbapenemases have emerged. Other carbapenems, such as ertapenem and meropenem, are not inactivated by dehydropeptidase and are not prescribed in combination with cilastatin.

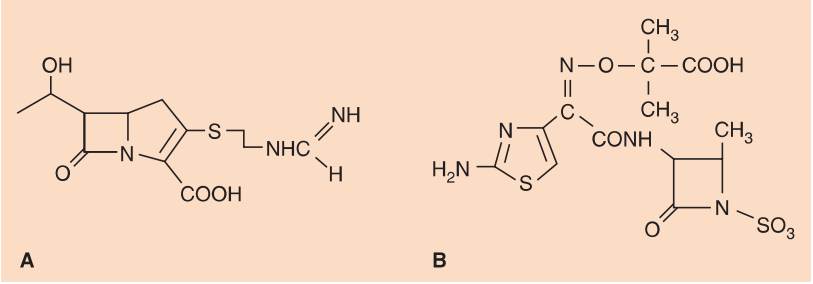


FIGURE 10–5 **A:** Imipenem. **B:** Aztreonam.

Monobactams

Monobactams are also β-lactam drugs that are structurally different from penicillins and cephalosporins. Monobactams are characterized by a β-lactam ring without an adjacent sulfur-containing ring structure (i.e., they are monocyclic) (see Figure 10–5). Aztreonam, currently the most useful monobactam, has excellent activity against many gram-negative rods, such as Enterobacteriaceae and *Pseudomonas,* but is inactive against gram-positive and anaerobic bacteria. It is resistant to most β-lactamases. It is very useful in patients who are hypersensitive to penicillin because there is no cross-reactivity.

Vancomycin

Vancomycin is a glycopeptide that **inhibits cell wall peptidoglycan synthesis by blocking transpeptidation** but by a mechanism different from that of the β-lactam drugs. Vancomycin binds directly to the D-alanyl-D-alanine portion of the pentapeptide, which blocks the transpeptidase from binding, whereas the β-lactam drugs bind to the transpeptidase itself. Vancomycin also inhibits a second enzyme, the bacterial transglycosylase, which also functions in synthesizing the peptidoglycan, but this appears to be less important than inhibition of the transpeptidase.

Vancomycin is a bactericidal agent **effective against certain gram-positive bacteria.** Its most important use is in the treatment of infections caused by *S. aureus* strains that are resistant to the penicillinase-resistant penicillins such as nafcillin and methicillin (e.g., methicillin-resistant *S. aureus* [MRSA]). Note that vancomycin is not a β-lactam drug and, therefore, is not degraded by β-lactamase.

Vancomycin is also used in the treatment of infections caused by *Staphylococcus epidermidis*, penicillin-resistant *Streptococcus pneumoniae*, and enterococci. Strains of *S. aureus, S. epidermidis,* and enterococci with partial or complete resistance to vancomycin have been recovered from patients.

A well-known adverse effect of vancomycin is "red man" syndrome. "Red" refers to the flushing caused by vasodilation induced by histamine release from mast cells and basophils. This is a direct effect of vancomycin on these cells and is not an IgE-mediated response.

Telavancin is a synthetic derivative of vancomycin that both inhibits peptidoglycan synthesis and disrupts bacterial cell membranes. It is used for the treatment of skin and soft tissue infections, especially those caused by MRSA. Oritavancin and dalbavancin are lipoglycopeptide derivatives of vancomycin and teicoplanin, respectively. These drugs inhibit the transpeptidases and transglycosylases required to synthesize the peptidoglycan of gram-positive bacteria. They are effective in the treatment of infections caused by *S. aureus*, including MRSA, and *Enterococcus*, including vancomycin-resistant enterococci (VRE).

Cycloserine & Bacitracin

Cycloserine is a structural analogue of D-alanine that inhibits the synthesis of the cell wall dipeptide D-alanyl-D-alanine. It is used as a second-line drug in the treatment of tuberculosis. Bacitracin is a cyclic polypeptide antibiotic that prevents the dephosphorylation of the phospholipid that carries the peptidoglycan subunit across the cell membrane. This blocks the regeneration of the lipid carrier and inhibits cell wall synthesis. Bacitracin is a bactericidal drug useful in the treatment of superficial skin infections but too toxic for systemic use.

INHIBITION OF PROTEIN SYNTHESIS

Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells. This selectivity is due to the differences between bacterial and human ribosomal proteins, RNAs, and associated enzymes. Bacteria have 70S[1] ribosomes with 50S and 30S subunits, whereas human cells have 80S ribosomes with 60S and 40S subunits.

Chloramphenicol, macrolides such as azithromycin and erythromycin, clindamycin, and linezolid act on the 50S subunit, whereas tetracyclines such as doxycycline and aminoglycosides such as gentamicin act on the 30S subunit. A summary of the modes of action of these drugs is presented in Table 10–5, and a summary of their clinically useful activity is presented in Table 10–6.

1. Drugs That Act on the 30S Subunit

Aminoglycosides

Aminoglycosides are bactericidal drugs especially useful against many gram-negative rods. Certain aminoglycosides are used against other organisms (e.g., streptomycin is used in the multidrug therapy of tuberculosis, and gentamicin is used in combination with penicillin G against enterococci). Aminoglycosides are named for the amino sugar component of the molecule, which is connected by a glycosidic linkage to other sugar derivatives (Figure 10–6).

The two important modes of action of aminoglycosides have been documented best for streptomycin; other aminoglycosides probably act similarly. Both **inhibition of the initiation complex** and **misreading of messenger RNA** (mRNA) occur; the former is probably more important for the bactericidal activity of the drug. An initiation complex composed of a streptomycin-treated 30S subunit, a 50S subunit, and mRNA will not function—that is, no peptide bonds are formed, no polysomes are made, and a frozen "streptomycin monosome" results. Misreading of the triplet codon of mRNA so that the wrong amino acid is inserted into the protein also occurs in streptomycin-treated bacteria. The site of action on the 30S subunit includes both a ribosomal protein and the ribosomal RNA (rRNA). As a result of inhibition of initiation and misreading, membrane damage occurs and the bacterium dies. (In 1993, another possible mode of action was described, namely, that aminoglycosides inhibit ribozyme-mediated self-splicing of rRNA.)

[1]"S" stands for Svedberg units, a measure of sedimentation rate in a density gradient. The rate of sedimentation is proportionate to the mass of the particle.

TABLE 10–5 Mode of Action of Antibiotics That Inhibit Protein Synthesis

Antibiotic	Ribosomal Subunit	Mode of Action	Bactericidal or Bacteriostatic
Aminoglycosides	30S	Blocks functioning of initiation complex and causes misreading of mRNA	Bactericidal
Tetracyclines	30S	Blocks tRNA binding to ribosome	Bacteriostatic
Chloramphenicol	50S	Blocks peptidyltransferase	Both[1]
Macrolides	50S	Blocks translocation	Primarily bacteriostatic
Clindamycin	50S	Blocks peptide bond formation	Primarily bacteriostatic
Linezolid	50S	Blocks early step in ribosome formation	Both[1]
Telithromycin	50S	Same as other macrolides (e.g., erythromycin)	Both[1]
Streptogramins	50S	Causes premature release of peptide chain	Both[1]

[1]Can be either bactericidal or bacteriostatic, depending on the organism.

Aminoglycosides have certain limitations in their use: (1) They have a toxic effect both on the kidneys and on the auditory and vestibular portions of the eighth cranial nerve. To avoid toxicity, serum levels of the drug, blood urea nitrogen, and creatinine should be measured. (2) They are poorly absorbed from the gastrointestinal tract and cannot be given orally. (3) They penetrate the spinal fluid poorly and must be given intrathecally in the treatment of meningitis. (4) They are ineffective against anaerobes, because their transport into the bacterial cell requires oxygen.

Tetracyclines

Tetracyclines are a family of antibiotics with bacteriostatic activity against a variety of gram-positive and gram-negative bacteria, mycoplasmas, chlamydiae, and rickettsiae. They inhibit protein synthesis by binding to the 30S ribosomal subunit and by

TABLE 10–6 Spectrum of Activity of Antibiotics That Inhibit Protein Synthesis[1]

Antibiotic	Clinically Useful Activity	Comments
Aminoglycosides		
Streptomycin	Tuberculosis, tularemia, plague, brucellosis	Ototoxic and nephrotoxic
Gentamicin and tobramycin	Many gram-negative rod infections including *Pseudomonas aeruginosa*	Most widely used aminoglycosides
Amikacin	Same as gentamicin and tobramycin	Effective against some organisms resistant to gentamicin and tobramycin
Neomycin	Preoperative bowel preparation	Too toxic to be used systemically; use orally since not absorbed
Tetracyclines	Rickettsial and chlamydial infections, *Mycoplasma pneumoniae*	Not given during pregnancy or to young children
Tigecycline	Skin infections caused by various gram-positive cocci and intra-abdominal infections caused by various facultative and anaerobic bacteria (see text)	Adverse effects similar to tetracyclines
Chloramphenicol	*Haemophilus influenzae* meningitis, typhoid fever, anaerobic infections (especially *Bacteroides fragilis*)	Bone marrow toxicity limits use to severe infections
Macrolides	Pneumonia caused by *Mycoplasma* and *Legionella,* infections by gram-positive cocci in penicillin-allergic patients	Generally well tolerated but some diarrhea
Clindamycin	Anaerobes such as *Clostridium perfringens* and *B. fragilis*	Pseudomembranous colitis is a major side effect
Linezolid	Vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus* and *Staphylococcus epidermidis,* and penicillin-resistant pneumococci	Generally well tolerated
Telithromycin	Community-acquired pneumonia caused by various bacteria, including multidrug-resistant *Streptococcus pneumoniae*	Many bacteria that are resistant to other macrolides are susceptible to telithromycin
Streptogramins	Bacteremia caused by vancomycin-resistant *Enterococcus faecium*	No cross-resistance between streptogramins and other drugs that inhibit protein synthesis
Retapamulin	Skin infections caused by *Streptococcus pyogenes* and methicillin-sensitive *S. aureus*	

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to illustrate the expanded coverage of gram-negative organisms with successive generations and does not cover all possible clinical uses.

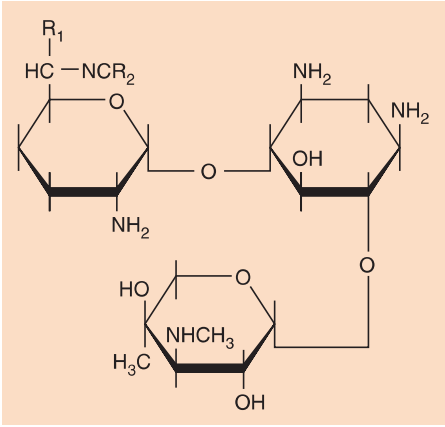


FIGURE 10–6 Aminoglycosides. Aminoglycosides consist of amino sugars joined by a glycosidic linkage. The structure of gentamicin is shown.

blocking the aminoacyl transfer RNA (tRNA) from entering the acceptor site on the ribosome. However, the selective action of tetracycline on bacteria is not at the level of the ribosome, because tetracycline in vitro will inhibit protein synthesis equally well in purified ribosomes from both bacterial and human cells. Its selectivity is based on its greatly increased uptake into susceptible bacterial cells compared with human cells.

Tetracyclines, as the name indicates, have four cyclic rings with different substituents at the three R groups (Figure 10–7). The various tetracyclines (e.g., doxycycline, minocycline, oxytetracycline) have similar antimicrobial activity but different pharmacologic properties. In general, tetracyclines have low toxicity but are associated with some important side effects. One is suppression of the normal flora of the intestinal tract, which can lead to diarrhea and overgrowth by drug-resistant bacteria and fungi. Second is that suppression of *Lactobacillus* in the vaginal normal flora results in a rise in pH, which allows *Candida albicans* to grow and cause vaginitis. Third is brown staining of the teeth of fetuses and young children as a result of deposition of the drug in developing teeth; tetracyclines are avid calcium chelators. For this reason, tetracyclines are contraindicated for use in pregnant women and in children younger than 8 years of age. Tetracyclines also chelate iron, and so products containing iron, such as iron-containing vitamins, should not be taken during therapy with tetracyclines. Photosensitivity (rash upon exposure to sunlight) can also occur during tetracycline therapy.

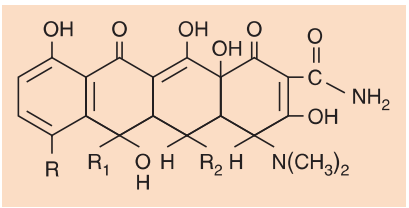


FIGURE 10–7 Tetracycline structure. The four-ring structure is depicted with its three R sites. Chlortetracycline, for example, has R = Cl, $R_1 = CH_3$, and $R_2 = H$.

Tigecycline (Tygacil) is the first clinically available member of the glycylcycline class of antibiotics. They have a structure similar to tetracyclines and have the same mechanism of action as tetracyclines; namely, they bind to the 30S ribosomal subunit and inhibit bacterial protein synthesis. They have a similar range of adverse effects. Tigecycline is used to treat skin and skin structure infections caused by methicillin-sensitive and methicillin-resistant *S. aureus*, group A and group B streptococci, vancomycin-resistant enterococci, *E. coli*, and *Bacteroides fragilis*. It is also used to treat complicated intra-abdominal infections caused by a variety of facultative and anaerobic bacteria.

2. Drugs That Act on the 50S Subunit

Chloramphenicol

Chloramphenicol is active against a broad range of organisms, including gram-positive and gram-negative bacteria (including anaerobes). It is bacteriostatic against certain organisms, such as *Salmonella typhi,* but has bactericidal activity against the three important encapsulated organisms that cause meningitis: *Haemophilus influenzae, S. pneumoniae,* and *Neisseria meningitidis.*

Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and **blocking the action of peptidyltransferase;** this prevents the synthesis of new peptide bonds. It inhibits bacterial protein synthesis selectively, because it binds to the catalytic site of the transferase in the 50S bacterial ribosomal subunit but not to the transferase in the 60S human ribosomal subunit. Chloramphenicol inhibits protein synthesis in the mitochondria of human cells to some extent, since mitochondria have a 50S subunit (mitochondria are thought to have evolved from bacteria). This inhibition may be the cause of the dose-dependent toxicity of chloramphenicol to bone marrow (see next paragraph).

Chloramphenicol is a comparatively simple molecule with a nitrobenzene nucleus (Figure 10–8). Nitrobenzene is a bone marrow depressant and is likely to be involved in the hematologic problems reported with this drug. The most important side effect of chloramphenicol is bone marrow toxicity, of which there are two types. One is a dose-dependent suppression, which is more likely to occur in patients receiving high doses for long periods and which is reversible when administration of the drug is stopped. The other is aplastic anemia, which is caused by an idiosyncratic reaction to the drug. This reaction is not dose-dependent, can occur weeks after administration of the drug has been stopped, and is not reversible. Fortunately, this reaction is rare, occurring in about 1 in 30,000 patients.

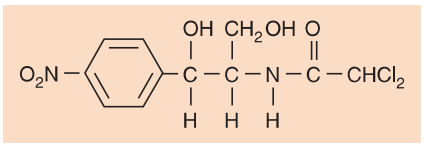


FIGURE 10–8 Chloramphenicol.

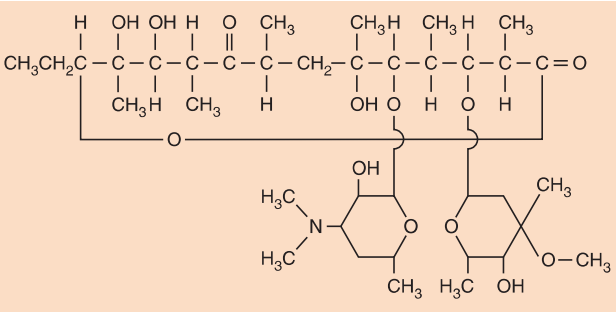


FIGURE 10–9 Erythromycin.

One specific toxic manifestation of chloramphenicol is "gray baby" syndrome, in which the infant's skin appears gray and vomiting and shock occur. This is due to reduced glucuronyl transferase activity in infants, resulting in a toxic concentration of chloramphenicol. Glucuronyl transferase is the enzyme responsible for detoxification of chloramphenicol.

Macrolides

Macrolides are a group of bacteriostatic drugs with a wide spectrum of activity. The name *macrolide* refers to their large (13–16 carbon) ring structure (Figure 10–9). Azithromycin, erythromycin, and clarithromycin are the main macrolides in clinical use. Azithromycin is used to treat genital tract infections caused by *Chlamydia trachomatis* and respiratory tract infections caused by *Legionella, Mycoplasma, Chlamydia pneumoniae,* and *S. pneumoniae.* Erythromycin has a similar spectrum of activity but has a shorter half-life and so must be taken more frequently and has more adverse effects, especially on the gastrointestinal tract. Clarithromycin is used primarily in the treatment of *Helicobacter* infections and in the treatment and prevention of *Mycobacterium avium-intracellulare* infections. An important adverse effect of clarithromycin is prolongation of the QT interval, which may increase the risk of cardiac death.

Macrolides inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit and blocking translocation. They prevent the release of the uncharged tRNA after it has transferred its amino acid to the growing peptide chain. The donor site remains occupied, a new tRNA cannot attach, and protein synthesis stops.

Clindamycin

The most useful clinical activity of this bacteriostatic drug is against anaerobes, both gram-positive bacteria such as *Clostridium perfringens* and gram-negative bacteria such as *B. fragilis.*

Clindamycin binds to the 50S subunit and blocks peptide bond formation by an undetermined mechanism. Its specificity for bacteria arises from its inability to bind to the 60S subunit of human ribosomes.

The most important side effect of clindamycin is pseudomembranous colitis, which, in fact, can occur with virtually any antibiotic, whether taken orally or parenterally. The pathogenesis of this potentially severe complication is suppression of the normal flora of the bowel by the drug and overgrowth of a drug-resistant strain of *C. difficile.* The organism secretes an exotoxin that produces the pseudomembrane in the colon and severe, often bloody diarrhea.

Linezolid

Linezolid is useful for the treatment of vancomycin-resistant enterococci, methicillin-resistant *S. aureus* and *S. epidermidis,* and penicillin-resistant pneumococci. It is bacteriostatic against enterococci and staphylococci but bactericidal against pneumococci.

Linezolid binds to the 23S ribosomal RNA in the 50S subunit and inhibits protein synthesis, but the precise mechanism is unknown. It appears to block some early step (initiation) in ribosome formation. Tedizolid is a second-generation drug in the same class as linezolid but is approximately 10 times more effective. It is used for the treatment of skin and soft tissue infections caused by a similar range of bacteria as linezolid and has a similar mechanism of action.

Telithromycin

Telithromycin (Ketek) is the first clinically useful member of the ketolide group of antibiotics. It is similar to the macrolides in general structure and mode of action but is sufficiently different chemically such that organisms resistant to macrolides may be sensitive to telithromycin. It has a wide spectrum of activity against a variety of gram-positive and gram-negative bacteria (including macrolide-resistant pneumococci) and is used in the treatment of community-acquired pneumonia, bronchitis, and sinusitis.

Streptogramins

A combination of two streptogramins, quinupristin and dalfopristin (Synercid), is used for the treatment of bloodstream infections caused by vancomycin-resistant *Enterococcus faecium* (but not vancomycin-resistant *Enterococcus faecalis*). It is also approved for use in infections caused by *Streptococcus pyogenes,* penicillin-resistant *S. pneumoniae,* methicillin-resistant *S. aureus,* and methicillin-resistant *S. epidermidis.*

Streptogramins cause premature release of the growing peptide chain from the 50S ribosomal subunit. The structure and mode of action of streptogramins are different from all other drugs that inhibit protein synthesis, and there is no cross-resistance between streptogramins and these other drugs.

Retapamulin

Retapamulin (Altabax) is the first clinically available member of a new class of antibiotics called pleuromutilins. These drugs inhibit bacterial protein synthesis by binding to the 23S RNA of the 50S subunit and blocking attachment of the donor tRNA. Retapamulin is a topical antibiotic used in the treatment of skin infections, such as impetigo, caused by *S. pyogenes* and methicillin-sensitive *S. aureus.*

INHIBITION OF NUCLEIC ACID SYNTHESIS

The mode of action and clinically useful activity of the important drugs that act by inhibiting nucleic acid synthesis are summarized in Table 10–7.

1. Inhibition of Precursor Synthesis

Sulfonamides

Either alone or in combination with trimethoprim, sulfonamides are useful in a variety of bacterial diseases such as urinary tract infections caused by *E. coli,* otitis media caused by *S. pneumoniae* or *H. influenzae* in children, shigellosis, nocardiosis, and chancroid. In combination, they are also the drugs of choice for two additional diseases, toxoplasmosis and *Pneumocystis* pneumonia. The sulfonamides are a large family of bacteriostatic drugs that are produced by chemical synthesis. In 1935, the parent compound, sulfanilamide, became the first clinically effective antimicrobial agent.

The mode of action of sulfonamides is to block the synthesis of tetrahydrofolic acid, which is required as a methyl donor in the synthesis of the nucleic acid precursors adenine, guanine, and thymine. Sulfonamides are **structural analogues of *p*-aminobenzoic acid** (PABA). PABA condenses with a pteridine compound to form dihydropteroic acid, a precursor of tetrahydrofolic acid (Figure 10–10). Sulfonamides compete with PABA for the active site of the enzyme dihydropteroate synthetase. This competitive inhibition can be overcome by an excess of PABA.

The basis of the selective action of sulfonamides on bacteria is that many bacteria synthesize their folic acid from PABA-containing precursors, whereas human cells require preformed folic acid as an exogenous nutrient because they lack the enzymes to synthesize it. Human cells therefore bypass the step at which sulfonamides act. Bacteria that can use preformed folic acid are similarly resistant to sulfonamides.

The *p*-amino group on the sulfonamide is essential for its activity. Modifications are therefore made on the sulfonic acid side chain. Sulfonamides are inexpensive and infrequently cause side effects. However, drug-related fever, rashes, photosensitivity (rash upon exposure to sunlight), and bone marrow suppression can occur. They are the most common group of drugs that cause erythema multiforme and its more severe forms, Stevens-Johnson syndrome and toxic epidermal necrolysis.

Trimethoprim

Trimethoprim also inhibits the production of tetrahydrofolic acid but by a mechanism different from that of the sulfonamides (i.e., it inhibits the enzyme **dihydrofolate reductase**) (see Figure 10–10). Its specificity for bacteria is based on its much greater affinity for bacterial reductase than for the human enzyme.

Trimethoprim is used most frequently together with sulfamethoxazole. Note that both drugs act on the same pathway—but at different sites—to inhibit the synthesis of tetrahydrofolate. The advantages of the combination are that (1) bacterial mutants resistant to one drug will be inhibited by the other and (2) the two drugs can act **synergistically** (i.e., when used together, they cause significantly greater inhibition than the sum of the inhibition caused by each drug separately).

Trimethoprim-sulfamethoxazole is clinically useful in the treatment of urinary tract infections, *Pneumocystis* pneumonia, and shigellosis. It also is used for prophylaxis in granulopenic patients to prevent opportunistic infections.

2. Inhibition of DNA Synthesis

Fluoroquinolones

Fluoroquinolones are bactericidal drugs that block bacterial DNA synthesis by inhibiting **DNA gyrase (topoisomerase)**. Fluoroquinolones, such as ciprofloxacin (Figure 10–11), levofloxacin, norfloxacin, ofloxacin, and others, are active against a broad range of organisms that cause infections of the lower

TABLE 10–7 Mode of Action and Activity of Selected Nucleic Acid Inhibitors[1]

Drug	Mode of Action	Clinically Useful Activity
Sulfonamides (e.g., sulfamethoxazole)	Inhibit folic acid synthesis; act as a competitive inhibitor of PABA	Used in combination with trimethoprim for UTI caused by *Escherichia coli*; otitis media and sinusitis caused by *Haemophilus influenzae*; MRSA; *Pneumocystis* pneumonia
Trimethoprim	Inhibits folic acid synthesis by inhibiting DHFR	Used in combination with sulfonamides for the uses described above
Fluoroquinolones (e.g., ciprofloxacin, levofloxacin)	Inhibit DNA synthesis by inhibiting DNA gyrase	Ciprofloxacin is used to treat GI tract infections caused by *Shigella* and *Salmonella*, and UTI caused by enteric gram-negative rods. Levofloxacin is used to treat respiratory tract infections, especially those caused by penicillin-resistant *Streptococcus pneumoniae*
Rifampin	Inhibits mRNA synthesis by inhibiting RNA polymerase	Used in combination with isoniazid and other drugs to treat tuberculosis

DHFR = dihydrofolate reductase; GI = gastrointestinal; MRSA = methicillin-resistant *Staphylococcus aureus*; PABA = para-aminobenzoic acid; UTI = urinary tract infection.

[1]The spectrum of activity is intentionally incomplete. It is simplified for the beginning student to emphasize the most common uses.

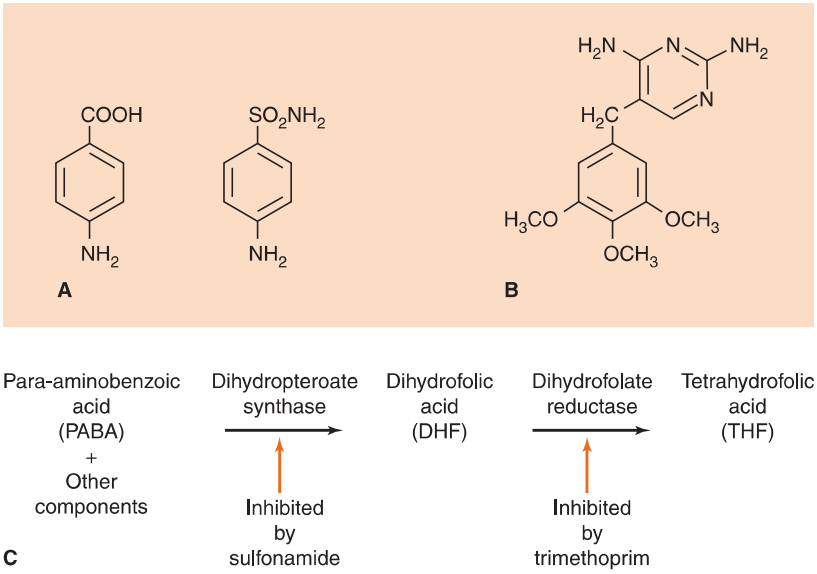


FIGURE 10–10 Mechanism of action of sulfonamides and trimethoprim. **A:** Comparison of the structures of *p*-aminobenzoic acid (PABA) and sulfanilamide. Note that the only difference is that PABA has a carboxyl (COOH) group, whereas sulfanilamide has sulfonamide (SO_2NH_2) group. **B:** Structure of trimethoprim. **C:** Inhibition of the folic acid pathway by sulfonamide and trimethoprim. Sulfonamides inhibit the synthesis of dihydrofolic acid (DHF) from its precursor PABA. Trimethoprim inhibits the synthesis of tetrahydrofolic acid (THF) from its precursor DHF. Loss of THF inhibits DNA synthesis because THF is required to transfer a methyl group onto uracil to produce thymidine, an essential component of DNA. (Adapted from Corcoran JW, Hahn FE, eds. *Mechanism of Action of Antimicrobial Agents. Vol. 3 of Antibiotics*. Springer-Verlag; 1975.)

respiratory tract, intestinal tract, urinary tract, and skeletal and soft tissues. Nalidixic acid, which is a quinolone but not a fluoroquinolone, is much less active and is used only for the treatment of urinary tract infections. Fluoroquinolones should not be given to pregnant women and children under the age of 18 years because they damage growing bone and cartilage.

The FDA has issued a warning regarding the possibility of Achilles tendonitis and tendon rupture associated with fluoroquinolone use, especially in those over 60 years of age and in patients receiving corticosteroids, such as prednisone. In view of this, the FDA recommends that fluoroquinolones *not* be used in the treatment of acute sinusitis and uncomplicated urinary tract infections. Another important adverse effect of fluoroquinolones is peripheral neuropathy, the symptoms of which include pain, burning, numbness, or tingling in the arms or legs.

3. Inhibition of mRNA Synthesis

Rifampin is used primarily for the treatment of tuberculosis in combination with other drugs and for prophylaxis in close contacts of patients with meningitis caused by either *N. meningitidis* or *H. influenzae*. It is also used in combination with other drugs in the treatment of prosthetic-valve endocarditis caused by *S. epidermidis*. With the exception of the short-term prophylaxis of meningitis, rifampin is given in combination with other drugs because resistant mutants appear at a high rate when it is used alone.

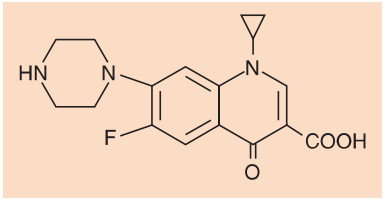


FIGURE 10–11 Ciprofloxacin. The triangle indicates a cyclopropyl group.

The selective mode of action of rifampin is based on **blocking mRNA synthesis** by bacterial RNA polymerase without affecting the RNA polymerase of human cells. Rifampin is red, and the urine, saliva, and sweat of patients taking rifampin often turn orange; this is disturbing but harmless. Rifampin is excreted in high concentration in saliva, which accounts for its success in the prophylaxis of bacterial meningitis since the organisms are carried in the throat.

Rifabutin, a rifampin derivative with the same mode of action as rifampin, is useful in the prevention of disease caused by *Mycobacterium avium-intracellulare* in patients with severely reduced numbers of helper T cells (e.g., acquired immunodeficiency syndrome [AIDS] patients). Note that rifabutin does not increase cytochrome P450 as much as rifampin, so rifabutin is used in human immunodeficiency virus (HIV)/AIDS patients taking protease inhibitors or non-reverse transcriptase inhibitors (NRTI).

Fidaxomicin (Dificid) inhibits the RNA polymerase of *C. difficile*. It is used in the treatment of pseudomembranous colitis and in preventing relapses of this disease. It specifically inhibits *C. difficile* and does not affect the gram-negative normal flora of the colon.

ALTERATION OF CELL MEMBRANE FUNCTION

There are few antimicrobial compounds that act on the cell membrane because the structural and chemical similarities of bacterial and human cell membranes make it difficult to provide sufficient selective toxicity.

Polymyxins are a family of polypeptide antibiotics of which the clinically most useful compound is polymyxin E (colistin). Colistin is active against gram-negative rods, especially *P. aeruginosa, Acinetobacter baumannii,* and carbapenemase-producing Enterobacteriaceae. Most strains of these highly antibiotic-resistant bacteria are sensitive to colistin, although rare isolates from patients are resistant.

Polymyxins are cyclic peptides composed of 10 amino acids, six of which are diaminobutyric acid. The positively charged free amino groups act like a cationic detergent to disrupt the phospholipid structure of the cell membrane.

Daptomycin is a cyclic lipopeptide that disrupts the cell membranes of gram-positive cocci. It is bactericidal for organisms such as *S. aureus, S. epidermidis, S. pyogenes, E. faecalis,* and *E. faecium,* including methicillin-resistant strains of *S. aureus* and *S. epidermidis,* vancomycin-resistant strains of *S. aureus*, and vancomycin-resistant strains of *E. faecalis* and *E. faecium.* It is approved for use in complicated skin and soft tissue infections caused by these bacteria.

ADDITIONAL DRUG MECHANISMS

Isoniazid, or isonicotinic acid hydrazide (INH), is a bactericidal drug highly specific for *Mycobacterium tuberculosis* and other mycobacteria. It is used in combination with other drugs to treat tuberculosis and by itself to prevent tuberculosis in exposed persons. Because it penetrates human cells well, it is effective against the organisms residing within macrophages. The structure of isoniazid is shown in Figure 10–12.

INH **inhibits mycolic acid synthesis,** which explains why it is specific for mycobacteria and relatively nontoxic for humans. The drug inhibits a reductase required for the synthesis of the long-chain fatty acids called mycolic acids that are an essential constituent of mycobacterial cell walls. The active drug is probably a metabolite of INH formed by the action of catalase peroxidase because deletion of the gene for these enzymes results in resistance to the drug. Its main side effect is liver toxicity. It is given with pyridoxine to prevent neurologic complications.

Metronidazole (Flagyl) is bactericidal against anaerobic bacteria such as *B. fragilis* and *C. difficile*, and it is used to treat bacterial vaginosis. (It is also effective against certain protozoa such as *Giardia* and *Trichomonas.*) Metronidazole is a prodrug

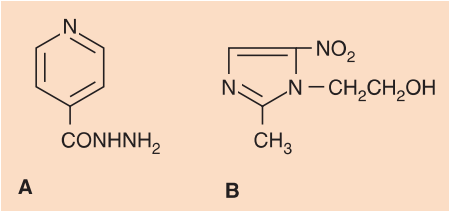


FIGURE 10–12 **A:** Isoniazid. **B:** Metronidazole.

that is activated to the active compound within anaerobic bacteria by ferredoxin-mediated reduction of its nitro group.

This drug has two possible mechanisms of action, and it is unclear which is more important. The first, which explains its specificity for anaerobes, is its ability to act as an **electron sink.** By accepting electrons, the drug deprives the organism of required reducing power. In addition, when electrons are acquired, the drug ring is cleaved and a toxic intermediate is formed that damages DNA. The precise nature of the intermediate and its action is unknown. The structure of metronidazole is shown in Figure 10–12.

The second mode of action of metronidazole relates to its ability to inhibit DNA synthesis. The drug binds to DNA and causes strand breakage, which prevents its proper functioning as a template for DNA polymerase.

Nitrofurantoin is a urinary tract antiseptic that is useful in the treatment of uncomplicated lower urinary tract infections. It is concentrated in the urine to reach bactericidal levels but does not reach cidal levels systemically so is not useful for infections outside the urinary tract.

Nitrofurantoin acts by binding to DNA. Its selective toxicity for bacteria is dependent upon the ability of bacteria to form larger amounts of the highly reactive reduced form of the drug compared to the amount formed in human cells.

Ethambutol is a bacteriostatic drug used in the treatment of infections caused by *M. tuberculosis* and many of the atypical mycobacteria. It acts by inhibiting the synthesis of arabinogalactan, which functions as a link between the mycolic acids and the peptidoglycan of the organism.

Pyrazinamide (PZA) is a bactericidal drug used in the treatment of tuberculosis but not in the treatment of most atypical mycobacterial infections. PZA is particularly effective against semidormant organisms in the lesion, which are not affected by INH or rifampin. The mechanism of action of PZA is uncertain, but there is evidence that it acts by inhibiting a fatty acid synthetase that prevents the synthesis of mycolic acid. It is converted to the active intermediate, pyrazinoic acid, by an amidase in the mycobacteria.

CHEMOPROPHYLAXIS

In most instances, the antimicrobial agents described in this chapter are used for the *treatment* of infectious diseases. However, there are times when they are used to *prevent* diseases from occurring—a process called **chemoprophylaxis.**

Chemoprophylaxis is used in three circumstances: prior to surgery, in immunocompromised patients, and in people with normal immunity who have been exposed to certain pathogens. Table 10–8 describes the drugs and the situations in which they

TABLE 10–8 Chemoprophylactic Use of Drugs Described in This Chapter

Drug	Use	Number of Chapter(s) for Additional Information
Penicillin	1. Prevent recurrent pharyngitis in high-risk patients who have had rheumatic fever 2. Prevent syphilis in high-risk patients exposed to *Treponema pallidum* 3. Prevent pneumococcal sepsis in splenectomized young children	15 24 15
Ampicillin	Prevent neonatal sepsis and meningitis in children born of mothers carrying group B streptococci	15
Amoxicillin	Prevent endocarditis caused by viridans streptococci in high-risk patients with damaged heart valves undergoing dental surgery	15
Cefazolin	Prevent staphylococcal surgical wound infections	15
Ceftriaxone	Prevent gonorrhea in high-risk patients exposed to *Neisseria gonorrhoeae*	16
Ciprofloxacin	1. Prevent meningitis in high-risk patients exposed to *Neisseria meningitidis* 2. Prevent anthrax in high-risk patients exposed to *Bacillus anthracis* 3. Prevent infection in neutropenic patients	16 17 68
Rifampin	Prevent meningitis in high-risk patients exposed to *N. meningitidis* and *Haemophilus influenzae*	16, 19
Isoniazid	Prevent progression of *Mycobacterium tuberculosis* in high-risk patients recently infected who are asymptomatic[1]	21
Erythromycin	1. Prevent pertussis in high-risk patients exposed to *Bordetella pertussis* 2. Prevent gonococcal and chlamydial conjunctivitis in newborns	19 16, 25
Tetracycline	Prevent plague in high-risk patients exposed to *Yersinia pestis*	20
Trimethoprim-sulfamethoxazole	Prevent recurrent urinary tract infections	18

[1]Chemoprophylaxis with isoniazid is also viewed as treatment of asymptomatic individuals (see Chapter 21).

are used. For more information, see the chapters on the individual organisms.

Of particular importance is the **prevention of endocarditis in high-risk patients undergoing dental surgery** by using amoxicillin perioperatively. High-risk patients include those who have unrepaired damage to their heart valve, have a prosthetic heart valve, or have previously had infective endocarditis. Prophylaxis to prevent endocarditis in patients undergoing gastrointestinal or genitourinary tract surgery is not recommended.

Cefazolin is often used to prevent staphylococcal infections in patients undergoing orthopedic surgery, including prosthetic joint implants, and in vascular graft surgery. Chemoprophylaxis is unnecessary in those with an implanted dialysis catheter, a cardiac pacemaker, or a ventriculoperitoneal shunt.

PROBIOTICS

In contrast to the chemical antibiotics previously described in this chapter, probiotics are live, nonpathogenic bacteria (or yeasts) that may be effective in the treatment or prevention of certain human diseases. The suggested basis for the possible beneficial effect lies either in providing colonization resistance by which the nonpathogen excludes the pathogen from binding sites on the mucosa, in enhancing the immune response against the pathogen, or in reducing the inflammatory response against the pathogen. For example, the oral administration of live *Lactobacillus rhamnosus* strain GG significantly reduces the number of cases of nosocomial diarrhea in young children. Also, the yeast *Saccharomyces boulardii* reduces the risk of antibiotic-associated diarrhea caused by *C. difficile*. Adverse effects are few; however, serious complications have arisen in highly immunosuppressed patients and in patients with indwelling vascular catheters.

PEARLS

- For an antibiotic to be clinically useful, it must exhibit **selective toxicity** (i.e., it must inhibit bacterial processes significantly more than it inhibits human cell processes).
- There are four main targets of antibacterial drugs: **cell wall, ribosomes, cell membrane,** and **nucleic acids.** Human cells are not affected by these drugs because our cells do not have a cell wall, and our cells have different ribosomes, nucleic acid enzymes, and sterols in the membranes.
- **Bactericidal** drugs kill bacteria, whereas **bacteriostatic** drugs inhibit the growth of the bacteria but do not kill. Bacteriostatic drugs depend on the phagocytes of the patient to kill the organism. If a patient has too few neutrophils, then bactericidal drugs should be used.

Inhibition of Cell Wall Synthesis

- **Penicillins** and **cephalosporins** act by inhibiting **transpeptidases,** the enzymes that cross-link peptidoglycan. Transpeptidases are also referred to as **penicillin-binding proteins.** Several medically important bacteria (e.g., *S. pneumoniae*) manifest resistance to penicillins based on mutations in the genes encoding penicillin-binding proteins.
- Exposure to penicillins activates **autolytic enzymes** that degrade the bacteria. If these autolytic enzymes are not activated (e.g., in certain strains of *S. aureus*), the bacteria are not killed and the strain is said to be **tolerant.**
- Penicillins kill bacteria when they are growing (i.e., when they are synthesizing new peptidoglycan). Penicillins are therefore **more active during the log phase** of bacterial growth than during the lag phase or the stationary phase.
- Penicillins and cephalosporins are **β-lactam drugs** (i.e., an intact **β-lactam ring** is required for activity). **β-Lactamases** (e.g., penicillinases and cephalosporinases) cleave the β-lactam ring and inactivate the drug.
- **Modification of the side chain** adjacent to the β-lactam ring endows these drugs with **new properties,** such as expanded activity against gram-negative rods, ability to be taken orally, and protection against degradation by β-lactamases. For example, the original penicillin (benzyl penicillin, penicillin G) cannot be taken orally because stomach acid hydrolyzes the bond between the β-lactam ring and the side chain. But ampicillin and amoxicillin can be taken orally because they have a different side chain.
- **Hypersensitivity** to penicillins, especially **IgE-mediated anaphylaxis,** remains a significant concern.
- **Cephalosporins** are structurally similar to penicillins: both have a β-lactam ring. The first-generation cephalosporins are active primarily against gram-positive cocci, and the second, third, and fourth generations have expanded coverage against gram-negative rods.
- Carbapenems, such as imipenem, and monobactams, such as aztreonam, are also β-lactam drugs but are structurally different from penicillins and cephalosporins.
- **Vancomycin** is a **glycopeptide** (i.e., it is not a β-lactam drug), but its mode of action is very similar to that of penicillins and cephalosporins (i.e., it **inhibits transpeptidases**).

Inhibition of Protein Synthesis

- **Aminoglycosides** and **tetracyclines** act at the level of the 30S ribosomal subunit, whereas **chloramphenicol, erythromycins,** and **clindamycin** act at the level of the 50S ribosomal subunit.
- **Aminoglycosides** inhibit bacterial protein synthesis by binding to the 30S subunit, which **blocks the initiation complex.** No peptide bonds are formed, and no polysomes are made. Aminoglycosides are a family of drugs that includes gentamicin, tobramycin, and streptomycin.
- **Tetracyclines** inhibit bacterial protein synthesis by **blocking the binding of aminoacyl tRNA** to the 30S ribosomal subunit. The tetracyclines are a family of drugs; doxycycline is used most often.
- **Chloramphenicol** inhibits bacterial protein synthesis by **blocking peptidyl transferase,** the enzyme that adds the new amino acid to the growing polypeptide. Chloramphenicol can cause bone marrow suppression.
- **Erythromycin** inhibits bacterial protein synthesis by **blocking the release of the tRNA** after it has delivered its amino acid to the growing polypeptide. Erythromycin is a member of the macrolide family of drugs that includes azithromycin and clarithromycin.
- **Clindamycin** binds to the same site on the ribosome as does erythromycin and is thought to act in the same manner. It is effective against many anaerobic bacteria. Clindamycin is one of the antibiotics that predisposes to pseudomembranous colitis caused by *C. difficile* and is infrequently used.

Inhibition of Nucleic Acid Synthesis

- **Sulfonamides** and **trimethoprim** inhibit **nucleotide** synthesis, **quinolones** inhibit **DNA** synthesis, and **rifampin** inhibits **RNA** synthesis.
- **Sulfonamides** and **trimethoprim** inhibit the **synthesis of tetrahydrofolic acid—**the main donor of the methyl groups that are required to synthesize adenine, guanine, and thymine. **Sulfonamides** are structural analogues of *p*-aminobenzoic acid, which is a component of folic acid. **Trimethoprim** inhibits **dihydrofolate reductase—**the enzyme that reduces dihydrofolic acid to tetrahydrofolic acid. A combination of sulfamethoxazole and trimethoprim is often used because bacteria resistant to one drug will be inhibited by the other.
- **Quinolones** inhibit DNA synthesis in bacteria by **blocking DNA gyrase** (topoisomerase)—the enzyme that unwinds DNA

CHAPTER

18

Gram-Negative Rods Related to the Enteric Tract

CHAPTER CONTENTS

INTRODUCTION

Gram-negative rods are a large group of diverse organisms (Figures 18–1, 18–2, and 19–1). In this book, these bacteria are subdivided into three clinically relevant categories, each in a separate chapter, according to whether the organism is related primarily to the enteric or the respiratory tract or to animal sources (Table 18–1). Although this approach leads to some overlap, it should be helpful because it allows general concepts to be emphasized.

Gram-negative rods related to the enteric tract include a large number of genera. These genera have therefore been divided into three groups depending on the major anatomic location of disease, namely, (1) pathogens both within and outside the enteric tract, (2) pathogens primarily within the enteric tract, and (3) pathogens outside the enteric tract (see Table 18–1).

The frequency with which the organisms related to the enteric tract cause disease in the United States is shown in Table 18–2. *Salmonella, Shigella*, and *Campylobacter* are frequent pathogens in the gastrointestinal tract, whereas *Escherichia, Vibrio*, and *Yersinia* are less so. Enterotoxigenic strains of *Escherichia coli* are a common cause of diarrhea in developing countries but are less common in the United States. The medically important gram-negative rods that cause diarrhea are described in Table 18–3. Urinary tract infections are caused primarily by *E. coli;* the other organisms occur less commonly. The medically important gram-negative rods that cause urinary tract infections are described in Table 18–4.

Additional information regarding the clinical aspects of infections caused by the organisms in this chapter is provided in Part IX entitled Infectious Diseases beginning on page 589.

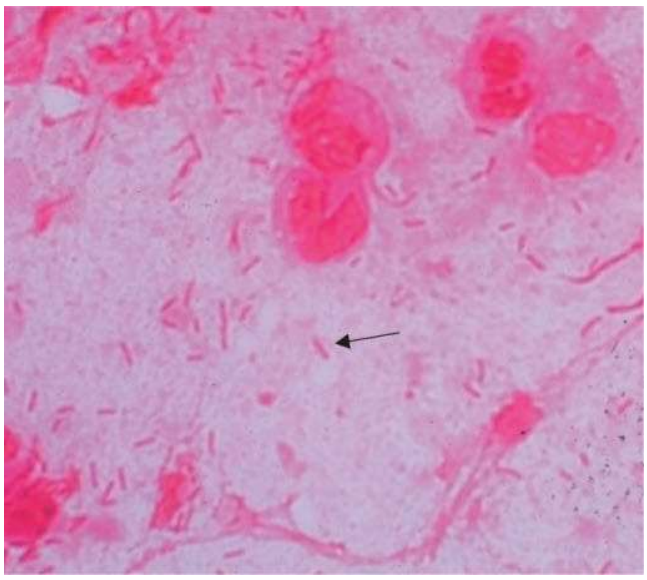


FIGURE 18–1 *Escherichia coli*—Gram stain. Arrow points to a gram-negative rod. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

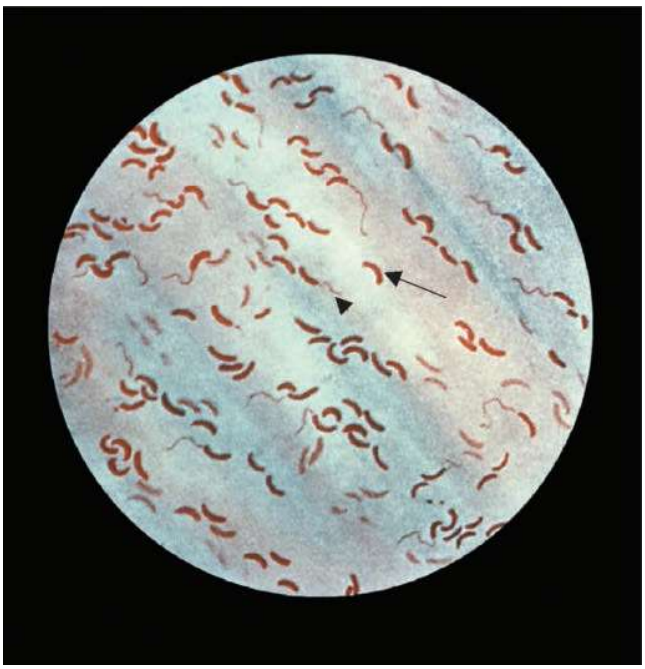


FIGURE 18–2 *Vibrio cholerae*—Gram stain. Long arrow points to a curved gram-negative rod. Arrowhead points to a flagellum at one end of a curved gram-negative rod. (Source: Public Health Image Library, Centers for Disease Control and Prevention.)

Patients infected with such enteric pathogens as *Shigella, Salmonella, Campylobacter*, and *Yersinia* have a high incidence of certain autoimmune diseases such as reactive arthritis and Reiter syndrome (see Chapter 66). In addition, infection with *Campylobacter jejuni* predisposes to Guillain-Barré syndrome.

Before describing the specific organisms, it is appropriate to describe the family Enterobacteriaceae, to which many of these gram-negative rods belong.

TABLE 18–1 Categories of Gram-Negative Rods

Chapter	Source of Site of Infection	Genus
18	Enteric tract	
	1. Both within and outside	*Escherichia, Salmonella*
	2. Primarily within	*Shigella, Vibrio, Campylobacter, Helicobacter*
	3. Outside only	*Klebsiella–Enterobacter–Serratia* group, *Proteus–Providencia–Morganella* group, *Pseudomonas, Bacteroides, Prevotella, Fusobacterium*
19	Respiratory tract	*Haemophilus, Legionella, Bordetella*
20	Animal sources	*Brucella, Francisella, Pasteurella, Yersinia*

TABLE 18–2 Frequency of Diseases Caused in the United States by Gram-Negative Rods Related to the Enteric Tract

Site of Infection	Frequent Pathogens	Less Frequent Pathogens
Enteric tract	*Salmonella, Shigella, Campylobacter*	*Escherichia, Vibrio, Yersinia*
Urinary tract	*Escherichia*	*Enterobacter, Klebsiella, Proteus, Pseudomonas*

ENTEROBACTERIACEAE & RELATED ORGANISMS

The Enterobacteriaceae is a large family of gram-negative rods found primarily in the colon of humans and other animals, many as part of the normal flora. These organisms are the major facultative anaerobes in the large intestine but are present in relatively small numbers compared with anaerobes such as *Bacteroides*. Although the members of the Enterobacteriaceae are classified together taxonomically, they cause a variety of diseases with different pathogenetic mechanisms. The organisms and some of the diseases they cause are listed in Table 18–5.

Features common to all members of this heterogeneous family are their anatomic location and the following four metabolic processes: (1) they are all facultative anaerobes; (2) they all ferment glucose (fermentation of other sugars varies); (3) none have cytochrome oxidase (i.e., they are oxidase-negative); and (4) they reduce nitrates to nitrites as part of their energy-generating processes.

These four reactions can be used to distinguish the Enterobacteriaceae from another medically significant group of organisms—the nonfermenting gram-negative rods, the most important of which is *Pseudomonas aeruginosa*.[1]

Pseudomonas aeruginosa, a significant cause of urinary tract infection and sepsis in hospitalized patients, does not ferment glucose or reduce nitrates and is oxidase-positive. In contrast to the Enterobacteriaceae, it is a strict aerobe and derives its energy from oxidation, not fermentation.

Pathogenesis

All members of the Enterobacteriaceae, being gram-negative, contain endotoxin in their cell walls. In addition, several exotoxins are produced (e.g., *E. coli* and *Vibrio cholerae* secrete exotoxins, called *enterotoxins*, that activate adenylate cyclase within the cells of the small intestine, causing diarrhea) (see Chapter 7). In addition, *E. coli* O157 produces Shiga toxin that causes hemolytic-uremic syndrome.

[1]The other less frequently isolated organisms in this group are members of the following genera: *Achromobacter, Alcaligenes, Eikenella, Flavobacterium, Kingella,* and *Moraxella* (see Chapter 27) and Acinetobacter (see Chapter 19).

TABLE 18–3 Gram-Negative Rods Causing Diarrhea

Species	Fever	Leukocytes in Stool	Infective Dose	Typical Bacteriologic or Epidemiologic Findings
Enterotoxin-mediated				
1. *Escherichia coli*	–	–	?	Ferments lactose
2. *Vibrio cholerae*	–	–	10^7	Comma-shaped bacteria
Invasive-inflammatory				
1. *Salmonella* (e.g., *S. enterica)*	+	+	10^5	Does not ferment lactose
2. *Shigella* (e.g., *S. dysenteriae*)	+	+	10^2	Does not ferment lactose
3. *Campylobacter jejuni*	+	+	10^4	Comma- or S-shaped bacteria; growth at 42°C
4. *E. coli* (enteropathic strains)	+	+	?	
5. *E. coli* O157:H7	+	+/–	?	Transmitted by undercooked hamburger; causes hemolytic-uremic syndrome
Mechanism uncertain				
1. *Vibrio parahaemolyticus*[1]	+	+	?	Transmitted by seafood
2. *Yersinia enterocolitica*[1]	+	+	10^8	Usually transmitted from pets (e.g., puppies)

[1]Some strains produce enterotoxin, but its pathogenic role is not clear.

TABLE 18–4 Gram-Negative Rods Causing Urinary Tract Infection[1] or Sepsis[2]

Species	Lactose Fermented	Features of the Organism
Escherichia coli	+	Colonies show green sheen on EMB agars
Enterobacter cloacae	+	Causes nosocomial infections and often drug-resistant
Klebsiella pneumoniae	+	Has large mucoid capsule and hence viscous colonies
Serratia marcescens	–	Red pigment produced; causes nosocomial infections and often drug resistant
Proteus mirabilis	–	Motility causes "swarming" on agar; produces urease
Pseudomonas aeruginosa	–	Blue-green pigment and fruity odor produced; causes nosocomial infections and often drug-resistant

EMB = eosin-methylene blue.

[1]Diagnosed by quantitative culture of urine.

[2]Diagnosed by culture of blood or pus.

TABLE 18–5 Diseases Caused by Members of the Enterobacteriaceae

Major Pathogen	Representative Diseases	Minor Related Genera
Escherichia	Urinary tract infection, traveler's diarrhea, neonatal meningitis	
Shigella	Dysentery	
Salmonella	Typhoid fever, enterocolitis	*Arizona, Citrobacter, Edwardsiella*
Klebsiella	Pneumonia, urinary tract infection	
Enterobacter	Pneumonia, urinary tract infection	*Hafnia*
Serratia	Pneumonia, urinary tract infection	
Proteus	Urinary tract infection	*Providencia, Morganella*
Yersinia	Plague, enterocolitis, mesenteric adenitis	

Antigens

The antigens of several members of the Enterobacteriaceae, especially *Salmonella* and *Shigella*, are important; they are used for identification purposes both in the clinical laboratory and in epidemiologic investigations. The three surface antigens are as follows:

(1) The cell wall antigen (also known as the somatic, or O, antigen) is the outer polysaccharide portion of the lipopolysaccharide (see Figure 2–6). The O antigen, which is composed of repeating oligosaccharides consisting of three or four sugars repeated 15 or 20 times, is the basis for the serologic typing of many enteric rods. The number of different O antigens is very large (e.g., there are approximately 1,500 types of *Salmonella* and 150 types of *E. coli*).

(2) The H antigen is on the flagellar protein. Only flagellated organisms, such as *Escherichia* and *Salmonella*, have H antigens, whereas the nonmotile ones, such as *Klebsiella* and *Shigella*, do not. The H antigens of certain *Salmonella* species are unusual because the organisms can reversibly alternate between two types of H antigens called phase 1 and phase 2. The organisms may use this change in antigenicity to evade the immune response.

(3) The capsular or K polysaccharide antigen is particularly prominent in heavily encapsulated organisms such as *Klebsiella*. The K antigen is identified by the quellung (capsular swelling) reaction in the presence of specific antisera and is used to serotype *E. coli* and *Salmonella typhi* for epidemiologic purposes. In *S. typhi*, the cause of typhoid fever, it is called the Vi (or virulence) antigen.

Laboratory Diagnosis

Specimens suspected of containing members of the Enterobacteriaceae and related organisms are usually inoculated onto two media, a blood agar plate and a selective differential medium such as MacConkey's agar or eosin-methylene blue (EMB) agar. The *differential* ability of these latter media is based on **lactose fermentation,** which is the most important metabolic criterion used in the identification of these organisms (Table 18–6). On these media, the non–lactose fermenters (e.g., *Salmonella* and *Shigella*) form colorless colonies, whereas the lactose fermenters (e.g., *E. coli*) form colored colonies. On EMB agar, *E. coli* colonies have a characteristic **green sheen.** The *selective* effect of the media in suppressing unwanted gram-positive organisms is exerted by bile salts or bacteriostatic dyes in the agar.

An additional set of screening tests, consisting of triple sugar iron (TSI) agar and urea agar, is performed prior to the definitive identification procedures. The rationale for the use of these media and the reactions of several important organisms are presented in the box titled "Agar Media for Enteric Gram-Negative Rods" and in Table 18–7. The results of the screening process are often sufficient to identify the genus of an organism; however, an array of 20 or more biochemical tests is required to identify the species.

TABLE 18–6 Lactose Fermentation by Members of the Enterobacteriaceae and Related Organisms

Lactose Fermentation	Organisms
Occurs	*Escherichia, Klebsiella, Enterobacter*
Does not occur	*Shigella, Salmonella, Proteus, Pseudomonas*
Occurs slowly	*Serratia, Vibrio*

TABLE 18–7 Triple Sugar Iron (TSI) Agar Reactions

Reactions[1]				
Slant	Butt	Gas	H_2S	Representative Genera
Acid	Acid	+	–	*Escherichia, Enterobacter, Klebsiella*
Alkaline	Acid	–	–	*Shigella, Serratia*
Alkaline	Acid	+	+	*Salmonella, Proteus*
Alkaline	Alkaline	–	–	*Pseudomonas*[2]

[1]Acid production causes the phenol red indicator to turn yellow; the indicator is red under alkaline conditions. The presence of black FeS in the butt indicates H_2S production. Not every species within the various genera will give the above appearance on TSI agar. For example, some *Serratia* strains can ferment lactose slowly and give an acid reaction on the slant.

[2]*Pseudomonas,* although not a member of the Enterobacteriaceae, is included in this table because its reaction on TSI agar is a useful diagnostic criterion.

Another valuable piece of information used to identify some of these organisms is their motility, which is dependent on the presence of flagella. *Proteus* species are very motile and characteristically **swarm** over the blood agar plate, obscuring the colonies of other organisms. Motility is also an important diagnostic criterion in the differentiation of *Enterobacter cloacae*, which is motile, from *K. pneumoniae*, which is nonmotile.

If the results of the screening tests suggest the presence of a *Salmonella* or *Shigella* strain, an agglutination test can be used to identify the genus of the organism and to determine whether it is a member of group A, B, C, or D.

Coliforms & Public Health

Contamination of the public water supply system by sewage is detected by the presence of coliforms in the water. In a general sense, the term *coliform* includes not only *E. coli* but also other inhabitants of the colon such as *Enterobacter* and *Klebsiella*. However, because only *E. coli* is exclusively a large intestine organism, whereas the others are found in the environment also, it is used as the indicator of fecal contamination. In water quality testing, *E. coli* is identified by its ability to ferment lactose with the production of acid and gas, its ability to grow at 44.5°C, and its characteristic colony type on EMB agar. An *E. coli* colony count above 4/dL in municipal drinking water is indicative of unacceptable fecal contamination. Because *E. coli* and the enteric pathogens are killed by chlorination of the drinking water, there is rarely a problem with meeting this standard. Disinfection of the public water supply is one of the most important advances of public health in the twentieth century.

Antibiotic Therapy

The appropriate treatment for infections caused by members of the Enterobacteriaceae and related organisms must be individually tailored to the antibiotic sensitivity of the organism.

AGAR MEDIA FOR ENTERIC GRAM-NEGATIVE RODS

Triple Sugar Iron (TSI) Agar

The important components of this medium are ferrous sulfate and the three sugars glucose, lactose, and sucrose. Glucose is present in one-tenth the concentration of the other two sugars. The medium in the tube has a solid, poorly oxygenated area on the bottom, called the butt, and an angled, well-oxygenated area on top, called the slant. The organism is inoculated into the butt and across the surface of the slant.

The interpretation of the test results is as follows: (1) If lactose (or sucrose) is fermented, a large amount of acid is produced, which turns the phenol red indicator yellow both in the butt and on the slant. Some organisms generate gases, which produce bubbles in the butt. (2) If lactose is not fermented but the small amount of glucose is, the oxygen-deficient butt will be yellow, but on the slant, the acid will be oxidized to CO_2 and H_2O by the organism and the slant will be red (neutral or alkaline). (3) If neither lactose nor glucose is fermented, both the butt and the slant will be red. The slant can become a deeper red-purple (more alkaline) as a result of the production of ammonia from the oxidative deamination of amino acids. (4) If H_2S is produced, the black color of ferrous sulfide is seen.

The reactions of some of the important organisms are presented in Table 18–7. Because several organisms can give the same reaction, TSI agar is only a screening device.

Urea Agar

The important components of this medium are urea and the pH indicator phenol red. If the organism produces urease, the urea is hydrolyzed to NH_3 and CO_2. Ammonia turns the medium alkaline, and the color of the phenol red changes from light orange to reddish purple. The important organisms that are urease-positive are *Proteus* species and *K. pneumoniae*.

Generally speaking, a wide range of antimicrobial agents are potentially effective (e.g., some penicillins and cephalosporins, aminoglycosides, chloramphenicol, tetracyclines, quinolones, and sulfonamides). The specific choice usually depends on the results of antibiotic sensitivity tests.

Note that many isolates of these enteric gram-negative rods are **highly antibiotic resistant** because of the production of β-lactamases, including extended-spectrum β-lactamases (ESBL; see Chapter 11) and other drug-modifying enzymes. These organisms undergo conjugation frequently, at which time they acquire plasmids (R factors) that mediate multiple drug resistance. For example, plasmid-encoded New Delhi metallo-β-lactamase causes resistance to penicillins, cephalosporins, monobactams, and carbapenems.

PATHOGENS BOTH WITHIN & OUTSIDE THE ENTERIC TRACT

ESCHERICHIA

Diseases

Escherichia coli is the most common cause of urinary tract infection and gram-negative rod sepsis. It is one of the two important causes of neonatal meningitis and is the bacterium most frequently associated with "traveler's diarrhea," a watery diarrhea. Some strains of *E. coli* cause bloody diarrhea and hemolytic-uremic syndrome.

Important Properties

Escherichia coli is a straight gram-negative rod (see Figure 18–1), in contrast to the curved gram-negative rods of the genera *Vibrio*, *Campylobacter*, and *Helicobacter*.

Escherichia coli is the most abundant facultative anaerobe in the colon and feces. It is, however, greatly outnumbered by the obligate anaerobes such as *Bacteroides*.

Escherichia coli **ferments lactose,** a property that distinguishes it from the two major intestinal pathogens, *Shigella* and *Salmonella*. It has three antigens that are used to identify the organism in epidemiologic investigations: the O, or cell wall, antigen; the H, or flagellar, antigen; and the K, or capsular, antigen. Because there are more than 150 O, 50 H, and 90 K antigens, the various combinations result in more than 1,000 antigenic types of *E. coli*. Specific serotypes are associated with certain diseases (e.g., O55 and O111 cause outbreaks of neonatal diarrhea).

Pathogenesis

The reservoir of *E. coli* includes both humans and animals. The source of the *E. coli* that causes urinary tract infections is the patient's own colonic flora that colonizes the urogenital area. The source of the *E. coli* that causes neonatal meningitis is the mother's birth canal; the infection is acquired during birth. In contrast, the *E. coli* that causes traveler's diarrhea is acquired by ingestion of food or water contaminated with human feces. Note that the main reservoir of enterohemorrhagic *E. coli* O157 is cattle and the organism is acquired in undercooked beef, for example, hamburgers.

Escherichia coli has several clearly identified components that contribute to its ability to cause disease: pili, a capsule, endotoxin, and three exotoxins (enterotoxins), two that cause watery diarrhea and one that causes bloody diarrhea and hemolytic-uremic syndrome.

Intestinal Tract Infection

The first step is the adherence of the organism to the cells of the jejunum and ileum by means of **pili** that protrude from the bacterial surface. Once attached, the bacteria synthesize **enterotoxins** (exotoxins that act in the enteric tract), which act on the cells of the jejunum and ileum to cause diarrhea. The toxins are strikingly cell-specific; the cells of the colon are not susceptible, probably because they lack receptors for the toxin. Enterotoxigenic strains of *E. coli* (ETEC) can produce either or both of two enterotoxins.

(1) The heat-labile toxin (LT) acts by stimulating **adenylate cyclase.** Both LT and cholera toxin act by catalyzing the addition of adenosine diphosphate-ribose (a process called ADP-ribosylation) to the G protein that stimulates the cyclase. This irreversibly activates the cyclase. The resultant increase in intracellular cyclic adenosine monophosphate (AMP) concentration stimulates cyclic AMP–dependent protein kinase, which phosphorylates ion transporters in the membrane. The transporters export ions, which cause an outpouring of fluid, potassium, and chloride from the enterocytes into the lumen of the gut, resulting in watery diarrhea. Note that cholera toxin has the same mode of action.

(2) The other enterotoxin is a low-molecular-weight, heat-stable toxin (ST), which stimulates guanylate cyclase.

The enterotoxin-producing strains **do not cause inflammation,** do not invade the intestinal mucosa, and cause a watery, nonbloody diarrhea. However, certain strains of *E. coli* are enteropathic (enteroinvasive) and cause disease not by enterotoxin formation but by invasion of the epithelium of the large intestine, causing bloody diarrhea (dysentery) accompanied by inflammatory cells (neutrophils) in the stool.

Certain enterohemorrhagic strains of *E. coli* (i.e., those with the O157:H7 serotype) (Shiga toxin–producing *E. coli* [STEC]) also cause bloody diarrhea by producing an exotoxin called **Shiga toxin,** so called because it is very similar to that produced by *Shigella* species. Shiga toxin acts by removing an adenine from the large (28S) ribosomal RNA, thereby stopping protein synthesis. Shiga toxin is encoded by temperate (lysogenic) bacteriophages. Shiga toxin is also called verotoxin because it has a cytopathic effect on Vero (monkey) cells in culture.

These O157:H7 strains are associated with outbreaks of bloody diarrhea following ingestion of undercooked hamburger, often at fast-food restaurants. The bacteria on the surface of the hamburger are killed by the cooking, but those in the interior, which is undercooked, survive. Also, direct contact with animals (e.g., visits to farms and petting zoos) has resulted in bloody diarrhea caused by O157:H7 strains. *E. coli* O157 has a low ID_{50} of approximately 100 organisms.

Hemolytic-Uremic Syndrome

Some patients with bloody diarrhea caused by O157:H7 strains also have a life-threatening complication called **hemolytic-uremic syndrome (HUS),** which occurs when Shiga toxin enters the bloodstream. This syndrome consists of hemolytic anemia, thrombocytopenia, and acute renal failure. The hemolytic anemia and renal failure occur because there are receptors for Shiga toxin on the surface of the endothelium of small blood vessels and on the surface of kidney epithelium. Death of the endothelial cells of small blood vessels results in a microangiopathic hemolytic anemia in which the red cells passing through the damaged area become grossly distorted (**schistocytes**) and then lyse. Thrombocytopenia occurs because platelets adhere to the damaged endothelial surface. Death of the kidney epithelial cells leads to renal failure. Treatment of diarrhea caused by O157:H7 strains with antibiotics, such as ciprofloxacin, increases the risk of developing HUS by increasing the amount of Shiga toxin released by the dying bacteria.

Urinary Tract Infections

Certain O serotypes of *E. coli* preferentially cause urinary tract infections. These **uropathic** strains are characterized by pili with adhesin proteins that bind to specific receptors on the urinary tract epithelium. The binding site on these receptors consists of dimers of galactose (**Gal-Gal dimers**). These pili are also called P fimbria or pyelonephritis-associated pili (PAP).

Cranberry juice contains flavonoids that inhibit the binding of pili to receptors and may be useful in the prevention of recurrent urinary tract infections. The motility of *E. coli* may aid its ability to ascend the urethra into the bladder and ascend the ureter into the kidney.

Systemic Infection

The other two structural components, the **capsule** and the **endotoxin,** play a more prominent role in the pathogenesis of systemic, rather than intestinal tract, disease. The capsular polysaccharide interferes with phagocytosis, thereby enhancing the organism's ability to cause infections in various organs. For example, *E. coli* strains that cause neonatal meningitis usually have a specific capsular type called the K1 antigen. The endotoxin of *E. coli* is the cell wall lipopolysaccharide, which causes several features of gram-negative sepsis such as fever, hypotension, and disseminated intravascular coagulation.

Th-17 helper T cells that produce interleukin-17 are an important host defense against sepsis caused by enteric bacteria such as *E. coli* and *Klebsiella*. Patients infected with human immunodeficiency virus (HIV) experience a loss of Th-17 cells and are predisposed to sepsis caused by *E. coli* and *Klebsiella*.

Clinical Findings

Escherichia coli causes a variety of diseases both within and outside the intestinal tract. The main clinical findings, the major pathogenetic factors, and the main laboratory results are described in Table 18–8.

TABLE 18–8 Clinical Aspects of *Escherichia coli*

Clinical Finding/Disease	Major Pathogenetic Factor	Main Laboratory Result
Findings within the intestinal tract		
Watery, nonbloody diarrhea (traveler's diarrhea)	Enterotoxin that increases cyclic AMP	No RBC or WBC in stool
Bloody diarrhea caused by *E. coli* O-157; hemolytic-uremic syndrome (HUS)	Shiga toxin (verotoxin) inhibits protein synthesis	RBC in stool; schistocytes in blood smear
Findings outside of intestinal tract		
Urinary tract infection	Gal-gal pili bind to bladder mucosa	WBC in urine, positive urine culture
Neonatal meningitis	K-1 capsular polysaccharide is antiphagocytic	WBC in spinal fluid, positive CSF culture
Sepsis, especially in hospital	Endotoxin induces fever, hypotension, and DIC	Leukocytosis, positive blood culture

AMP = adenosine monophosphate; CSF = cerebrospinal fluid; DIC = disseminated intravascular coagulation; RBC = red blood cell; WBC = white blood cell.

(1) **Clinical findings within the intestinal tract:**

Diarrhea caused by **enterotoxigenic *E. coli* (ETEC)** is usually **watery,** nonbloody, self-limited, and of short duration (1–3 days). It is frequently associated with travel (traveler's diarrhea, or "turista").[2]

Infection with enterohemorrhagic *E. coli* **(EHEC),** on the other hand, results in a dysentery-like syndrome characterized by **bloody diarrhea,** abdominal cramping, and fever similar to that caused by *Shigella*.

The O157:H7 strains of *E. coli* **(STEC)** also cause bloody diarrhea, which can be complicated by **HUS.** This syndrome is characterized by kidney failure, hemolytic anemia, and thrombocytopenia. The hemolytic anemia is caused by exotoxin-induced capillary damage, which results in damage to the red cells as they pass through the capillaries. These distorted, fragmented red cells called **schistocytes** can be seen on blood smear and are characteristic of a microangiopathic hemolytic anemia.

In 2011, an outbreak of diarrhea and HUS in Germany was caused by a Shiga toxin–producing strain of *E. coli* that was typed as O104:H4, not O157:H7. This indicates that strains of *E. coli* other than O157:H7 can also cause HUS.

HUS occurs particularly in children who have been treated with fluoroquinolones or other antibiotics for their diarrhea. For this reason, antibiotics should not be used to treat diarrhea caused by EHEC.

(2) **Clinical findings outside of the intestinal tract:**

Escherichia coli is the leading cause of community-acquired **urinary tract infections.** These infections occur primarily in women; this finding is attributed to three features that facilitate ascending infection into the bladder, namely, a short urethra, the proximity of the urethra to the anus, and colonization of the vagina by members of the fecal flora. It is also the most frequent cause of nosocomial (hospital-acquired) urinary tract infections, which occur equally frequently in both men and women and are associated with the use of indwelling urinary catheters.

[2]Enterotoxigenic *E. coli* is the most common cause of traveler's diarrhea, but other bacteria (e.g., *Salmonella, Shigella, Campylobacter,* and *Vibrio* species), viruses such as Norwalk virus, and protozoa such as *Giardia* and *Cryptosporidium species* are also involved.

Urinary tract infections can be limited to the bladder or extend up the collecting system to the kidneys. If only the bladder is involved, the disease is called *cystitis*, whereas infection of the kidney is called *pyelonephritis*. The most prominent symptoms of cystitis are pain (dysuria) and frequency of urination; patients are usually afebrile. Pyelonephritis is characterized by fever, flank pain, and costovertebral angle tenderness; dysuria and frequency may or may not occur.

Escherichia coli is also a major cause, along with the group B streptococci, of **meningitis** and sepsis in neonates. Exposure of the newborn to *E. coli* and group B streptococci occurs during birth as a result of colonization of the vagina by these organisms in approximately 25% of pregnant women. *Escherichia coli* is the organism isolated most frequently from patients with hospital-acquired sepsis, which arises primarily from urinary, biliary, or peritoneal infections. Peritonitis is usually a mixed infection caused by *E. coli* or other facultative enteric gram-negative rod plus anaerobic members of the colonic flora such as *Bacteroides* and *Fusobacterium.*

Laboratory Diagnosis

Specimens suspected of containing enteric gram-negative rods, such as *E. coli*, are grown initially on a blood agar plate and on a differential medium, such as EMB agar or MacConkey's agar. *Escherichia coli*, which ferments lactose, forms pink colonies, whereas lactose-negative organisms are colorless. On EMB agar, *E. coli* colonies have a characteristic **green sheen.** Some of the important features that help distinguish *E. coli* from other lactose-fermenting gram-negative rods are as follows: (1) it produces indole from tryptophan, (2) it decarboxylates lysine, (3) it uses acetate as its only source of carbon, and (4) it is motile. *Escherichia coli* O157:H7 does not ferment sorbitol, which serves as an important criterion that distinguishes it from other strains of *E. coli*. The isolation of enterotoxigenic or enteropathogenic *E. coli* from patients with diarrhea is not a routine diagnostic procedure.

Treatment

Treatment of *E. coli* infections depends on the site of disease and the resistance pattern of the specific isolate. For example,

an uncomplicated lower urinary tract infection (cystitis) can be treated using oral trimethoprim-sulfamethoxazole or nitrofurantoin. Pyelonephritis can be treated with ciprofloxacin or ceftriaxone. However, *E. coli* sepsis requires treatment with parenteral antibiotics (e.g., a third-generation cephalosporin, such as cefotaxime, with or without an aminoglycoside, such as gentamicin). For the treatment of neonatal meningitis, a combination of ampicillin and cefotaxime is usually given.

Antibiotic therapy is usually *not* indicated in *E. coli* diarrheal diseases. However, administration of trimethoprim-sulfamethoxazole or loperamide (Imodium) may shorten the duration of symptoms. Rehydration is typically all that is necessary in this self-limited disease. However, diarrhea caused by *E. coli* O157 should *not* be treated with antimotility drugs as their use increases the risk of HUS. Giving antibiotics to patients with *E. coli* O157 diarrhea is controversial as it may increase the risk of HUS.

Treatment of HUS is typically supportive but dialysis may be necessary if renal failure occurs. If severe anemia or thrombocytopenia occurs, transfusions may be necessary. Plasma exchange does not alter the course of HUS.

Prevention

There is no specific prevention for *E. coli* infections, such as active or passive immunization. However, various general measures can be taken to prevent certain infections caused by *E. coli* and other organisms. For example, the incidence of urinary tract infections can be lowered by the judicious use and prompt withdrawal of catheters and, in recurrent infections, by prolonged prophylaxis with urinary antiseptic drugs (e.g., nitrofurantoin or trimethoprim-sulfamethoxazole). The use of cranberry juice to prevent recurrent urinary tract infections appears to be based on the ability of flavonoids in the juice to inhibit the binding of the pili of the uropathic strains of *E. coli* to the bladder epithelium rather than to acidification of the urine, which was the previous explanation.

Some cases of sepsis can be prevented by prompt removal of or switching the site of intravenous lines. Traveler's diarrhea can sometimes be prevented by the prophylactic use of doxycycline, ciprofloxacin, trimethoprim-sulfamethoxazole, or Pepto-Bismol. Ingestion of uncooked foods and unpurified water should be avoided while traveling in certain countries.

SALMONELLA

Diseases

Salmonella species cause enterocolitis, enteric fevers such as typhoid fever, and septicemia with metastatic infections such as osteomyelitis. They are one of the most common causes of bacterial enterocolitis in the United States.

Important Properties

Salmonellae are gram-negative rods that **do not ferment lactose** but do produce H_2S—features that are used in their laboratory identification. Their antigens—cell wall O, flagellar H, and capsular Vi (virulence)—are important for taxonomic and epidemiologic purposes. The O antigens, which are the outer polysaccharides of the cell wall, are used to subdivide the salmonellae into groups A–I. There are two forms of the H antigens, phases 1 and 2. Only one of the two H proteins is synthesized at any one time, depending on which gene sequence is in the correct alignment for transcription into mRNA. The Vi antigens (capsular polysaccharides) are antiphagocytic and are an important virulence factor for *S. typhi*, the agent of typhoid fever. The Vi antigens are also used for the serotyping of *S. typhi* in the clinical laboratory.

There are three methods for naming the salmonellae. Ewing divides the genus into three species: *S. typhi, Salmonella choleraesuis*, and *Salmonella enteritidis*. In this scheme there is one serotype in each of the first two species and 1,500 serotypes in the third. Kaufman and White assign different species names to each serotype; there are roughly 1,500 different species, usually named for the city in which they were isolated. *Salmonella dublin* according to Kaufman and White would be *S. enteritidis* serotype *dublin* according to Ewing. The third approach to naming the salmonellae is based on relatedness determined by DNA hybridization analysis. In this scheme, *S. typhi* is not a distinct species but is classified as *Salmonella enterica* serotype (or serovar) *typhi*. All three of these naming systems are in current use.

Clinically, the *Salmonella* species are often thought of in two distinct categories, namely, the typhoidal species (i.e., those that cause typhoid fever) and the nontyphoidal species (i.e., those that cause diarrhea [enterocolitis] and metastatic infections, such as osteomyelitis). The typhoidal species are *S. typhi* and *S. paratyphi*. The nontyphoidal species are the many serotypes of *S. enterica*. Of the serotypes, *S. enterica* serotype *choleraesuis* is the species most often involved in metastatic infections.

Pathogenesis & Epidemiology

The three types of *Salmonella* infections (enterocolitis, enteric fevers, and septicemia) have different pathogenic features.

(1) **Enterocolitis** is characterized by an invasion of the epithelial and subepithelial tissue of the small and large intestines. Strains that do not invade do not cause disease. The organisms penetrate both through and between the mucosal cells into the lamina propria, with resulting inflammation and diarrhea. Neutrophils limit the infection to the gut and the adjacent mesenteric lymph nodes; bacteremia is infrequent in enterocolitis. In contrast to *Shigella* enterocolitis, in which the infectious dose is very small (on the order of 100 organisms), the dose of *Salmonella* required is much higher, at least 100,000 organisms. Various properties of salmonellae and shigellae are compared in Table 18–9. Gastric acid is an important host defense; gastrectomy or use of antacids lowers the infectious dose significantly.

(2) In **typhoid** and other enteric fevers, infection begins in the small intestine, but few gastrointestinal symptoms occur. The organisms enter, multiply in the mononuclear phagocytes of Peyer's patches, and then spread to the phagocytes of the liver, gallbladder, and spleen. This leads to bacteremia, which is

TABLE 18–9 Comparison of Important Features of *Salmonella* and *Shigella*

Feature	*Shigella*	*Salmonella* Except *Salmonella typhi*	*Salmonella typhi*
Reservoir	Humans	Animals, especially poultry and eggs	Humans
Infectious dose (ID_{50})	Low[1]	High	High
Diarrhea as a prominent feature	Yes	Yes	No
Invasion of bloodstream	No	Yes	Yes
Chronic carrier state	No	Infrequent	Yes
Lactose fermentation	No	No	No
H_2S production	No	Yes	Yes
Vaccine available	No	No	Yes

[1]An organism with a low ID_{50} requires very few bacteria to cause disease.

associated with the onset of fever and other symptoms, probably caused by endotoxin. Survival and growth of the organism within phagosomes in phagocytic cells are a striking feature of this disease, as is the predilection for invasion of the gallbladder, which can result in establishment of the **carrier state** and excretion of the bacteria in the feces for long periods.

(3) **Septicemia** accounts for only about 5–10% of *Salmonella* infections and occurs in one of two settings: a patient with an underlying chronic disease, such as **sickle cell anemia** or cancer, or a child with enterocolitis. The septic course is more indolent than that seen with many other gram-negative rods. Bacteremia results in the seeding of many organs, with **osteomyelitis,** pneumonia, and meningitis as the most common sequelae. **Osteomyelitis in a child with sickle cell anemia** is an important example of this type of salmonella infection. Previously damaged tissues, such as infarcts and **aneurysms,** especially aortic aneurysms, are the most frequent sites of metastatic abscesses. *Salmonella* are also an important cause of vascular graft infections.

The epidemiology of *Salmonella* infections is related to the ingestion of food and water contaminated by human and animal wastes. *Salmonella typhi*, the cause of typhoid fever, is **transmitted only by humans,** but all other species have a significant animal as well as human reservoir. Human sources are either persons who temporarily excrete the organism during or shortly after an attack of enterocolitis or chronic carriers who excrete the organism for years. The most frequent **animal source is poultry and eggs,** but meat products that are inadequately cooked have been implicated as well. Dogs and other pets, including turtles, snakes, lizards, and iguanas, are additional sources.

Clinical Findings

After an incubation period of 12 to 48 hours, enterocolitis begins with nausea and vomiting and then progresses to abdominal pain and diarrhea, which can vary from mild to severe, with or without blood. Usually the disease lasts a few days, is self-limited, causes nonbloody diarrhea, and does not require medical care except in the very young and very old. HIV-infected individuals, especially those with a low CD4 count, have a much greater number of *Salmonella* infections, including more severe diarrhea and more serious metastatic infections than those who are not infected with HIV. *Salmonella typhimurium* is the most common species of *Salmonella* to cause enterocolitis in the United States, but almost every species has been involved.

In typhoid fever, caused by *S. typhi*, and in enteric fever, caused by organisms such as *S. paratyphi* A, B, and C (*S. paratyphi* B and C are also known as *Salmonella schottmuelleri* and *Salmonella hirschfeldii*, respectively), the onset of illness is slow, with fever and constipation rather than vomiting and diarrhea predominating. Diarrhea may occur early but usually disappears by the time the fever and bacteremia occur. After the first week, as the bacteremia becomes sustained, high fever, delirium, tender abdomen, and enlarged spleen occur. **Rose spots** (i.e., rose-colored macules on the abdomen) are associated with typhoid fever but occur only rarely. Leukopenia and anemia are often seen. Liver function tests are often abnormal, indicating hepatic involvement.

The disease begins to resolve by the third week, but severe complications such as intestinal hemorrhage or perforation can occur. About 3% of typhoid fever patients become chronic carriers. The carrier rate is higher among women, especially those with previous gallbladder disease and gallstones.

Septicemia is most often caused by *S. choleraesuis*. The symptoms begin with fever but little or no enterocolitis and then proceed to focal symptoms associated with the affected organ, frequently bone, lung, or meninges.

Laboratory Diagnosis

In enterocolitis, the organism is most easily isolated from a stool sample. However, in the enteric fevers, a blood culture is the procedure most likely to reveal the organism during the first 2 weeks of illness. Bone marrow cultures are often positive. Stool cultures may also be positive, especially in chronic carriers in whom the organism is secreted in the bile into the intestinal tract.

Salmonellae form non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. On TSI agar, an alkaline slant and an acid butt, frequently with both gas and H_2S (black color in the butt), are produced. *S. typhi* is the major exception; it does not form gas and produces only a small amount of H_2S. If the organism is urease-negative (*Proteus* organisms, which can produce a similar reaction on TSI agar, are urease-positive), the *Salmonella* isolate can be identified and grouped by the slide agglutination test into serogroup A, B, C, D, or E based on its O antigen. Definitive serotyping of the O, H, and Vi antigens is performed by special public health laboratories for epidemiologic purposes.

Salmonellosis is a notifiable disease, and an investigation to determine its source should be undertaken. In certain cases

of enteric fever and sepsis, when the organism is difficult to recover, the diagnosis can be made serologically by detecting a rise in antibody titer in the patient's serum (Widal test).

Treatment

Enterocolitis caused by *Salmonella* is usually a self-limited disease that resolves without treatment. Fluid and electrolyte replacement may be required. Antibiotic treatment does not shorten the illness or reduce the symptoms; in fact, it may prolong excretion of the organisms, increase the frequency of the carrier state, and select mutants resistant to the antibiotic. Antimicrobial agents are indicated only for neonates or persons with chronic diseases who are at risk for septicemia and disseminated abscesses. Plasmid-mediated antibiotic resistance is common, and antibiotic sensitivity tests should be done. Drugs that retard intestinal motility (i.e., that reduce diarrhea) appear to prolong the duration of symptoms and the fecal excretion of the organisms.

The treatment of choice for enteric fevers such as typhoid fever and septicemia with metastatic infection is either ceftriaxone or ciprofloxacin. Ampicillin or ciprofloxacin should be used in patients who are chronic carriers of *S. typhi*. Cholecystectomy may be necessary to abolish the chronic carrier state. Focal abscesses should be drained surgically when feasible.

Prevention

Salmonella infections are prevented mainly by public health and personal hygiene measures. Proper sewage treatment, a chlorinated water supply that is monitored for contamination by coliform bacteria, cultures of stool samples from food handlers to detect carriers, handwashing prior to food handling, pasteurization of milk, and proper cooking of poultry, eggs, and meat are all important.

Two vaccines are available, but they confer limited (50–80%) protection against *S. typhi*. One contains the Vi capsular polysaccharide of *S. typhi* (given intramuscularly), and the other contains a live, attenuated strain (Ty21a) of *S. typhi* (given orally). The two vaccines are equally effective. The vaccine is recommended for those who will travel or reside in high-risk areas and for those whose occupation brings them in contact with the organism. A new conjugate vaccine against typhoid fever containing the capsular polysaccharide (Vi) antigen coupled to a carrier protein is safe and immunogenic in young children but is not available in the United States at this time.

PATHOGENS PRIMARILY WITHIN THE ENTERIC TRACT

SHIGELLA

Disease

Shigella species cause enterocolitis. Enterocolitis caused by *Shigella* is often called bacillary dysentery. The term *dysentery* refers to bloody diarrhea.

Important Properties

Shigellae are **non–lactose-fermenting,** gram-negative rods that can be distinguished from salmonellae by three criteria: they produce no gas from the fermentation of glucose, they **do not produce H_2S,** and they are **nonmotile.** All shigellae have O antigens (polysaccharide) in their cell walls, and these antigens are used to divide the genus into four groups: A, B, C, and D.

Pathogenesis & Epidemiology

Shigellae are the most effective pathogens among the enteric bacteria. They have a **very low ID_{50}** (see page 31). Ingestion of as few as 100 organisms causes disease, whereas at least 10^5 *V. cholerae* or *Salmonella* organisms are required to produce symptoms. Various properties of shigellae and salmonellae are compared in Table 18–9.

Shigellosis is only a **human disease** (i.e., there is no animal reservoir). The organism is transmitted by the fecal–oral route. The four Fs—fingers, flies, food, and feces—are the principal factors in transmission. Foodborne outbreaks outnumber waterborne outbreaks by 2 to 1. Outbreaks occur in day care nurseries and in mental hospitals, where **fecal–oral** transmission is likely to occur. Children younger than 10 years account for approximately half of *Shigella*-positive stool cultures. There is no prolonged carrier state with *Shigella* infections, unlike that seen with *S. typhi* infections.

Shigellae cause disease almost exclusively in the gastrointestinal tract. They produce bloody diarrhea (dysentery) by invading the cells of the mucosa of the distal ileum and colon. Local inflammation accompanied by ulceration occurs, but the organisms rarely penetrate through the wall or enter the bloodstream, unlike salmonellae. Although some strains produce an enterotoxin (called *Shiga toxin*), invasion is the critical factor in pathogenesis. The evidence for this is that mutants that fail to produce enterotoxin but are invasive can still cause disease, whereas noninvasive mutants are nonpathogenic.

Shiga toxins are encoded by lysogenic bacteriophages. Shiga toxins very similar to those produced by *Shigella* are produced by enterohemorrhagic *E. coli* O157:H7 strains that cause enterocolitis and HUS.

Clinical Findings

After an incubation period of 1 to 4 days, symptoms begin with fever and abdominal cramps, followed by diarrhea, which may be watery at first but later contains blood and mucus. The disease varies from mild to severe depending on two major factors: the species of *Shigella* and the age of the patient, with young children and elderly people being the most severely affected. *Shigella dysenteriae*, which causes the most severe disease, is

usually seen in the United States only in travelers returning from abroad. *Shigella sonnei*, which causes mild disease, is isolated from approximately 75% of all individuals with shigellosis in the United States. The diarrhea frequently resolves in 2 or 3 days; in severe cases, antibiotics can shorten the course. Serum agglutinins appear after recovery but are not protective because the organism does not enter the blood. The role of intestinal IgA in protection is uncertain.

Laboratory Diagnosis

Shigellae form non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. On TSI agar, they cause an alkaline slant and an acid butt, with no gas and no H_2S. Confirmation of the organism as *Shigella* and determination of its group are done by slide agglutination.

One important adjunct to laboratory diagnosis is a methylene blue stain of a fecal sample to determine whether neutrophils are present. If they are found, an invasive organism such as *Shigella, Salmonella*, or *Campylobacter* is involved rather than a toxin-producing organism such as *V. cholerae, E. coli*, or *Clostridium perfringens*. (Certain viruses also cause diarrhea without neutrophils in the stool.)

Treatment

The main treatment for shigellosis is fluid and electrolyte replacement. In mild cases, no antibiotics are indicated. In severe cases, a fluoroquinolone (e.g., ciprofloxacin) is the drug of choice, but strains resistant to fluoroquinolones have emerged and antibiotic sensitivity tests must be performed. Trimethoprim-sulfamethoxazole is an alternative choice. Antiperistaltic drugs are contraindicated in shigellosis, because they prolong the fever, diarrhea, and excretion of the organism.

Prevention

Prevention of shigellosis is dependent on interruption of fecal–oral transmission by proper sewage disposal, chlorination of water, and personal hygiene (handwashing by food handlers). There is no vaccine, and prophylactic antibiotics are not recommended.

VIBRIO

Diseases

Vibrio cholerae, the major pathogen in this genus, is the cause of cholera. *Vibrio parahaemolyticus* causes diarrhea associated with eating raw or improperly cooked seafood. *Vibrio vulnificus* causes cellulitis and sepsis. Important features of pathogenesis by *V. cholerae, C. jejuni*, and *Helicobacter pylori* are described in Table 18–10.

Important Properties

Vibrios are curved, **comma-shaped,** gram-negative rods (see Figure 18–2). *V. cholerae* is divided into serogroups according to the nature of its O cell wall antigen. Members of the O1 and O139 serogroups cause epidemic disease, whereas non-O1 organisms either cause sporadic disease or are nonpathogens. The O1 organisms have two biotypes, called classic and El Tor, and three serotypes, called Ogawa, Inaba, and Hikojima. (Biotypes are based on differences in biochemical reactions, whereas serotypes are based on antigenic differences.) These features are used to characterize isolates in epidemiologic investigations. Serogroup O139 organisms, which caused a major epidemic in 1992, are identified by their reaction to antisera to the O139 polysaccharide antigens (O antigen).

Note that only the O1 and O139 organisms cause cholera because only they produce cholera toxin. They produce cholera toxin because they are lysogenized by a bacteriophage that carries the gene for the toxin (see below). The non-O1 strains can cause milder outbreaks of diarrhea but not cholera.

Vibrio parahaemolyticus and *V. vulnificus* are **marine organisms;** they live primarily in the ocean, especially in warm salt water. They are **halophilic** (i.e., they require a high NaCl concentration to grow).

1. Vibrio cholerae

Pathogenesis & Epidemiology

Vibrio cholerae is transmitted by **fecal contamination** of water and food, primarily from human sources. Human carriers are frequently asymptomatic and include individuals who are either in the incubation period or convalescing. The main animal reservoirs are marine shellfish, such as shrimp and oysters. Ingestion of these without adequate cooking can transmit the disease.

A major epidemic of cholera, which spanned the 1960s and 1970s, began in Southeast Asia and spread over three continents to areas of Africa, Europe, and the rest of Asia. Another epidemic of cholera began in Peru in 1991 and has spread to many countries in Central and South America. The organism isolated most frequently was the El Tor biotype of O1 *V. cholerae*,

TABLE 18–10 Important Features of Pathogenesis by Curved Gram-Negative Rods Affecting the Gastrointestinal Tract

Organism	Type of Pathogenesis	Typical Disease	Site of Infection	Main Approach to Therapy
Vibrio cholerae	Toxigenic	Watery diarrhea	Small intestine	Fluid replacement
Campylobacter jejuni	Inflammatory	Bloody diarrhea	Colon	Antibiotics[1]
Helicobacter pylori	Inflammatory	Gastritis; peptic ulcer	Stomach; duodenum	Antibiotics[1]

[1]See text for specific antibiotics.

usually of the Ogawa serotype. The factors that predispose to epidemics are poor sanitation, malnutrition, overcrowding, and inadequate medical services. Quarantine measures failed to prevent the spread of the disease because there were many asymptomatic carriers. In 1992, *V. cholerae* serogroup O139 emerged and caused a widespread epidemic of cholera in India and Bangladesh.

The pathogenesis of cholera is dependent on colonization of the small intestine by the organism and secretion of enterotoxin. For colonization to occur, large numbers of bacteria must be ingested because the organism is particularly sensitive to stomach acid. Persons with little or no stomach acid, such as those taking antacids or those who have had gastrectomy, are much more susceptible. Adherence to the cells of the brush border of the gut, which is a requirement for colonization, is related to secretion of the bacterial enzyme mucinase, which dissolves the protective glycoprotein coating over the intestinal cells.

After adhering, the organism multiplies and secretes an **enterotoxin** called choleragen (cholera toxin). This exotoxin can reproduce the symptoms of cholera even in the absence of the *Vibrio* organisms. The mode of action of cholera toxin is described in the next paragraph and in Figure 7–3 in the chapter on bacterial pathogenesis.

Choleragen consists of an A (active) subunit and a B (binding) subunit. The B subunit, which is a pentamer composed of five identical proteins, binds to a ganglioside receptor on the surface of the enterocyte. The A subunit is inserted into the cytosol, where it catalyzes the addition of ADP-ribose to the G_s protein (G_s is the stimulatory G protein). This locks the G_s protein in the "on" position, which causes the persistent stimulation of **adenylate cyclase.** The resulting overproduction of cyclic AMP activates cyclic AMP–dependent protein kinase, an enzyme that phosphorylates ion transporters in the cell membrane, resulting in the loss of water and ions from the cell. The watery efflux enters the lumen of the gut, resulting in a massive watery diarrhea that contains neither neutrophils nor red blood cells. Morbidity and death are due to **dehydration** and **electrolyte imbalance.** However, if treatment is instituted promptly, the disease runs a self-limited course in up to 7 days.

The genes for cholera toxin and other virulence factors are carried on a single-stranded DNA bacteriophage called CTX. Lysogenic conversion of non–toxin-producing strains to toxin-producing ones can occur when the CTX phage transduces these genes. The pili that attach the organism to the gut mucosa are the receptors for the phage.

Non-O1 *V. cholerae* is an occasional cause of diarrhea associated with eating shellfish obtained from the coastal waters of the United States.

Clinical Findings

Watery diarrhea in large volumes is the hallmark of cholera. There are no red blood cells or white blood cells in the stool. **Rice-water stool** is the term often applied to the nonbloody effluent. There is no abdominal pain, and subsequent symptoms are referable to the marked dehydration. The loss of fluid and electrolytes leads to cardiac and renal failure. Acidosis and hypokalemia also occur as a result of loss of bicarbonate and potassium in the stool. The mortality rate without treatment is 40%.

Laboratory Diagnosis

The approach to laboratory diagnosis depends on the situation. During an epidemic, a clinical judgment is made and there is little need for the laboratory. In an area where the disease is endemic or for the detection of carriers, a variety of selective media[3] that are not in common use in the United States are used in the laboratory.

For diagnosis of sporadic cases in this country, a culture of the diarrhea stool containing *V. cholerae* will show colorless colonies on MacConkey's agar because lactose is fermented slowly. The organism is oxidase-positive, which distinguishes it from members of the Enterobacteriaceae. On TSI agar, an acid slant and an acid butt without gas or H_2S are seen because the organism ferments sucrose. A presumptive diagnosis of *V. cholerae* can be confirmed by agglutination of the organism by polyvalent O1 or non-O1 antiserum. A retrospective diagnosis can be made serologically by detecting a rise in antibody titer in acute- and convalescent-phase sera.

Treatment

Treatment consists of prompt, adequate replacement of water and electrolytes, either orally or intravenously. Glucose is added to the solution to enhance the uptake of water and electrolytes. Antibiotics such as tetracycline are not necessary, but they do shorten the duration of symptoms and reduce the time of excretion of the organisms.

Prevention

Prevention is achieved mainly by public health measures that ensure a clean water and food supply. An oral, live attenuated vaccine called Vaxchora is available in the United States for travelers to areas where cholera caused by serogroup O1 is endemic. Other oral vaccines containing killed organisms are available in countries where cholera epidemics occur.

The use of tetracycline for prevention is effective in close contacts but does not prevent the spread of a major epidemic. Prompt detection of carriers is important in limiting outbreaks.

2. Vibrio parahaemolyticus

Vibrio parahaemolyticus is a marine organism transmitted by **ingestion of raw or undercooked seafood,** especially shellfish such as oysters. It is a major cause of diarrhea in Japan, where raw fish is eaten in large quantities, but is an infrequent pathogen in the United States, although several outbreaks have occurred aboard cruise ships in the Caribbean. Little is known

[3]Media such as thiosulfate-citrate-bile salts agar or tellurite-taurocholate-gelatin are used.

about its pathogenesis, except that an enterotoxin similar to choleragen is secreted and limited invasion sometimes occurs.

The clinical picture caused by *V. parahaemolyticus* varies from mild to quite severe watery diarrhea, nausea and vomiting, abdominal cramps, and fever. The illness is self-limited, lasting about 3 days. *Vibrio parahaemolyticus* is distinguished from *V. cholerae* mainly on the basis of growth in NaCl: *V. parahaemolyticus* grows in 8% NaCl solution (as befits a marine organism), whereas *V. cholerae* does not. No specific treatment is indicated, because the disease is relatively mild and self-limited. Disease can be prevented by proper refrigeration and cooking of seafood.

3. Vibrio vulnificus

Vibrio vulnificus is also a marine organism (i.e., it is found in warm salt waters such as the Caribbean Sea). It causes severe skin and soft tissue infections (**cellulitis**), **especially in shellfish handlers**, who often sustain skin wounds. It can also cause a rapidly fatal **septicemia in immunocompromised people who have eaten raw shellfish** containing the organism. Hemorrhagic bullae in the skin often occur in patients with sepsis caused by *V. vulnificus*. Chronic liver disease (e.g., cirrhosis) predisposes to severe infections. The recommended treatment is doxycycline.

CAMPYLOBACTER

Diseases

Campylobacter jejuni is a frequent cause of enterocolitis, especially in children. *C. jejuni* infection is a common antecedent to Guillain-Barré syndrome. Other *Campylobacter* species are rare causes of systemic infection, particularly bacteremia.

Important Properties

Campylobacters are curved, gram-negative rods that appear either **comma**- or **S-shaped.** They are **microaerophilic,** growing best in 5% oxygen rather than in the 20% present in the atmosphere. *Campylobacter jejuni* grows well at 42°C, whereas *Campylobacter intestinalis*[4] does not—an observation that is useful in microbiologic diagnosis.

Pathogenesis & Epidemiology

Domestic animals such as cattle, chickens, and dogs serve as a source of the organisms for humans. Transmission is usually **fecal–oral.** Food and water contaminated with animal feces are the major sources of human infection. Foods, such as poultry, meat, and unpasteurized milk, are commonly involved. Puppies with diarrhea are a common source for children. Human-to-human transmission occurs but is less frequent than animal-to-human transmission. *Campylobacter jejuni* is a major cause of diarrhea in the United States; it was recovered in 4.6% of patients with diarrhea, compared with 2.3% and 1% for *Salmonella* and *Shigella*, respectively. *Campylobacter jejuni* is the leading cause of diarrhea associated with consumption of unpasteurized milk.

Features of pathogenesis by *Campylobacter* are described in Table 18–10. Inflammation of the intestinal mucosa often occurs, accompanied by blood in stools. Systemic infections (e.g., bacteremia) occur most often in neonates or debilitated adults.

Clinical Findings

Enterocolitis, caused primarily by *C. jejuni*, begins as watery, foul-smelling diarrhea followed by bloody stools accompanied by fever and severe abdominal pain. Systemic infections, most commonly bacteremia, are caused more often by *C. intestinalis*. The symptoms of bacteremia (e.g., fever and malaise) are associated with no specific physical findings.

Gastrointestinal infection with *C. jejuni* is associated with Guillain-Barré syndrome, the most common cause of acute neuromuscular paralysis. Guillain-Barré syndrome is an autoimmune disease attributed to the formation of antibodies against *C. jejuni* that cross-react with antigens on neurons (see Chapter 66). Infection with *Campylobacter* is also associated with two other autoimmune diseases: reactive arthritis and Reiter syndrome. These are also described in Chapter 66.

Laboratory Diagnosis

If the patient has diarrhea, a stool specimen is cultured on a blood agar plate containing antibiotics[5] that inhibit most other fecal flora.

The plate is incubated at 42°C in a microaerophilic atmosphere containing 5% oxygen and 10% carbon dioxide, which favors the growth of *C. jejuni*. It is identified by failure to grow at 25°C, oxidase positivity, and sensitivity to nalidixic acid. Unlike *Shigella* and *Salmonella*, lactose fermentation is not used as a distinguishing feature. If bacteremia is suspected, a blood culture incubated under standard temperature and atmospheric conditions will reveal the growth of the characteristically comma- or S-shaped, motile, gram-negative rods. Identification of the organism as *C. intestinalis* is confirmed by its failure to grow at 42°C, its ability to grow at 25°C, and its resistance to nalidixic acid.

Treatment

Erythromycin or ciprofloxacin is used successfully in *C. jejuni* enterocolitis. The treatment of choice for *C. intestinalis* bacteremia is an aminoglycoside.

Prevention

There is no vaccine or other specific preventive measure. Proper sewage disposal and personal hygiene (handwashing) are important.

[4]Also known as *Campylobacter fetus* subsp. *fetus*.

[5]For example, Skirrow's medium contains vancomycin, trimethoprim, cephalothin, polymyxin, and amphotericin B.

HELICOBACTER

Diseases

Helicobacter pylori causes gastritis and peptic ulcers. Infection with *H. pylori* is a risk factor for gastric carcinoma and is linked to mucosal-associated lymphoid tissue (MALT) lymphomas.

Important Properties

Helicobacters are curved gram-negative rods similar in appearance to campylobacters, but because they differ sufficiently in certain biochemical and flagellar characteristics, they are classified as a separate genus. In particular, helicobacters are strongly urease-positive, whereas campylobacters are urease-negative.

Pathogenesis & Epidemiology

Helicobacter pylori attaches to the mucus-secreting cells of the gastric mucosa. The production of large amounts of ammonia from urea by the organism's urease, coupled with an inflammatory response, leads to damage to the mucosa. Loss of the protective mucus coating predisposes to gastritis and peptic ulcer (see Table 18–10). The ammonia also neutralizes stomach acid, allowing the organism to survive. Epidemiologically, most patients with these diseases show *H. pylori* in biopsy specimens of the gastric epithelium.

The natural habitat of *H. pylori* is the human stomach, and it is probably acquired by ingestion. However, it has not been isolated from stool, food, water, or animals. Person-to-person transmission probably occurs because there is clustering of infection within families. The rate of infection with *H. pylori* in developing countries is very high—a finding that is in accord with the high rate of gastric carcinoma in those countries.

MALT lymphomas are B-cell tumors located typically in the stomach, but they occur elsewhere in the gastrointestinal tract as well. *Helicobacter pylori* is often found in the MALT lesion, and the chronic inflammation induced by the organism is thought to stimulate B-cell proliferation and eventually a B-cell lymphoma. Antibiotic treatment directed against the organism often causes the tumor to regress.

Clinical Findings

Gastritis and peptic ulcer are characterized by recurrent pain in the upper abdomen, frequently accompanied by bleeding into the gastrointestinal tract. No bacteremia or disseminated disease occurs.

Laboratory Diagnosis

The organism can be seen on Gram-stained smears of biopsy specimens of the gastric mucosa. It can be cultured on the same media as campylobacters. In contrast to *C. jejuni, H. pylori* is urease-positive. Urease production is the basis for a noninvasive diagnostic test called the "urea breath" test. In this test, radiolabeled urea is ingested. If the organism is present, urease will cleave the ingested urea, radiolabeled CO_2 is evolved, and the radioactivity is detected in the breath.

A test for *Helicobacter* antigen in the stool can be used for diagnosis and for confirmation that treatment has eliminated the organism. The presence of IgG antibodies in the patient's serum can also be used as evidence of infection.

Treatment & Prevention

The concept that underlies the choice of drugs is to use antibiotics to eliminate *Helicobacter* plus a drug to reduce gastric acidity. A combination of two antibiotics is used because resistance, especially to metronidazole, has emerged. Treatment of duodenal ulcers with antibiotics (e.g., amoxicillin and metronidazole) and bismuth salts (Pepto-Bismol) results in a greatly decreased recurrence rate. Tetracycline can be used instead of amoxicillin. There is no vaccine or other specific preventive measure.

PATHOGENS OUTSIDE THE ENTERIC TRACT

KLEBSIELLA–ENTEROBACTER–SERRATIA GROUP

Diseases

These organisms are usually opportunistic pathogens that cause nosocomial infections, especially pneumonia and urinary tract infections. *Klebsiella pneumoniae* is an important respiratory tract pathogen outside hospitals as well.

Important Properties

Klebsiella pneumoniae, Enterobacter cloacae, and *Serratia marcescens* are the species most often involved in human infections. They are frequently found in the **large intestine** but are also present in soil and water. These organisms have very similar properties and are usually distinguished on the basis of several biochemical reactions and motility. *Klebsiella pneumoniae* has a **very large polysaccharide capsule,** which gives its colonies a striking mucoid appearance. *Serratia marcescens* produces **red-pigmented colonies** (Figure 18–3).

Pathogenesis & Epidemiology

Of the three organisms, *K. pneumoniae* is most likely to be a primary, nonopportunistic pathogen; this property is related to its antiphagocytic capsule. Although this organism is a primary pathogen, patients with *K. pneumoniae* infections frequently have predisposing conditions such as advanced age, chronic respiratory disease, diabetes, or alcoholism. The organism is carried in the respiratory tract of about 10% of healthy people, who are prone to pneumonia if host defenses are lowered.

Enterobacter and *Serratia* infections are clearly related to hospitalization, especially to invasive procedures such as

FIGURE 18–3 *Serratia marcescens*—red-pigmented colonies. Arrow points to a red-pigmented colony of *S. marcescens*. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

intravenous catheterization, respiratory intubation, and urinary tract manipulations. In addition, outbreaks of *Serratia* pneumonia have been associated with contamination of the water in respiratory therapy devices. Prior to the extensive use of these procedures, *S. marcescens* was a harmless organism most frequently isolated from environmental sources such as water.

Serratia also causes endocarditis in users of injection drugs. As with many other gram-negative rods, the pathogenesis of septic shock caused by these organisms is related to the endotoxins in their cell walls.

Clinical Findings

Urinary tract infections and pneumonia are the usual clinical entities associated with these three bacteria, but bacteremia and secondary spread to other areas such as the meninges and liver occur. It is difficult to distinguish infections caused by these organisms on clinical grounds, with the exception of pneumonia caused by *Klebsiella*, which produces a thick, mucoid, bloody sputum ("currant-jelly" sputum) and can progress to necrosis and abscess formation.

There are two other species of *Klebsiella* that cause unusual human infections rarely seen in the United States. *Klebsiella ozaenae* is associated with atrophic rhinitis, and *Klebsiella rhinoscleromatis* causes a destructive granuloma of the nose and pharynx.

Laboratory Diagnosis

Organisms of this group produce lactose-fermenting (colored) colonies on differential agar such as MacConkey's or EMB, although *Serratia*, which is a late lactose fermenter, can produce a negative reaction. These organisms are differentiated by the use of biochemical tests.

Treatment

Because the antibiotic resistance of these organisms can vary greatly, the choice of drug depends on the results of sensitivity testing. Isolates from hospital-acquired infections are frequently resistant to multiple antibiotics. ESBL strains of *K. pneumoniae* that produce extended-spectrum β-lactamases are an important cause of hospital-acquired infections and are resistant to almost all known antibiotics. An aminoglycoside (e.g., gentamicin) and a cephalosporin (e.g., cefotaxime) are used empirically until the results of testing are known. In severe *Enterobacter* infections, a combination of imipenem and gentamicin is often used.

Prevention

Some hospital-acquired infections caused by gram-negative rods can be prevented by such general measures as changing the site of intravenous catheters, removing urinary catheters when they are no longer needed, and taking proper care of respiratory therapy devices. There is no vaccine.

PROTEUS–PROVIDENCIA–MORGANELLA GROUP

Diseases

These organisms primarily cause urinary tract infections, both community- and hospital-acquired.

Important Properties

These gram-negative rods are distinguished from other members of the Enterobacteriaceae by their ability to produce the enzyme phenylalanine deaminase. In addition, they produce the enzyme **urease,** which cleaves urea to form NH_3 and CO_2. Certain species are very motile and produce a striking **swarming** effect on blood agar, characterized by expanding rings (waves) of organisms over the surface of the agar (Figure 18–4).

The cell wall O antigens of certain strains of *Proteus*, such as OX-2, OX-19, and OX-K, cross-react with antigens of several species of rickettsiae. These *Proteus* antigens can be used in laboratory tests to detect the presence of antibodies against certain rickettsiae in patients' serum. This test, called the Weil-Felix reaction after its originators, is being used less frequently as more specific procedures are developed.

In the past, there were four medically important species of *Proteus*. However, molecular studies of DNA relatedness showed that two of the four were significantly different. These species have been renamed: *Proteus morganii* is now *Morganella morganii*, and *Proteus rettgeri* is now *Providencia rettgeri*.

FIGURE 18–4 *Proteus* species—swarming motility on blood agar. Arrowhead points to the site where *Proteus* bacteria were placed on the blood agar. Short arrow points to the edge of the first ring of swarming motility. Long arrow points to the edge of the second ring of swarming motility. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

In the clinical laboratory, these organisms are distinguished from *Proteus vulgaris* and *Proteus mirabilis* on the basis of several biochemical tests.

Pathogenesis & Epidemiology

The organisms are present in the human colon as well as in soil and water. Their tendency to cause urinary tract infections is probably due to their presence in the colon and to colonization of the urethra, especially in women. The vigorous motility of *Proteus* organisms may contribute to their ability to invade the urinary tract.

Production of the enzyme urease is an important feature of the pathogenesis of urinary tract infections by this group. Urease hydrolyzes the urea in urine to form ammonia, which raises the pH, producing an alkaline urine. This encourages the formation of stones (calculi) called **"struvite"** composed of magnesium ammonium phosphate. Struvite stones often manifest as staghorn calculi in the renal pelvis. They obstruct urine flow, damage urinary epithelium, and serve as a nidus for recurrent infection by trapping bacteria within the stone. Because alkaline urine also favors growth of the organisms and more extensive renal damage, treatment involves keeping the urine at a low pH.

Clinical Findings

The signs and symptoms of urinary tract infections caused by these organisms cannot be distinguished from those caused by *E. coli* or other members of the Enterobacteriaceae. *Proteus* species can also cause pneumonia, wound infections, and septicemia. *Proteus mirabilis* is the species of *Proteus* that causes most community- and hospital-acquired infections, but *P. rettgeri* is emerging as an important agent of nosocomial infections.

Laboratory Diagnosis

These organisms usually are highly motile and produce a "swarming" overgrowth on blood agar, which can frustrate efforts to recover pure cultures of other organisms. Growth on blood agar containing phenylethyl alcohol inhibits swarming, thus allowing isolated colonies of *Proteus* and other organisms to be obtained. They produce non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. *Proteus vulgaris* and *P. mirabilis* produce H_2S, which blackens the butt of TSI agar, whereas neither *M. morganii* nor *P. rettgeri* does. *Proteus mirabilis* is indole-negative, whereas the other three species are indole-positive—a distinction that can be used clinically to guide the choice of antibiotics. These four medically important species are urease-positive. Identification of these organisms in the clinical laboratory is based on a variety of biochemical reactions.

Treatment

Most strains are sensitive to aminoglycosides and trimethoprim-sulfamethoxazole, but because individual isolates can vary, antibiotic sensitivity tests should be performed. *Proteus mirabilis* is the species most frequently sensitive to ampicillin. The indole-positive species (*P. vulgaris, M. morganii*, and *P. rettgeri*) are more resistant to antibiotics than is *P. mirabilis*, which is indole-negative. The treatment of choice for the indole-positive species is a cephalosporin (e.g., cefotaxime). *Proteus rettgeri* is frequently resistant to multiple antibiotics.

Prevention

There are no specific preventive measures, but many hospital-acquired urinary tract infections can be prevented by prompt removal of urinary catheters.

PSEUDOMONAS

Diseases

Pseudomonas aeruginosa causes infections (e.g., sepsis, pneumonia, and urinary tract infections) primarily in patients with lowered host defenses. It also causes chronic lower respiratory tract infections in patients with cystic fibrosis, wound infections (cellulitis) in burn patients (Figure 18–5), and malignant otitis externa in diabetic patients. It is the most common cause of ventilator-associated pneumonia. (*P. aeruginosa* is also known as *Burkholderia aeruginosa*.) *Pseudomonas cepacia* (renamed *Burkholderia cepacia*) and *Pseudomonas maltophilia* (renamed *Xanthomonas maltophilia* and now called *Stenotrophomonas maltophilia*) also cause these infections, but much less frequently. *Pseudomonas pseudomallei* (also known as *Burkholderia pseudomallei*), the cause of melioidosis, is described in Chapter 27.

Important Properties

Pseudomonads are gram-negative rods that resemble the members of the Enterobacteriaceae but differ in that they are strict aerobes (i.e., they derive their energy only by oxidation of sugars

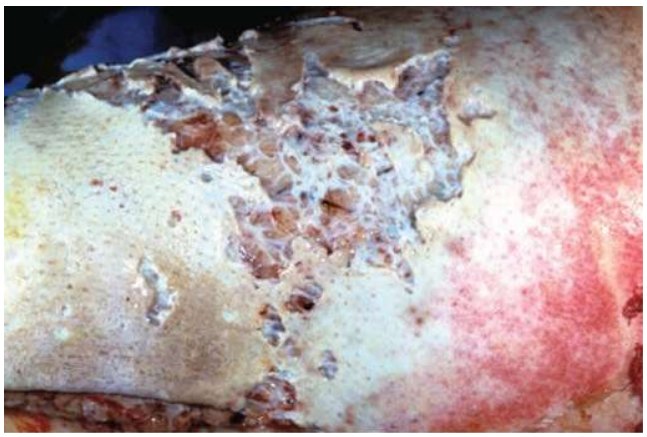


FIGURE 18–5 Cellulitis caused by *Pseudomonas aeruginosa*. Note the blue-green color of the pus in the burn wound infection. (Used with permission from Dr. Robert L. Sheridan.)

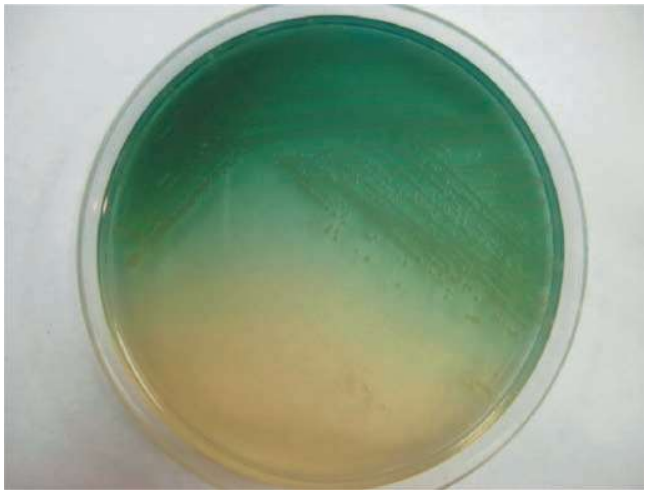


FIGURE 18–6 *Pseudomonas aeruginosa*—blue-green pigment. Blue-green pigment (pyocyanin) produced by *P. aeruginosa* diffuses into the agar. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

rather than by fermentation). Because they do not ferment glucose, they are called **nonfermenters,** in contrast to the members of the Enterobacteriaceae, which do ferment glucose. Oxidation involves electron transport by cytochrome c (i.e., they are **oxidase-positive**).

Pseudomonads are able to grow in **water** containing only traces of nutrients (e.g., tap water), and this favors their persistence in the hospital environment. *Pseudomonas aeruginosa* and *B. cepacia* have a remarkable ability to withstand disinfectants; this accounts in part for their role in hospital-acquired infections. They have been found growing in hexachlorophene-containing soap solutions, in antiseptics, and in detergents.

Pseudomonas aeruginosa produces two pigments useful in clinical and laboratory diagnosis: (1) **pyocyanin,** which can **color the pus in a wound blue** and (2) pyoverdin (fluorescein), a yellow-green pigment that fluoresces under ultraviolet light, a property that can be used in the early detection of skin infection in burn patients. In the laboratory, these **pigments diffuse into the agar, imparting a blue-green color** that is useful in identification. *Pseudomonas aeruginosa* is the only species of *Pseudomonas* that synthesizes pyocyanin (Figure 18–6).

Strains of *P. aeruginosa* isolated from cystic fibrosis patients have a prominent slime layer (glycocalyx), which gives their colonies a very mucoid appearance. The slime layer mediates adherence of the organism to mucous membranes of the respiratory tract and prevents antibody from binding to the organism.

Pathogenesis & Epidemiology

Pseudomonas aeruginosa is found chiefly in soil and water, although approximately 10% of people carry it in the normal flora of the colon. It is found on the skin in moist areas and can colonize the upper respiratory tract of hospitalized patients. Its ability to grow in simple aqueous solutions has resulted in contamination of respiratory therapy and anesthesia equipment, intravenous fluids, and even distilled water.

Pseudomonas aeruginosa is primarily an opportunistic pathogen that causes infections in hospitalized patients (e.g., those with extensive burns), in whom the skin host defenses are destroyed; in those with chronic respiratory disease (e.g., cystic fibrosis), in whom the normal clearance mechanisms are impaired; in those who are immunosuppressed; in those with neutrophil counts of less than 500/μL; and in those with indwelling catheters. It causes 10–20% of hospital-acquired infections and, in many hospitals, is the most common cause of gram-negative nosocomial pneumonia, especially ventilator-associated pneumonia.

Pathogenesis is based on multiple virulence factors: endotoxin, exotoxins, and enzymes. Its endotoxin, like that of other gram-negative bacteria, causes the symptoms of sepsis and septic shock. The best known of the exotoxins is exotoxin A, which causes tissue necrosis. It inhibits eukaryotic protein synthesis by the same mechanism as diphtheria exotoxin, namely, ADP-ribosylation of elongation factor-2. It also produces enzymes, such as elastase and proteases, that are histotoxic and facilitate invasion of the organism into the bloodstream. Pyocyanin damages the cilia and mucosal cells of the respiratory tract.

Strains of *P. aeruginosa* that have a "type III secretion system" are significantly more virulent than those that do not. This secretion system transfers the exotoxin from the bacterium directly into the adjacent human cell, which allows the toxin to avoid neutralizing antibody. Type III secretion systems are mediated by transport pumps in the bacterial cell membrane. Of the four exoenzymes known to be transported by this secretion system, Exo S is the one most clearly associated with virulence. Exo S has several modes of action, the most important of which is ADP-ribosylation of a Ras protein, leading to damage to the cytoskeleton.

Clinical Findings

Pseudomonas aeruginosa can cause infections virtually anywhere in the body, but urinary tract infections, pneumonia

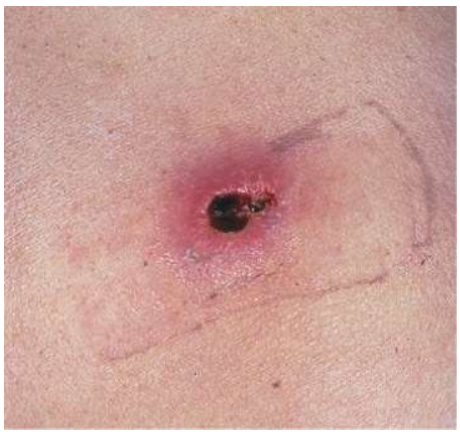


FIGURE 18–7 Ecthyma gangrenosum. Necrotic skin lesion caused by *Pseudomonas aeruginosa*. (Reproduced with permission from Wolff K, Johnson R, Saavedra A eds. *Fitzpatrick's Color Atlas & Synopsis of Clinical Dermatology*. 7th ed. New York, NY: McGraw-Hill; 2013.)

(especially in **cystic fibrosis** patients), and wound infections (especially burns) (see Figure 18–5) predominate. It is an important cause of hospital-acquired pneumonia, especially in those undergoing mechanical ventilation (ventilator-associated pneumonia). From these sites, the organism can enter the blood, causing sepsis. The bacteria can spread to the skin, where they cause black, necrotic lesions called **ecthyma gangrenosum** (Figure 18–7). Patients with *P. aeruginosa* sepsis have a mortality rate of greater than 50%. It is an important cause of endocarditis in intravenous drug users.

Severe external otitis (malignant otitis externa) and other skin lesions (e.g., folliculitis) occur in users of swimming pools and hot tubs (hot tub folliculitis) in which the chlorination is inadequate. *Pseudomonas aeruginosa* is the most common cause of osteomyelitis of the foot in those who sustain puncture wounds through the soles of gym shoes. Corneal infections caused by *P. aeruginosa* are seen in contact lens users.

In addition to *P. aeruginosa*, *Stenotrophomonas* and *Burkholderia* also cause chronic lung infections in patients with cystic fibrosis.

Laboratory Diagnosis

Pseudomonas aeruginosa grows as non–lactose-fermenting (colorless) colonies on MacConkey's or EMB agar. It is **oxidase-positive.** A typical metallic sheen of the growth on TSI agar, coupled with the blue-green pigment on ordinary nutrient agar (see Figure 18–6), and a fruity aroma are sufficient to make a presumptive diagnosis. The diagnosis is confirmed by biochemical reactions. Identification for epidemiologic purposes is done by bacteriophage or pyocin[6] typing.

[6]A pyocin is a type of bacteriocin produced by *P. aeruginosa*. Different strains produce various pyocins, which can serve to distinguish the organisms.

Treatment

Because *P. aeruginosa* is **resistant to many antibiotics,** treatment must be tailored to the sensitivity of each isolate and monitored frequently; resistant strains can emerge during therapy. The treatment of choice is an antipseudomonal penicillin (e.g., piperacillin/tazobactam or ticarcillin/clavulanate) plus an aminoglycoside (e.g., gentamicin or amikacin). Ceftazidime is also effective. For infections caused by highly resistant strains, colistin (polymyxin E) is useful. The drug of choice for urinary tract infections is ciprofloxacin. The drug of choice for infections caused by *B. cepacia* and *S. maltophilia* is trimethoprim-sulfamethoxazole.

Prevention

Prevention of *P. aeruginosa* infections involves keeping neutrophil counts above 500/μL, removing indwelling catheters promptly, taking special care of burned skin, and taking other similar measures to limit infection in patients with reduced host defenses.

BACTEROIDES & PREVOTELLA

Diseases

Members of the genus *Bacteroides* are the most common cause of serious anaerobic infections (e.g., sepsis, peritonitis, and abscesses). *Bacteroides fragilis* is the most frequent pathogen. *Prevotella melaninogenica* is also an important pathogen. It was formerly known as *Bacteroides melaninogenicus*, and both names are still encountered.

Important Properties

Bacteroides and *Prevotella* organisms are anaerobic, non–spore-forming, gram-negative rods. Of the many species of *Bacteroides*, two are human pathogens: *B. fragilis*[7] and *Bacteroides corrodens*.

Members of the *B. fragilis* group are the predominant organisms in the human colon, numbering approximately 10^{11}/g of feces and are found in the vagina of approximately 60% of women. *Prevotella melaninogenica* and *B. corrodens* occur primarily in the oral cavity.

Pathogenesis & Epidemiology

Because *Bacteroides* and *Prevotella* species are part of the normal flora, **infections** are endogenous, usually arising from a break in a mucosal surface, and are not communicable. These organisms cause a variety of infections, such as local abscesses at the site of a mucosal break, metastatic abscesses by hematogenous spread to distant organs, or lung abscesses by aspiration of oral flora.

[7]*B. fragilis* is divided into five subspecies, the most important of which is *B. fragilis* subsp. *fragilis*. The other four subspecies are *B. fragilis* subsp. *distasonis, ovatus, thetaiotamicron*, and *vulgatus*. It is proper, therefore, to speak of the *B. fragilis* group rather than simply *B. fragilis*.

Predisposing factors such as surgery, trauma, and chronic disease play an important role in pathogenesis. Local tissue necrosis, impaired blood supply, and growth of facultative anaerobes at the site contribute to anaerobic infections. The facultative anaerobes, such as *E. coli*, utilize the oxygen, thereby reducing it to a level that allows the anaerobic *Bacteroides* and *Prevotella* strains to grow. As a result, many anaerobic infections contain a mixed facultative and anaerobic flora. This has important implications for therapy; both the facultative anaerobes and the anaerobes should be treated.

The polysaccharide capsule of *B. fragilis* is an important virulence factor. The host response to the capsule plays a major role in abscess formation. Note also that the endotoxin of *B. fragilis* contains a variant lipid A that is missing one of the fatty acids and consequently is 1,000-fold less active than the typical endotoxin of bacteria such as *Neisseria meningitidis*.

Enzymes such as hyaluronidase, collagenase, and phospholipase are produced and contribute to tissue damage. Enterotoxin-producing strain of *B. fragilis* can cause diarrhea in both children and adults.

Clinical Findings

The *B. fragilis* group of organisms is most frequently associated with intra-abdominal infections, either peritonitis or localized abscesses. Pelvic abscesses, necrotizing fasciitis, and bacteremia occur as well. Abscesses of the mouth, pharynx, brain, and lung are more commonly caused by *P. melaninogenica*, a member of the normal oral flora, but *B. fragilis* is found in about 25% of lung abscesses. In general, *B. fragilis* causes disease below the diaphragm, whereas *P. melaninogenica* causes disease above the diaphragm. *Prevotella intermedia* is an important cause of gingivitis, periodontitis, and dental abscess.

Laboratory Diagnosis

Bacteroides species can be isolated anaerobically on blood agar plates containing kanamycin and vancomycin to inhibit unwanted organisms. They are identified by biochemical reactions (e.g., sugar fermentations) and by production of certain organic acids (e.g., formic, acetic, and propionic acids), which are detected by gas chromatography. *Prevotella melaninogenica* produces characteristic black colonies (Figure 18–8).

Treatment

Members of the *B. fragilis* group are resistant to penicillins, first-generation cephalosporins, and aminoglycosides, making them among the most antibiotic-resistant of the anaerobic bacteria. Penicillin resistance is the result of β-lactamase production. Metronidazole is the drug of choice, with cefoxitin, clindamycin, and chloramphenicol as alternatives. Aminoglycosides are frequently combined to treat the facultative gram-negative rods in mixed infections. The drug of choice for *P. melaninogenica* infections is either metronidazole or clindamycin. β-Lactamase–producing strains of *P. melaninogenica* have been isolated from patients. Surgical drainage of abscesses usually accompanies antibiotic therapy, but lung abscesses often heal without drainage.

FIGURE 18–8 *Prevotella melaninogenica*—black pigmented colonies. Arrow points to a black pigmented colony of *P. melaninogenica*. (Used with permission from Professor Shirley Lowe, University of California, San Francisco School of Medicine.)

Prevention

Prevention of *Bacteroides* and *Prevotella* infections centers on perioperative administration of a *cephalosporin*, frequently cefoxitin, for abdominal or pelvic surgery. There is no vaccine.

FUSOBACTERIUM

Fusobacterium species are long, anaerobic, gram-negative rods with pointed ends (Figure 18–9). They are part of the human normal flora of the mouth, colon, and female genital tract and are isolated from brain, pulmonary, intra-abdominal, and pelvic abscesses. They are frequently found in mixed infections with other anaerobes and facultative anaerobes.

Fusobacterium nucleatum occurs, along with various spirochetes, in cases of Vincent's angina (trench mouth), which is characterized by a necrotizing ulcerative gingivitis. *Fusobacterium necrophorum* causes Lemierre's disease, which is an anaerobic infection of the posterior pharyngeal space accompanied by thrombophlebitis of the internal jugular vein and metastatic infectious emboli to the lung.

The laboratory diagnosis is made by culturing the organism anaerobically. The drug of choice for *Fusobacterium* infections is either penicillin G, clindamycin, or metronidazole. There is no vaccine.

Introduction to Fungi

Third Edition

John Webster and
Roland W. S. Weber

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Introduction to Fungi

This new edition of the universally acclaimed and widely used textbook on fungal biology has been completely rewritten, drawing directly on the authors' research and teaching experience. The text takes account of the rapid and exciting progress that has been made in the taxonomy, cell and molecular biology, biochemistry, pathology and ecology of the fungi. Features of taxonomic significance are integrated with natural functions, including their relevance to human affairs. Special emphasis is placed on the biology and control of human and plant pathogens, providing a vital link between fundamental and applied mycology. The book is richly illustrated throughout with specially prepared drawings and photographs, based on living material. Illustrated life cycles are provided, and technical terms are clearly explained. Extensive reference is made to recent literature and developments, and the emphasis throughout is on whole-organism biology from an integrated, multi-disciplinary perspective.

John Webster is Professor Emeritus of the School of Biosciences at the University of Exeter, UK.

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Introduction to Fungi

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and

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To Philip M. Booth

Contents

Colour plate section appears between pages 412 and 413

Preface to the first edition

There are several available good textbooks of mycology, and some justification is needed for publishing another. I have long been convinced that the best way to teach mycology, and indeed all biology, is to make use, wherever possible, of living material. Fortunately with fungi, provided one chooses the right time of the year, a wealth of material is readily available. Also by use of cultures and by infecting material of plant pathogens in the glasshouse or by maintaining pathological plots in the garden, it is possible to produce material at almost any time. I have therefore tried to write an introduction to fungi which are easily available in the living state, and have tried to give some indication of where they can be obtained. In this way I hope to encourage students to go into the field and look for fungi themselves. The best way to begin is to go with an expert, or to attend a Fungus Foray such as those organized in the spring and autumn by mycological and biological societies. I owe much of my own mycological education to such friendly gatherings. A second aim has been to produce original illustrations of the kind that a student could make for himself from simple preparations of living material, and to illustrate things which he can verify for himself. For this reason I have chosen not to use electron micrographs, but to make drawings based on them.

The problem of what to include has been decided on the criterion of ready availability. Where an uncommon fungus has been included this is because it has been used to establish some important fact or principle. A criticism which I must accept is that no attempt has been made to deal with Fungi Imperfecti as a group. This is not because they are not common or important but that to have included them would have made the book much longer. To mitigate this shortcoming I have described the conidial states of some Ascomycotina rather fully, to include reference to some of the form-genera which have been linked with them. A more difficult problem has been to know which system of classification to adopt. I have finally chosen the 'General Purpose Classification' proposed by Ainsworth, which is adequate for the purpose of providing a framework of reference. I recognize that some might wish to classify fungi differently, but see no great merit in burdening the student with the arguments in favour of this or that system.

Because the evidence for the evolutionary origins of fungi is so meagre I have made only scant reference to the speculations which have been made on this topic. There are so many observations which can be verified, and for this reason I have preferred to leave aside those which never will.

The literature on fungi is enormous, and expanding rapidly. Many undergraduates do not have much time to check original publications. However, since the book is intended as an introduction I have tried to give references to some of the more recent literature, and at the same time to quote the origins of some of the statements made.

Exeter, 27 April 1970 J.W.

Preface to the second edition

In revising the first edition, which was first published about ten years ago, I have taken the opportunity to give a more complete account of the Myxomycota, and to give a more general introduction to the Eumycota. An account has also been given of some conidial fungi, as exemplified by aquatic Fungi Imperfecti, nematophagous fungi and seed-borne fungi. The taxonomic framework has been based on Volumes IVA and IVB of Ainsworth, Sparrow and Sussman's *The Fungi: An Advanced Treatise* (Academic Press, 1973).

Exeter, January 1979 J.W.

Preface to the third edition

Major advances, especially in DNA-based technology, have catalysed a sheer explosion of mycological knowledge since the second edition of *Introduction to Fungi* was published some 25 years ago. As judged by numbers of publications, the field of molecular phylogeny, i.e. the computer-aided comparison of homologous DNA or protein sequences, must be at the epicentre of these developments. As a result, information is now available to facilitate the establishment of taxonomic relationships between organisms or groups of organisms on a firmer basis than that previously assumed from morphological resemblance. This has in turn led to revised systems of classification and provided evidence on which to base opinions on the possible evolutionary origin of fungal groups. We have attempted to reflect some of these advances in this edition. In general we have followed the outline system of classification set out in *The Mycota* Volume VII (Springer-Verlag) and the *Dictionary of the Fungi* (ninth edition, CABI Publishing). However, the main emphasis of our book remains that of presenting the fungi in a sensible biological context which can be understood by students, and therefore some fungi have been treated along with taxonomically separate groups if these share fundamental biological principles. Examples include *Microbotryum*, which is treated together with smut fungi rather than the rusts to which it belongs taxonomically, or *Haptoglossa*, which we discuss alongside *Plasmodiophora* rather than with the Oomycota.

Molecular phylogeny has been instrumental in clarifying the relationships of anamorphic fungi (fungi imperfecti), presenting an opportunity to integrate their treatment with sexually reproducing relatives. There are only a few groups such as nematophagous fungi and the aquatic and aero-aquatic hyphomycetes which we continue to treat as ecological entities rather than scattered among ascomycetes and basidiomycetes. Similarly, the gasteromycetes, clearly an unnatural assemblage, are described together because of their unifying biological features. However, in all these cases taxonomic affinities are indicated where known. We have also included several groups now placed well outside the Fungi, such as the Oomycota (Straminipila) and Myxomycota and Plasmodiophoromycota (Protozoa). This is because of their biological and economic importance and because they have been and continue to be studied by mycologists.

There have been major advances in other areas of research, notably the molecular cell biology of the two yeasts *Saccharomyces* and *Schizosaccharomyces*, 'model organisms' which have a bearing far beyond mycology. Further, much exciting progress is being made in elucidating the molecular aspects of the infection biology of human and plant pathogens, and in developing fungi for biotechnology. These trends are represented in the current edition. Nevertheless, the fundamental concept of *Introduction to Fungi* remains that of the previous two editions: to place an organism in its taxonomic context while discussing as many relevant aspects of its biology as possible in a holistic manner. Many of the illustrations are based on original line drawings because we believe that these can readily portray an understanding of structure and that drawing as a record of interpretation is a good discipline. However, we have also extended the use of photographs, and we now provide illustrated life cycles because these are more easily understood. As before, our choice of illustrated species has been influenced by the ready availability of material, enabling students and their teachers to examine living fungi, which is a cornerstone of good teaching. At their first introduction most technical terms have been printed in bold, their meanings explained and their derivations given. The page numbers where these definitions are given have been highlighted in the index.

The discipline of mycology has evolved and diversified so enormously in recent decades that it is now a daunting task for individual authors to give a balanced, integrated account of the fungi. Of course, there will be omissions or

misrepresentations in a work of this scale, and we offer our apologies to those who feel that their work or that of others has not been adequately covered. At the same time, it has been a fascinating experience for us to write this book, and we have thoroughly enjoyed the immense diversity of approaches and ideas which make mycology such a vibrant discipline at present. We hope to have conveyed some of its fascination to the reader in the text and by referring to as many original publications as possible.

Exeter and Kaiserslautern, 1 March 2006

J.W. and R.W.S.W.

Acknowledgements

We are indebted to many people who have helped us in our extensive revisions to *Introduction to Fungi*. This edition is dedicated to Mr Philip M. Booth in profound gratitude for his financial support and his encouragement over many years. We have acknowledged in the figure legends the many friends and colleagues who have responded so enthusiastically to our call for help by providing us with illustrations, sometimes previously unpublished, and we thank numerous publishing houses for permission to include published figures. We thank Caroline Huxtable and Rob Ford (Exeter University Library) and Jennifer Mergel and Petra Tremmel (Kaiserslautern University Library) for help beyond the call of duty in obtaining interlibrary loans. Dr Wolf-Rüdiger Arendholz and Dr Roger T.A. Cook have read the entire manuscript or parts of it, and their feedback and corrections have been most valuable to us. We are immensely grateful to Professors Heidrun and Timm Anke (Kaiserslautern) for their support of this project, their encouragement and for providing such a stimulating environment for research and teaching of fungal biology.

By far the heaviest toll has been paid by our families and friends who have had only cursory sightings of us during the past six years. We owe a debt of gratitude to them for their patient forbearance and unwavering support.

1

Introduction

1.1 What are fungi?

About 80 000 to 120 000 species of fungi have been described to date, although the total number of species is estimated at around 1.5 million (Hawksworth, 2001; Kirk *et al*., 2001). This would render fungi one of the least-explored biodiversity resources of our planet. It is notoriously difficult to delimit fungi as a group against other eukaryotes, and debates over the inclusion or exclusion of certain groups have been going on for well over a century. In recent years, the main arguments have been between taxonomists striving towards a phylogenetic definition based especially on the similarity of relevant DNA sequences, and others who take a biological approach to the subject and regard fungi as organisms sharing all or many key ecological or physiological characteristics – the 'union of fungi' (Barr, 1992). Being interested mainly in the way fungi function in nature and in the laboratory, we take the latter approach and include several groups in this book which are now known to have arisen independently of the monophyletic 'true fungi' (**Eumycota**) and have been placed outside them in recent classification schemes (see Fig. 1.25). The most important of these 'pseudofungi' are the Oomycota (see Chapter 5). Based on their lifestyle, fungi may be circumscribed by the following set of characteristics (modified from Ainsworth, 1973):

1. *Nutrition*. Heterotrophic (lacking photosynthesis), feeding by absorption rather than ingestion.
2. *Vegetative state*. On or in the substratum, typically as a non-motile mycelium of hyphae showing internal protoplasmic streaming. Motile reproductive states may occur.
3. *Cell wall*. Typically present, usually based on glucans and chitin, rarely on glucans and cellulose (Oomycota).
4. *Nuclear status*. Eukaryotic, uni- or multinucleate, the thallus being homo- or heterokaryotic, haploid, dikaryotic or diploid, the latter usually of short duration (but exceptions are known from several taxonomic groups).
5. *Life cycle*. Simple or, more usually, complex.
6. *Reproduction*. The following reproductive events may occur: sexual (i.e. nuclear fusion and meiosis) and/or parasexual (i.e. involving nuclear fusion followed by gradual de-diploidization) and/or asexual (i.e. purely mitotic nuclear division).
7. *Propagules*. These are typically microscopically small spores produced in high numbers. Motile spores are confined to certain groups.
8. *Sporocarps*. Microscopic or macroscopic and showing characteristic shapes but only limited tissue differentiation.
9. *Habitat*. Ubiquitous in terrestrial and freshwater habitats, less so in the marine environment.
10. *Ecology*. Important ecological roles as saprotrophs, mutualistic symbionts, parasites, or hyperparasites.
11. *Distribution*. Cosmopolitan.

With photosynthetic pigments being absent, fungi have a heterotrophic mode of nutrition. In contrast to animals which typically feed by ingestion, fungi obtain their nutrients by extracellular digestion due to the activity of secreted enzymes, followed by absorption of the solubilized breakdown products. The combination of extracellular digestion and absorption can be seen as the ultimate determinant of the fungal lifestyle. In the course of evolution, fungi have conquered an astonishingly wide range of habitats, fulfilling important roles in diverse ecosystems (Dix & Webster, 1995). The conquest of new, often patchy resources is greatly facilitated by the production of numerous small spores rather than a few large propagules, whereas the colonization of a food source, once reached, is achieved most efficiently by growth as a system of branching tubes, the **hyphae** (Figs. 1.1a,b), which together make up the **mycelium**.

Hyphae are generally quite uniform in different taxonomic groups of fungi. One of the few features of distinction that they do offer is the presence or absence of cross-walls or **septa**. The Oomycota and Zygomycota generally have aseptate hyphae in which the nuclei lie in a common mass of cytoplasm (Fig. 1.1a). Such a condition is described as **coenocytic** (Gr. *koinos* = shared, in common; *kytos* = a hollow vessel, here meaning cell). In contrast, Asco- and Basidiomycota and their associated asexual states generally have septate hyphae (Fig. 1.1b) in which each segment contains one, two or more nuclei. If the nuclei are genetically identical, as in a mycelium derived from a single uninucleate spore, the mycelium is said to be **homokaryotic**, but where

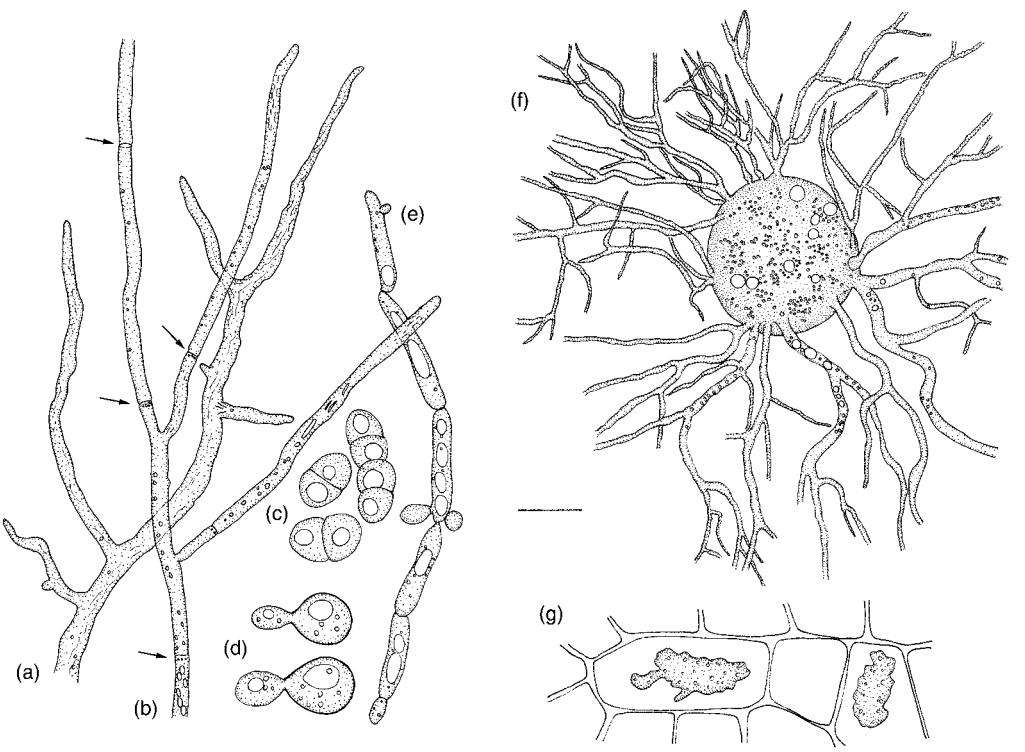

Fig 1.1 Various growth forms of fungi. (a) Aseptate hypha of *Mucor mucedo* (Zygomycota). The hypha branches to form a mycelium. (b) Septate branched hypha of *Trichoderma viride* (Ascomycota). Septa are indicated by arrows. (c) Yeast cells of *Schizosaccharomyces pombe* (Ascomycota) dividing by binary fission. (d) Yeast cells of *Dioszegia takashimae* (Basidiomycota) dividing by budding. (e) Pseudohypha of *Candida parapsilosis* (Ascomycota), which is regarded as an intermediate stage between yeast cells and true hyphae. (f) Thallus of *Rhizophlyctis rosea* (Chytridiomycota) from which a system of branching rhizoids extends into the substrate. (g) Plasmodia of *Plasmodiophora brassicae* (Plasmodiophoromycota) inside cabbage root cells. Scale bar = 20 μm (a,b,f,g) or 10 μm (c–e).

a cell or mycelium contains nuclei of different genotype, e.g. as a result of fusion (**anastomosis**) of genetically different hyphae, it is said to be **heterokaryotic**. A special condition is found in the mycelium of many Basidiomycota in which each cell contains two genetically distinct nuclei. This condition is **dikaryotic**, to distinguish it from mycelia which are **monokaryotic**. It should be noted that septa, where present, are usually perforated and allow for the exchange of cytoplasm or organelles.

Not all fungi grow as hyphae. Some grow as discrete **yeast** cells which divide by fission (Fig. 1.1c) or, more frequently, budding (Fig. 1.1d). Yeasts are common, especially in situations where efficient penetration of the substratum is not required, e.g. on plant surfaces or in the digestive tracts of animals (Carlile, 1995). A few species, including certain pathogens of humans and animals, are **dimorphic**, i.e. capable of switching between hyphal and yeast-like growth forms (Gow, 1995). Intermediate stages between yeast cells and true hyphae also occur and are termed **pseudohyphae** (Fig. 1.1e). Some lower fungi grow as a **thallus**, i.e. a walled structure in which the protoplasm is concentrated in one or more centres from which root-like branches (**rhizoids**) ramify (Fig. 1.1f). Certain obligately plant-pathogenic fungi and fungus-like organisms grow as a naked **plasmodium** (Fig. 1.1g), a uni- or multinucleate mass of protoplasm not surrounded by a cell wall of its own, or as a **pseudoplasmodium** of amoeboid cells which retain their individual plasma membranes. However, by far the most important device which accounts for the typical biological features of fungi is the hypha (Bartnicki-Garcia, 1996), which therefore seems an appropriate starting point for an exploration of these organisms.

1.2 Physiology of the growing hypha

1.2.1 Polarity of the hypha

By placing microscopic markers such as small glass beads beside a growing hypha, Reinhardt (1892) was able to show that cell wall extension, measured as an increase in the distance between two adjacent markers, occurred only at the extreme apex. Four years earlier, H.M. Ward (1888), in an equally simple experiment, had collected liquid droplets from the apex of hyphae of *Botrytis cinerea* and found that these 'ferment-drops' were capable of degrading plant cell walls. Thus, the two fundamental properties of the vegetative fungal hypha – the polarity of both growth and secretion of degradative enzymes – have been known for over a century. Numerous studies have subsequently confirmed that 'the key to the fungal hypha lies in the apex' (Robertson, 1965), although the detailed mechanisms determining hyphal polarity are still obscure.

Ultrastructural studies have shown that many organelles within the growing hyphal tip are distributed in steep gradients, as would be expected of a cell growing in a polarized mode (Girbardt, 1969; Howard, 1981). This is visible even with the light microscope by careful observation of an unstained hypha using phase-contrast optics (Reynaga-Peña *et al*., 1997), and more so with the aid of simple staining techniques (Figs. 1.2a–d). The cytoplasm of the extreme apex is occupied almost exclusively by secretory vesicles and microvesicles (Figs. 1.2a, 1.3). In the higher fungi (Asco- and Basidiomycota), the former are arranged as a spherical shell around the latter, and the entire formation is called the **Spitzenkörper** or 'apical body' (Fig. 1.4c; Bartnicki-Garcia, 1996). The Spitzenkörper may be seen in growing hyphae even with the light microscope. Hyphae of the Oomycota and some lower Eumycota (notably the Zygomycota) do not contain a recognizable Spitzenkörper, and the vesicles are instead distributed more loosely in the apical dome (Fig. 1.4a,b). Hyphal growth can be simulated by means of computer models based on the assumption that the emission of secretory vesicles is coordinated by a 'vesicle supply centre', regarded as the mathematical equivalent of the Spitzenkörper in higher fungi. By modifying certain parameters, it is even possible to generate the somewhat more pointed apex often found in hyphae of Oomycota and Zygomycota (Figs. 1.4a,b; Diéguez-Uribeondo *et al*., 2004).

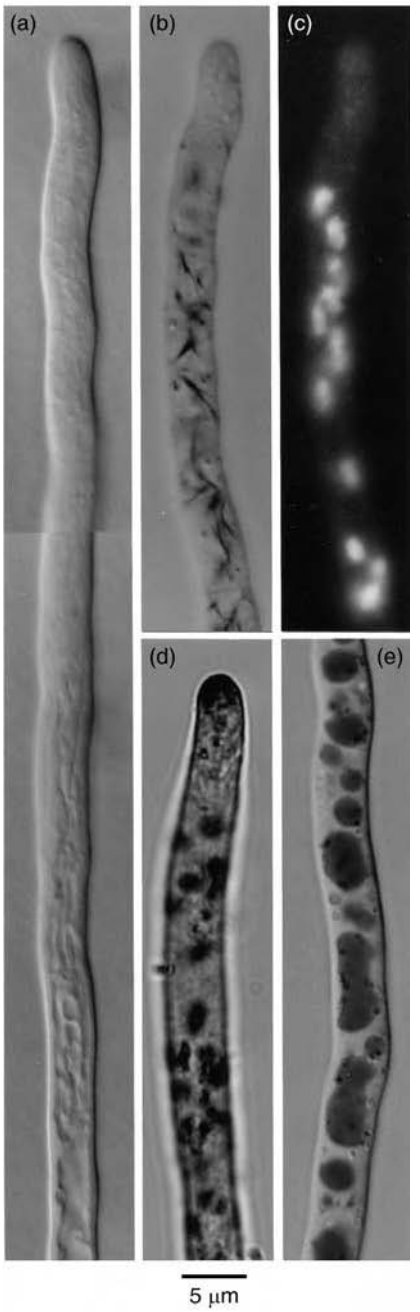

Fig I.2 The organization of vegetative hyphae as seen by light microscopy. (a) Growing hypha of *Galactomyces candidus* showing the transition from dense apical to vacuolate basal cytoplasm. Tubular vacuolar continuities are also visible. (b–e) Histochemistry in *Botrytis cinerea.* (b) Tetrazolium staining for mitochondrial succinate dehydrogenase. The mitochondria appear as dark filamentous structures in subapical and maturing regions. (c) Staining of the same hypha for nuclei with the fluorescent DNA-binding dye DAPI. The apical cell contains numerous nuclei. (d) Staining of acid phosphatase activity using the Gomori lead-salt method with a fixed hypha. Enzyme activity is localized both in the secretory vesicles forming the Spitzenkörper, and in vacuoles. (e) Uptake of Neutral Red into vacuoles in a mature hyphal segment. All images to same scale.

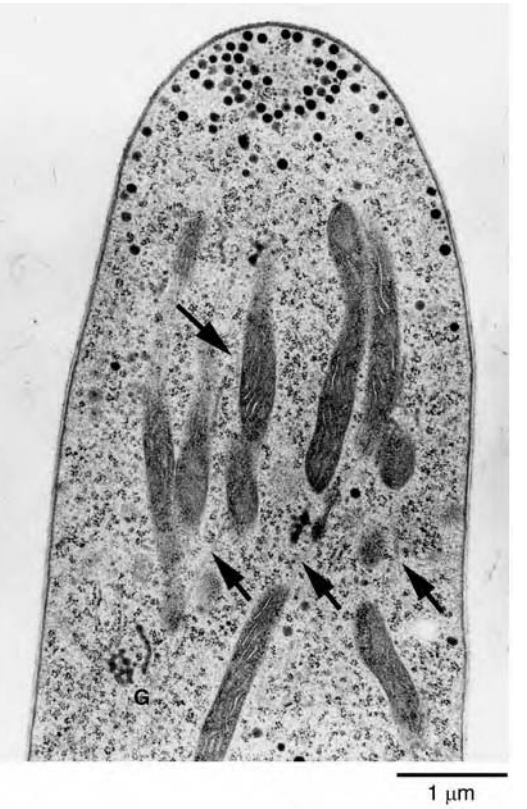

Fig I.3 Transmission electron microscopy of a hyphal tip of *Fusarium acuminatum* preserved by the freeze-substitution method to reveal ultrastructural details. The vesicles of the Spitzenkörper as well as mitochondria (dark elongated organelles), a Golgi-like element (G) and microtubules (arrows) are visible. Microtubules are closely associated with mitochondria. Reproduced from Howard and Aist (1980), by copyright permission of The Rockefeller University Press.

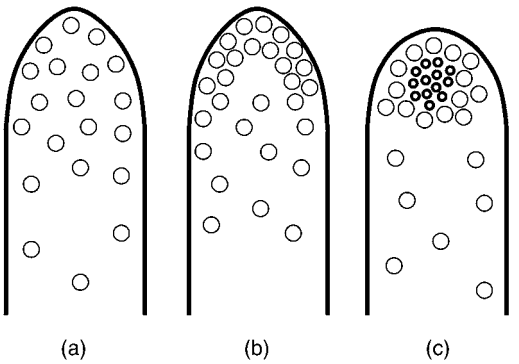

Fig 1.4 Schematic drawings of the arrangement of vesicles in growing hyphal tips. Secretory vesicles are visible in all hyphal tips, but the smaller microvesicles (chitosomes) are prominent only in Asco- and Basidiomycota and contribute to the Spitzenkörper morphology of the vesicle cluster. (a) Oomycota. (b) Zygomycota. (c) Ascomycota and Basidiomycota.

A little behind the apical dome, a region of intense biosynthetic activity and energy generation is indicated by parallel sheets of endoplasmic reticulum and an abundance of mitochondria (Figs. 1.2b, 1.3). The first nuclei usually appear just behind the biosynthetic zone (Fig. 1.2c), followed ultimately by a system of ever-enlarging vacuoles (Fig. 1.2d). These may fill almost the entire volume of mature hyphal regions, making them appear empty when viewed with the light microscope.

1.2.2 Architecture of the fungal cell wall

Although the chemical composition of cell walls can vary considerably between and within different groups of fungi (Table 1.1), the basic design seems to be universal. It consists of a structural scaffold of fibres which are cross-linked, and a matrix of gel-like or crystalline material (Hunsley & Burnett, 1970; Ruiz-Herrera, 1992; Sentandreu *et al*., 1994). The degree of cross-linking will determine the plasticity (extensibility) of the wall, whereas the pore size (permeability) is a property of the wall matrix. The scaffold forms the inner layer of the wall and the matrix is found predominantly in the outer layer (de Nobel *et al*., 2001).

In the Ascomycota and Basidiomycota, the fibres are **chitin** microfibrils, i.e. bundles of linear β-(1,4)-linked *N*-acetylglucosamine chains (Fig. 1.5), which are synthesized at the plasma membrane and extruded into the growing ('nascent') cell wall around the apical dome. The cell wall becomes rigid only after the microfibrils have been fixed in place by cross-linking. These cross-links consist of highly branched **glucans** (glucose polymers), especially those in which the glucose moieties are linked by β-(1,3)- and β-(1,6)-bonds (Suarit *et al*., 1988; Wessels *et al*., 1990; Sietsma & Wessels, 1994). Such β-glucans are typically insoluble in alkaline solutions (1 M KOH). In contrast, the alkali-soluble glucan fraction contains mainly α-(1,3)- and/or α-(1,4)-linked branched or unbranched chains (Wessels *et al*., 1972; Bobbitt & Nordin, 1982) and does not perform a structural role but instead contributes significantly to the cell wall matrix (Sietsma & Wessels, 1994). Proteins represent the third important chemical

Table 1.1 The chemical composition of cell walls of selected groups of fungi (dry weight of total cell wall fraction, in per cent). Data adapted from Ruiz-Herrera (1992) and Griffin (1994).

Group	Example	Chitin	Cellulose	Glucans	Protein	Lipid
Oomycota	*Phytophthora*	0	25	65	4	2
Chytridiomycota	*Allomyces*	58	0	16	10	?
Zygomycota	*Mucor*	9*	0	44	6	8
Ascomycota	*Saccharomyces*	1	0	60	13	8
	Fusarium	39	0	29	7	6
Basidiomycota	*Schizophyllum*	5	0	81	2	?
	Coprinus	33	0	50	10	?

*Mainly chitosan.

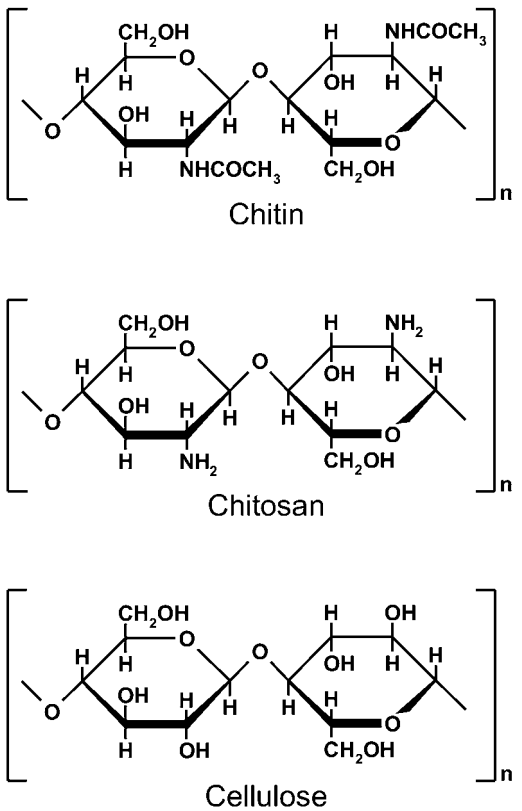

Fig 1.5 Structural formulae of the principal fibrous components of fungal cell walls.

constituent of fungal cell walls. In addition to enzymes involved in cell wall synthesis or lysis, or in extracellular digestion, there are also structural proteins. Many cell wall proteins are modified by glycosylation, i.e. the attachment of oligosaccharide chains to the polypeptide. The degree of glycosylation can be very high, especially in the yeast *Saccharomyces cerevisiae*, where up to 90% of the molecular weight of an extracellular protein may be contributed by its glycosylation chains (van Rinsum *et al*., 1991). Since mannose is the main component, such proteins are often called **mannoproteins** or mannans. In *S. cerevisiae*, the pore size of the cell wall is determined not by matrix glucans but by mannoproteins located close to the external wall surface (Zlotnik *et al*., 1984). Proteins exposed at the cell wall surface can also determine surface properties such as adhesion and recognition (Cormack *et al*., 1999). Structural proteins often contain a glycosylphosphatidylinositol anchor by which they are attached to the lumen of the rough endoplasmic reticulum (ER) and later to the external plasma membrane surface, or a modified anchor which covalently binds them to the β-(1,6)-glucan fraction of the cell wall (Kollár *et al*., 1997; de Nobel *et al*., 2001).

In the Zygomycota, the chitin fibres are modified after their synthesis by partial or complete deacetylation to produce poly-β-(1,4)-glucosamine, which is called **chitosan** (Fig. 1.5) (Calvo-Mendez & Ruiz-Herrera, 1987). Chitosan fibres are cross-linked by polysaccharides containing glucuronic acid and various neutral sugars (Datema *et al*., 1977). The cell wall matrix comprises glucans and proteins, as it does in members of the other fungal groups.

One traditional feature to distinguish the Oomycota from the 'true fungi' (Eumycota) has been the absence of chitin from their cell walls (Wessels & Sietsma, 1981), even though chitin is now known to be produced by certain species of Oomycota under certain conditions (Gay *et al*., 1993). By and large, however, in Oomycota, the structural role of chitin is filled by **cellulose**, an aggregate of linear β-(1,4)-glucan chains (Fig. 1.5). As in many other fungi, the fibres thus produced are cross-linked by an alkali-insoluble glucan containing β-(1,3)- and β-(1,6)-linkages. In addition to proteins, the main matrix component appears to be an alkali-soluble β-(1,3)-glucan (Wessels & Sietsma, 1981).

1.2.3 Synthesis of the cell wall

The synthesis of chitin is mediated by specialized organelles termed **chitosomes** (Bartnicki-Garcia *et al*., 1979; Sentandreu *et al*., 1994) in which inactive chitin synthases are delivered to the apical plasma membrane and become activated upon contact with the lipid bilayer (Montgomery & Gooday, 1985). Microvesicles, visible especially in the core region of the Spitzenkörper, are likely to be the ultrastructural manifestation of chitosomes (Fig. 1.6). In contrast, structural proteins and enzymes travel together in the larger secretory vesicles and are discharged into the environment when the vesicles fuse with the plasma membrane

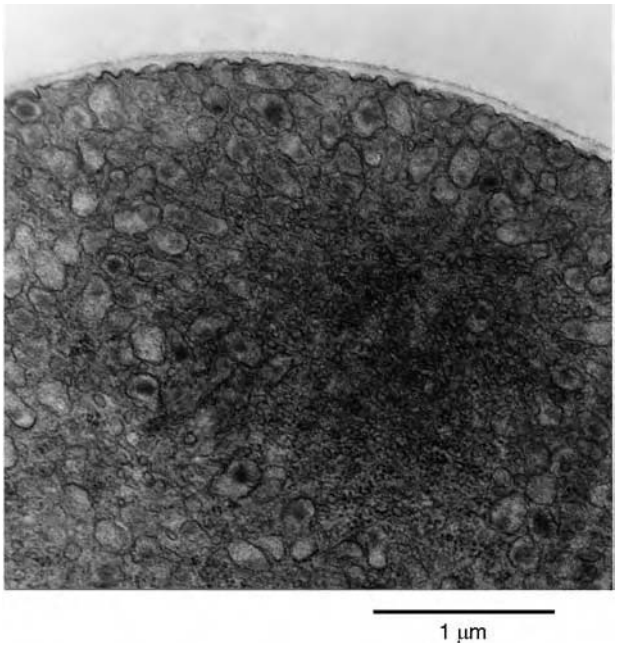

Fig 1.6 The Spitzenkörper of *Botrytis cinerea* which is differentiated into an electron-dense core consisting of microvesicles (chitosomes) and an outer region made up of larger secretory vesicles, some of which are located close to the plasma membrane. Reprinted from Weber and Pitt (2001), with permission from Elsevier.

(Fig. 1.6). Whereas most proteins are fully functional by the time they traverse the plasma membrane (see p. 10), the glucans are secreted by secretory vesicles as partly formed precursors (Wessels, 1993a) and undergo further polymerization in the nascent cell wall, or they are synthesized entirely at the plasma membrane (Sentandreu *et al*., 1994; de Nobel *et al*., 2001). Cross-linking of glucans with other components of the cell wall takes place after extrusion into the cell wall (Kollár *et al*., 1997; de Nobel *et al*., 2001).

Wessels *et al*. (1990) have provided experimental evidence to support a model for cell wall synthesis in *Schizophyllum commune* (Basidiomycota). The individual linear β-(1,4)-*N*-acetylglucosamine chains extruded from the plasma membrane are capable of undergoing self-assembly into chitin microfibrils, but this is subject to a certain delay during which cross-linking with glucans must occur. The glucans, in turn, become alkali-insoluble only after they have become covalently linked to chitin. Once the structural scaffold is in place, the wall matrix can be assembled. Wessels (1997) suggested that hyphal growth occurs as the result of a continuously replenished supply of soft wall material at the apex, but there is good evidence that the softness of the apical cell wall is also influenced by the activity of wall-lytic enzymes such as chitinases or glucanases (Fontaine *et al*., 1997; Horsch *et al*., 1997). Further, when certain Oomycota grow under conditions of hyperosmotic stress, their cell wall is measurably softer due to the secretion of an *endo*-β-(1,4)-glucanase, thus permitting continued growth when the turgor pressure is reduced or even absent (Money, 1994; Money & Hill, 1997). Since, in higher Eumycota, both cell wall material and synthetic as well as lytic enzymes are secreted together by the vesicles of the Spitzenkörper, the appearance, position and movement of this structure should influence the direction and speed of apical growth directly. This has indeed been shown to be the case (López-Franco *et al*., 1995; Bartnicki-Garcia, 1996; Riquelme *et al*., 1998).

Of course, cell wall-lytic enzymes are also necessary for the formation of hyphal branches, which usually arise by a localized weakening of the mature, fully polymerized cell wall. An *endo*-β-(1,4)-glucanase has also been shown to be involved in softening the mature regions of hyphae in the growing stipes of *Coprinus* fruit bodies, thus permitting intercalary hyphal extension (Kamada, 1994). Indeed, the expansion

of mushroom-type fruit bodies in general seems to be based mainly on non-apical extension of existing hyphae (see p. 22), which is a rare exception to the rule of apical growth in fungi.

The properties of the cell wall depend in many ways on the environment in which the hypha grows. Thus, when *Schizophyllum commune* is grown in liquid submerged culture, a significant part of the β-glucan fraction may diffuse into the liquid medium before it is captured by the cell wall, giving rise to mucilage (Sietsma *et al*., 1977). In addition to causing problems when growing fungi in liquid culture for experimental purposes, mucilage may cause economic losses when released by *Botrytis cinerea* in grapes to be used for wine production (Dubourdieu *et al*., 1978a). On the other hand, secreted polysaccharides, especially of Basidiomycota, may have interesting medicinal properties and are being promoted as anti-tumour medication both in conventional and in alternative medicine (Wasser, 2002).

Another difference between submerged and aerial hyphae is caused by the **hydrophobins**, which are structural cell wall proteins with specialized functions in physiology, morphogenesis and pathology (Wessels, 2000). Some hydrophobins are constitutively secreted by the hyphal apex. In submerged culture, they diffuse into the medium as monomers, whereas they polymerize by hydrophobic interactions on the surface of hyphae exposed to air, thereby effectively impregnating them and rendering them hydrophobic (Wessels, 1997, 2000). When freeze-fractured hydrophobic surfaces of hyphae or spores are viewed with the transmission electron microscope, polymerized hydrophobins may be visible as patches of rodlets running in parallel to each other. Other hydrophobins are produced only at particular developmental stages and are involved in inducing morphogenetic changes of the hypha, leading, for example, to the formation of spores or infection structures, or aggregation of hyphae into fruit bodies (Stringer *et al*., 1991; Wessels, 1997).

Some fungi are wall-less during the assimilative stage of their life cycle. This is true especially of certain plant pathogens such as the Plasmodiophoromycota (Chapter 3), insect pathogens (Entomophthorales; p. 202) and some members of the Chytridiomycota (Chapter 6). Since their protoplasts are in direct contact with the host cytoplasm, they are buffered against osmotic fluctuations. The motile spores (zoospores) of certain groups of fungi swim freely in water, and bursting due to osmotic inward movement of water is prevented by the constant activity of water-expulsion vacuoles.

1.2.4 The cytoskeleton

In contrast to the hyphae of certain Oomycota, which seem to grow even in the absence of measurable turgor pressure (Money & Hill, 1997), the hyphae of most fungi extend only when a threshold turgor pressure is exceeded. This can be generated even at a reduced external water potential by the accumulation of compatible solutes such as glycerol, mannitol or trehalose inside the hypha (Jennings, 1995). The correlation between turgor pressure and hyphal growth might be interpreted such that the former drives the latter, but this crude mechanism would lead to uncontrolled tip extension or even tip bursting. Further, when hyphal tips are made to burst by experimental manipulation, they often do so not at the extreme apex, but a little further behind (Sietsma & Wessels, 1994). It seems, therefore, that the soft wall at the apex is protected internally, and there is now good evidence that this is mediated by the cytoskeleton.

Both main elements of the cytoskeleton, i.e. microtobules (Figs. 1.7a,b) and actin filaments (Fig. 1.7c), are abundant in filamentous fungi and yeasts (Heath, 1994, 1995a). Intermediate filaments, which fulfil skeletal roles in animal cells, are probably of lesser significance in fungi. Microtubules are typically orientated longitudinally relative to the hypha (Fig. 1.7a) and are involved in long-distance transport of organelles such as secretory vesicles (Fig. 1.7b; Seiler *et al*., 1997) or nuclei (Steinberg, 1998), and in the positioning of mitochondria, nuclei or vacuoles (Howard & Aist, 1977; Steinberg *et al*., 1998). They therefore maintain the polarized distribution of many organelles in the hyphal tip.

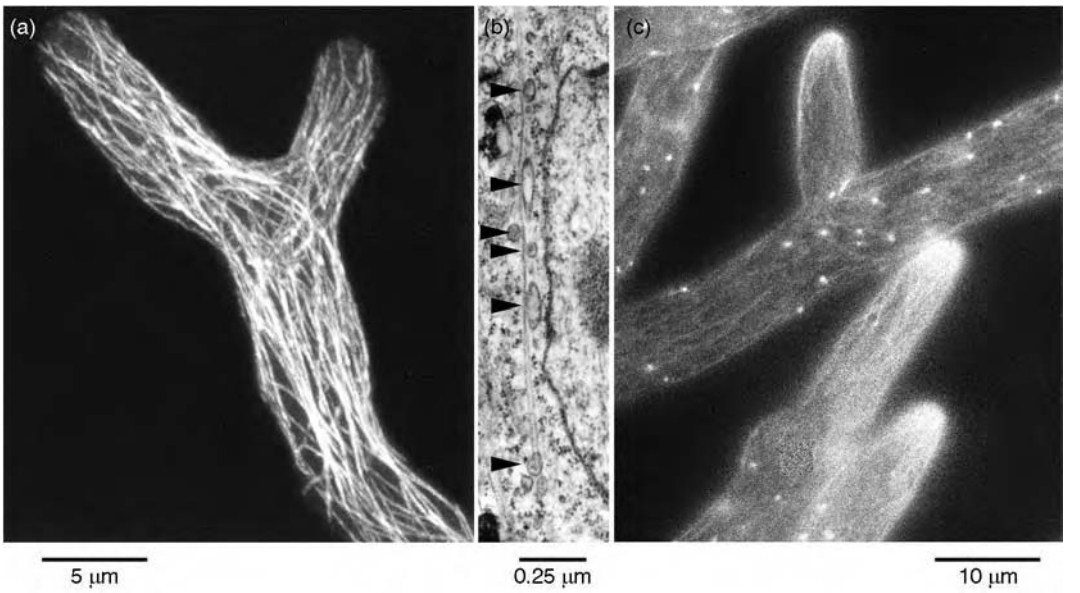

Fig I.7 The cytoskeleton in fungi. (a) Microtubules in *Rhizoctonia solani* (Basidiomycota) stained with an α-tubulin antibody. (b) Secretory vesicles (arrowheads) associated with a microtubule in *Botrytis cinerea* (Ascomycota). (c) The actin system of *Saprolegnia ferax* (Oomycota) stained with phalloidin–rhodamine. Note the dense actin cap in growing hyphal tips. (a) reproduced from Bourett *et al.* (1998), with permission from Elsevier; original print kindly provided by R. J. Howard. (b) reproduced from Weber and Pitt (2001), with permission from Elsevier. (c) reproduced from I. B. Heath (1987), by copyright permission of Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart; original print kindly provided by I. B. Heath.

Actin filaments are found in the centre of the Spitzenkörper, as discrete subapical patches, and as a cap lining the inside of the extreme hyphal apex (Heath, 1995a; Czymmek *et al*., 1996; Srinivasan *et al*., 1996). The apical actin cap is particularly pronounced in Oomycota such as *Saprolegnia* (Fig. 1.7c), and it now seems that the soft wall at the hyphal apex is actually being assembled on an internal scaffold consisting of actin and other structural proteins, such as spectrin (Heath, 1995b; Degousée *et al*., 2000). The rate of hyphal extension might be controlled, and bursting prevented, by the actin/spectrin cap being anchored to the rigid, subapical wall via rivet-like integrin attachments which traverse the membrane and might bind to wall matrix proteins (Fig. 1.8; Kaminskyj & Heath, 1996; Heath, 2001). Indeed, in *Saprolegnia* the cytoskeleton is probably responsible for pushing the hyphal tip forward, at least in the absence of turgor (Money, 1997), although it probably has a restraining function under normal physiological conditions. Heath (1995b) has proposed an ingenious if speculative model to explain how the actin cap might regulate the rate of hyphal tip extension in the Oomycota. Stretch-activated channels selective for Ca^{2+} ions are known to be concentrated in the apical plasma membrane of *Saprolegnia* (Garrill *et al*., 1993), and the fact that Ca^{2+} ions cause contractions of actin filaments is also well known. A stretched plasma membrane will admit Ca^{2+} ions into the apical cytoplasm where they cause localized contractions of the actin cap, thereby reducing the rate of apical growth which leads to closure of the stretch-activated Ca^{2+} channels. Sequestration of Ca^{2+} by various subapical organelles such as the ER or vacuoles lowers the concentration of free cytoplasmic Ca^{2+}, leading to a relaxation of the actin cap and of its restrictive effect on hyphal growth.

In the Eumycota, there is only indirect evidence for a similar role of actin, integrin and other structural proteins in protecting the apex and restraining its extension (Degousée *et al*., 2000; Heath, 2001), and the details of

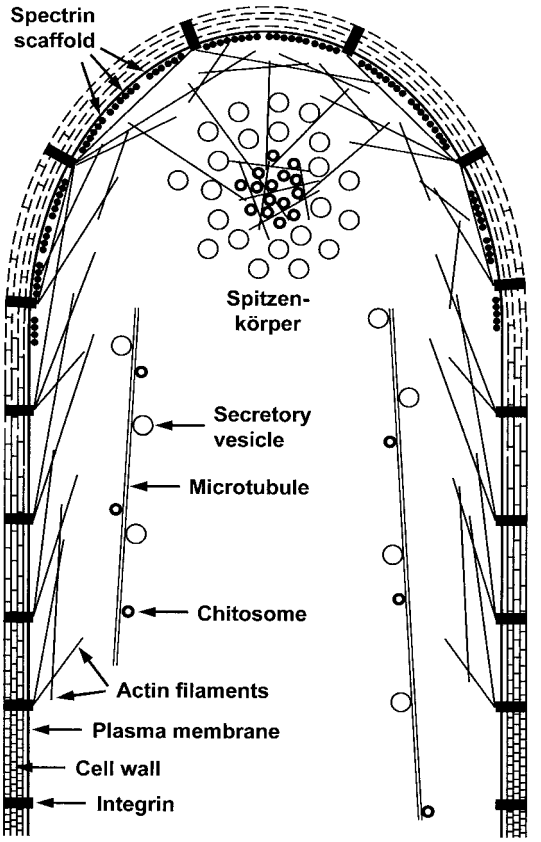

Fig 1.8 Diagrammatic representation of the internal scaffold model of tip growth in fungi proposed by Heath (1995b). Secretory vesicles and chitosomes are transported along microtubules from their subapical sites of synthesis to the growing apex. The Spitzenkörper forms around a cluster of actin filaments. An actin scaffold inside the extreme apex is linked to rivet-like integrin molecules which are anchored in the rigid subapical cell wall. The apex is further stabilized by spectrin molecules lining the cytoplasmic surface of the plasma membrane. Redrawn and modified from Weber and Pitt (2001).

regulation are likely to be different. Whereas a tip-high Ca^{2+} gradient is present and is required for growth, stretch-activated Ca^{2+} channels are not, and the apical Ca^{2+} seems to be of endogenous origin. Silverman-Gavrila and Lew (2001, 2002) have proposed that the signal molecule inositol-(1,4,5)-trisphosphate (IP_3), released by the action of a stretch-activated phospholipase C in the apical plasma membrane, acts on Ca^{2+}-rich secretory vesicles in the Spitzenkörper region. These would release Ca^{2+} from their lumen, leading to a contraction of the apical scaffold. As in the Oomycota, sequestration of Ca^{2+} occurs subapically by the ER from which secretory vesicles are formed. These therefore act as Ca^{2+} shuttles in the Eumycota (Torralba *et al*., 2001). Although hyphal tip growth appears to be a straightforward affair, none of the conflicting models accounts for all aspects of it. A good essay in hyphal tip diplomacy has been written by Bartnicki-Garcia (2002).

Numerous inhibitor studies have hinted at a role of the cytoskeleton in the transport of vesicles to the apex. Depolymerization of microtubules results in a disappearance of the Spitzenkörper, termination or at least severe reduction of apical growth and enzyme secretion, and an even redistribution of secretory vesicles and other organelles throughout the hypha (Howard & Aist, 1977; Rupeš *et al*., 1995; Horio & Oakley, 2005). In contrast, actin depolymerization leads to uncontrolled tip extension to form giant spheres (Srinivasan *et al*., 1996). Long-distance transport of secretory vesicles therefore seems to be brought about by microtubules, whereas the fine-tuning of vesicle fusion with the plasma membrane is controlled by actin (Fig. 1.8; Torralba *et al*., 1998). The integrity of the Spitzenkörper is maintained by an interplay between actin and tubulin. Not surprisingly, the yeast *S. cerevisiae*, which has a very short vesicle transport distance between the mother cell and the extending bud, reacts more sensitively to disruptions of the actin component than the microtubule component of its cytoskeleton; continued growth in the absence of the latter can be explained by Brownian motion of secretory vesicles (Govindan *et al*., 1995; Steinberg, 1998).

1.2.5 Secretion and membrane traffic

One of the most important ecological roles of fungi, that of decomposing dead plant matter, requires the secretion of large quantities of hydrolytic and oxidative enzymes into the environment. In liquid culture under optimized experimental conditions, certain fungi

are capable of secreting more than 20 g of a single enzyme or enzyme group per litre culture broth within a few days' growth (Sprey, 1988; Peberdy, 1994). Clearly, this aspect of fungal physiology holds considerable potential for biotechnological or pharmaceutical applications. However, for reasons not yet entirely understood, fungi often fail to secrete the heterologous proteins of introduced genes of commercial interest to the same high level as their own proteins (Gwynne, 1992). There are still great deficits in our understanding of the fundamental mechanisms of the secretory route in filamentous fungi, although much is known in the yeast *S. cerevisiae*. An overview is given in Fig. 1.10.

As in other eukaryotes, the secretory route in fungi begins in the ER. Ribosomes loaded with a suitable messenger RNA dock onto the ER membrane and translate the polypeptide product which enters the ER lumen during its synthesis unless specific internal signal sequences cause it to be retained in the ER membrane. As soon as the protein is in contact with the ER lumen, oligosaccharide chains may be added onto selected amino acids. These glycosylation chains are subject to successive modification steps as the protein traverses the secretory route, whereby the chains in *S. cerevisiae* become considerably larger than those in most filamentous fungi (Maras *et al*., 1997; Gemmill & Trimble, 1999). Paradoxically, even though filamentous fungi possess such powerful secretory systems, morphologically recognizable Golgi stacks have not generally been observed except for the Oomycota, Plasmodiophoromycota and related groups (Grove *et al*., 1968; Beakes & Glockling, 1998). In all other fungi, the Golgi apparatus seems to be much reduced to single cisternae (Howard, 1981; see Fig. 1.3), with images of fully fledged Golgi stacks only published occasionally (see e.g. Fig. 10.1). In *S. cerevisiae* and probably also in filamentous fungi, the transport of proteins from the ER to the Golgi system occurs via vesicular carriers (Schekman, 1992), although continuous membrane flow is also possible (see p. 272). Membrane lipids seem to be recycled to the ER by a different mechanism relying on tubular continuities (Rupeš *et al*., 1995; Akashi *et al*., 1997).

In the Golgi system, proteins are subjected to stepwise further modifications (Graham & Emr, 1991), and proteins destined for the vacuolar system are separated from those bound for secretion (Seeger & Payne, 1992). Both destinations are probably reached by vesicular carriers, the secretory vesicles moving along microtubules to reach the growing hyphal apex (Fig. 1.7b), which is the site for secretion of extracellular enzymes as well as new cell wall material (Peberdy, 1994). Collinge and Trinci (1974) estimated that 38 000 secretory vesicles per minute fuse with the plasma membrane of a single growing hypha of *Neurospora crassa*. Microvesicles (chitosomes) probably arise from a discrete population of Golgi cisternae (Howard, 1981).

There is mounting evidence that fungi, like most eukaryotes, are capable of performing endocytosis by the inward budding of the plasma membrane at subapical locations. Endocytosis may be necessary to retrieve membrane material in excess of that which is required for extension at the growing apex, i.e. endocytosis and exocytosis may be coupled (Steinberg & Fuchs, 2004). The prime destination of endocytosed membrane material or vital stains is the vacuole (Vida & Emr, 1995; Fischer-Parton *et al*., 2000; Weber, 2002). In fungi, large vacuoles (Figs. 1.2e, 1.9) represent the main element of the lytic system and are the sink not only for endocytosed material but also for autophagocytosis, i.e. the sequestration and degradation of organelles or cytoplasm. Autophagocytosis is especially prominent under starvation conditions (Takeshige *et al*., 1992). Careful ultrastructural studies have revealed that adjacent vacuoles may be linked by thin membranous tubes, thereby providing a potential means of transport (Rees *et al*., 1994). These tubes can extend even through the septal pores and show peristaltic movement, possibly explaining why especially mycorrhizal fungi are capable of rapid translocation of solutes over long hyphal distances (Fig. 1.9; Cole *et al*., 1998; Ashford *et al*., 2001).

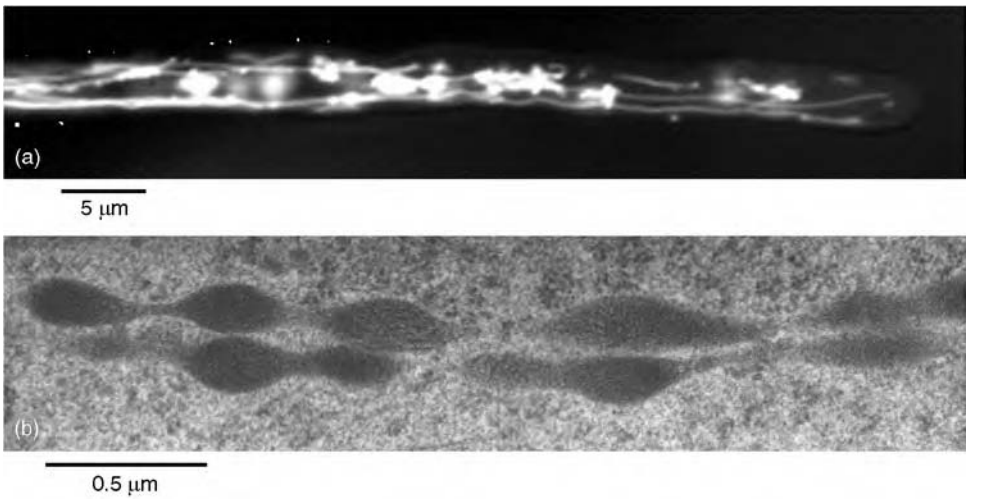

Fig 1.9 Tubular continuities linking adjacent vacuoles of *Pisolithus tinctorius*. (a) Light micrograph of the vacuolar system of *Pisolithus tinctorius* stained with a fluorescent dye. (b) TEM image of a freeze-substituted hypha. Reproduced from Ashford *et al*. (2001), with kind permission of Springer Science and Business Media. Original images kindly provided by A. E. Ashford.

1.2.6 Nutrient uptake

One of the hallmarks of fungi is their ability to take up organic or inorganic solutes from extremely dilute solutions in the environment, accumulating them 1000-fold or more against their concentration gradient (Griffin, 1994). The main barrier to the movement of water-soluble substances into the cell is the lipid bilayer of the plasma membrane. Uptake is mediated by proteinaceous pores in the plasma membrane which are always selective for particular solutes. The pores are termed **channels** (system I) if they facilitate the diffusion of a solute following its concentration gradient whilst they are called **porters** (system II) if they use metabolic energy to accumulate the solute across the plasma membrane against its gradient (Harold, 1994). Fungi often possess one channel and one porter for a given solute. The high-affinity porter system is repressed at high external solute concentrations such as those found in most laboratory media (Scarborough, 1970; Sanders, 1988).

In nature, however, the concentration of nutrients is often so low that the porter systems are active. Porters do not directly convert metabolic energy (ATP) into the uptake of solutes; rather, ATP is hydrolysed by ATPases which pump protons (H^+) to the outside of the plasma membrane, thus establishing a transmembrane pH gradient (acid outside). It has been estimated that one-third of the total cellular ATP is used for the establishment of the transmembrane H^+ gradient (Gradmann *et al*., 1978). The inward movement of H^+ following its electrochemical gradient is harnessed by the porters for solute uptake by means of solute–porter–H^+ complexes (Slayman & Slayman, 1974; Slayman, 1987; Garrill, 1995). Different types of porter exist, depending on the charge of the desired solute. Uniport and symport carriers couple the inward movement of H^+ with the uptake of uncharged or negatively charged solutes, respectively, whereas antiports harness the outward diffusion of cations such as K^+ for the uptake of other positively charged solutes. Charge imbalances can be rectified by the selective opening of K^+ channels. Porters have been described for NH_4^+, NO_3^-, amino acids, hexoses, orthophosphate and other solutes (Garrill, 1995; Jennings, 1995).

The ATPases fuelling active uptake mechanisms are located in subapical or mature regions of the plasma membrane, whereas the porter systems are typically situated in the apical membrane (Harold, 1994), closest to the site where the solutes may be released by the activity of extracellular enzymes. Thus, mature hyphal segments make a substantial direct contribution

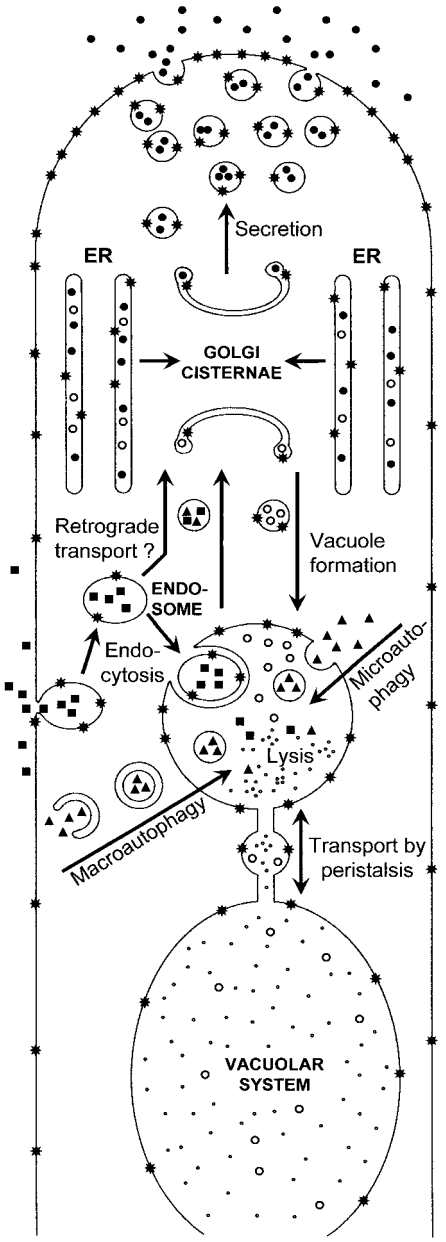

Fig 1.10 Schematic summary of the pathways of membrane flow in a growing hypha. Secretory proteins (•), vacuolar luminal proteins (○), membrane-bound proteins (✱), endocytosed (■) and autophagocytosed (▲) material is indicated, as are vacuolar degradation products (•). Redrawn and modified from Weber (2002).

to the growth of the hypha at its tip. The spatial separation of H^+ expulsion and re-entry generates an external electric field carried by protons (Fig. 1.11), which was at one time thought to be a causal factor of hyphal tip polarity but is now regarded as a consequence of it (Harold, 1994).

Proton pumps fuelled by ATP are prominent also in the vacuolar membrane, the tonoplast (Fig. 1.11), and their activity acidifies the vacuolar lumen (Klionsky *et al*., 1990). The principle of proton-coupled solute transport is utilized by the vacuole to fulfil its role as a system for the storage of nutrients, for example phosphate (Cramer & Davis, 1984) or amino acids such as arginine (Keenan & Weiss, 1997), or for the removal of toxic compounds from the cytoplasm, e.g. Ca^{2+} or heavy metal ions (Cornelius & Nakashima, 1987).

1.2.7 Hyphal branching

Assimilative hyphae of most fungi grow monopodially by a main axis (**leading hypha**) capable of potentially unlimited apical growth. Branches arise at some distance behind the apex, suggesting some form of apical dominance, i.e. the presence of a growing apex inhibits the development of lateral branches close to it. Dichotomous branching is rare, but does occur in *Allomyces* (see Fig. 6.20d) and *Galactomyces geotrichum*. In septate fungi, branches are often located immediately behind a septum. Branches usually arise singly in vegetative hyphae, although whorls of branches (i.e. branches arising near a common point) occur in reproductive structures. Branching may thus be under genetic or external control (Burnett, 1976). An even spacing between vegetative hyphae results from a combination of chemotropic growth towards a source of diffusible nutrients, and growth away from staling products secreted by other hyphae which have colonized a substratum. The circular appearance of fungal colonies in Petri dish cultures arises because certain lateral branches grow out and fill the space between the leading radial branches, keeping pace with their rate of growth. This **invasive growth** is the most efficient way to spread throughout a substratum. In nature, it may be obvious even to the naked eye, for example, in the shape of fairy rings (see Figs. 19.18a,b).

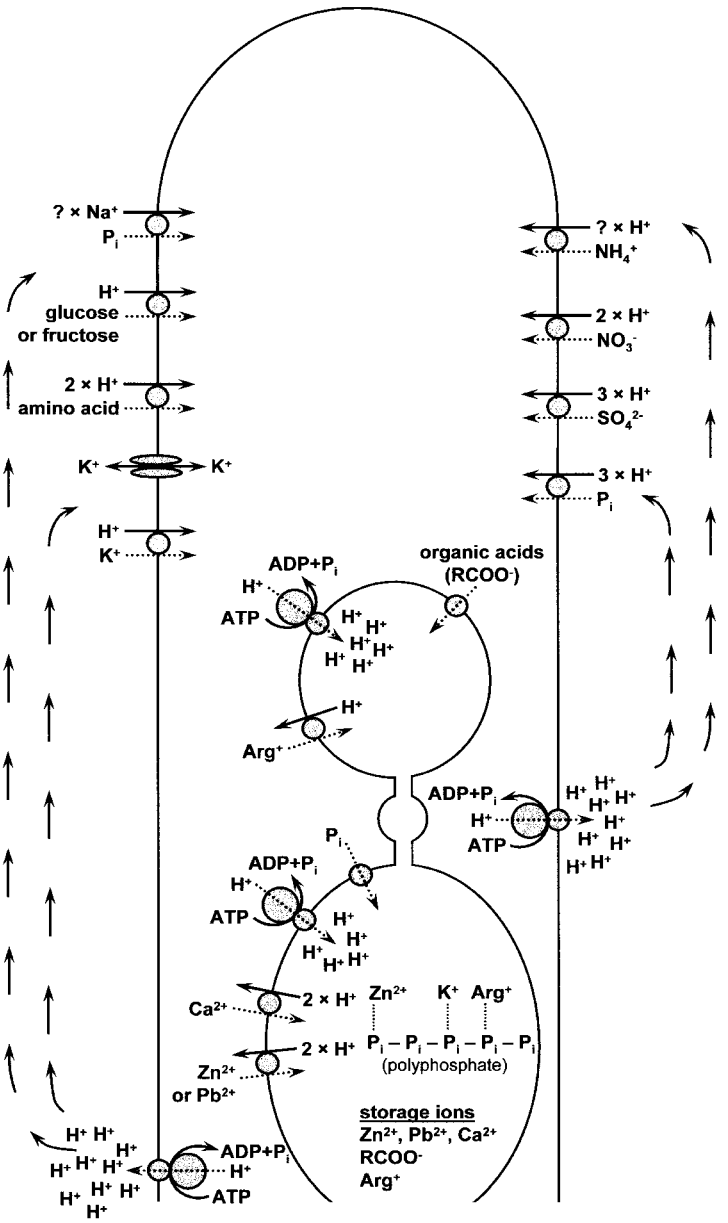

Fig 1.11 Ion fluxes in a growing hypha. The proton (H^+) gradient across the plasma membrane is generated by subapical ATP-driven expulsion of protons. It is used for the active uptake of nutrients by porters. Channels also exist for most of the nutrients but are not shown here, except for the K^+ channel which operates to compensate for charge imbalances. Dotted arrows indicate movement of a solute against its concentration gradient; solid arrows indicate movement from concentrated to dilute. For details, see Garrill (1995).

1.3 Hyphal aggregates

Whereas plants and animals form genuine tissues by their ability to perform cell divisions in all directions, fungi are limited by their growth as one-dimensional hyphae. None the less, fungi are capable of producing complex and characteristic multicellular structures which resemble the tissues of other eukaryotes. This must be controlled by the positioning, growth rate and growth direction of individual hyphal branches (Moore, 1994). Further, instead of spacing themselves apart as during invasive growth, hyphae must be made to aggregate. Very little is known about the signalling events

leading to the synchronized growth of groups of hyphae. However, it may be speculated that the diffusion of signalling molecules takes place between adjacent hyphae, i.e. that a given hypha is able to influence the gene expression of adjacent hyphae by secreting chemical messengers. This may be facilitated by an extrahyphal glucan matrix within which aggregating hyphae are typically embedded (Moore, 1994). Such matrices have been found in rhizomorphs (Rayner *et al*., 1985), sclerotia (Fig. 1.16c; Willetts & Bullock, 1992) and fruit bodies (Williams *et al*., 1985). The composition of proteins on the surface of hyphal walls may also play an important role in recognition and adhesion phenomena (de Nobel *et al*., 2001).

1.3.1 Mycelial strands

The formation of aggregates of parallel, relatively undifferentiated hyphae is quite common in the Basidiomycota and in some Ascomycota. For instance, mycelial strands form the familiar 'spawn' of the cultivated mushroom *Agaricus bisporus*. Strands arise most readily from a well-developed mycelium extending from an exhausted food base into nutrient-poor surroundings (Fig. 1.12a). When a strand encounters a source of nutrients exceeding its internal supply, coherence is lost and a spreading assimilative mycelium regrows (Moore, 1994). Alternatively, mycelial strands may be employed by fungi which produce their fructifications some distance away from the food base, as in the stinkhorn, *Phallus impudicus*. Here the mycelial strand is more tightly aggregated and is referred to as a **mycelial cord**. The tip of the mycelial cord, which arises from a buried tree stump, differentiates into an egg-like basidiocarp initially upon reaching the soil surface (Fig. 1.12b).

The development of *A. bisporus* strands has been described by Mathew (1961). Robust leading hyphae extend from the food base and branch at fairly wide intervals to form finer laterals, most of which grow away from the parent hypha. A few branch hyphae, however, form at an acute angle to the parent hypha and tend to grow parallel to it. Hyphae of many fungi occasionally

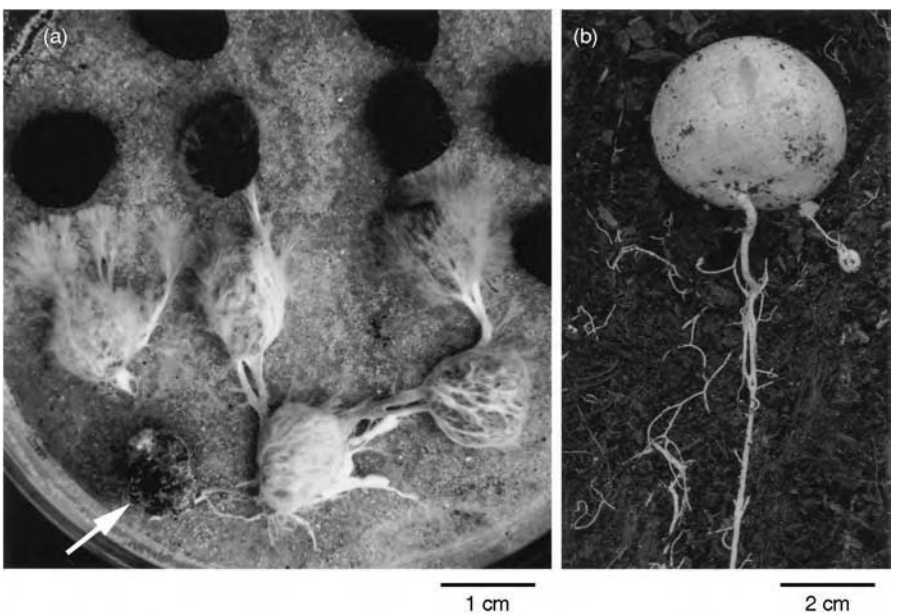

Fig 1.12 Mycelial strands. (a) Strands of *Podosordaria tulasnei* (Ascomycota) extending from a previously colonized rabbit pellet (arrow) over sand. Note the dissolution of the strand upon reaching a new nutrient source, in this case fresh sterile rabbit pellets. (b) Excavated mycelial cords of the stinkhorn *Phallus impudicus*, which can be traced back from the egg-like basidiocarp primordium to the base of an old tree stump (below the bottom of the picture, not shown).

grow alongside each other or another physical obstacle which they chance to encounter. A later and specific stage in strand development is characterized by the formation of numerous fine, aseptate 'tendril hyphae' as branches from the older regions of the main hyphae. The tendril hyphae, which may extend forwards or backwards, become appressed to the main hypha and branch frequently to form even finer tendrils which grow round the main hyphae and ensheath them. Major strands are consolidated by anastomoses between their hyphae, and they increase in thickness by the assimilation of minor strands. A similar development has been noted in the strands of *Serpula lacrymans*, the dry-rot fungus (Fig. 1.13), which are capable of extending for several metres across brickwork and other surfaces from a food base in decaying wood (Jennings & Watkinson, 1982; Nuss *et al*., 1991).

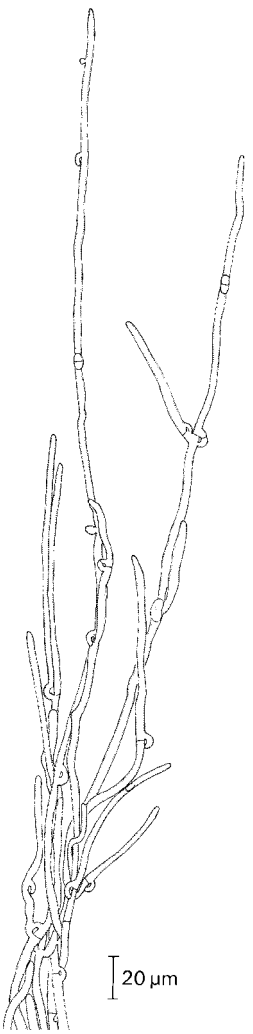

Fig 1.13 The tip of a hyphal strand of *Serpula lacrymans* (Basidiomycota). Note the formation of lateral branches which grow parallel to the direction of the main hyphae. The buckle-shaped structures at the septa are clamp connections.

By recovering the nutrients from obsolete strands and forming new strands, colonies can move about and explore their vicinity in the search for new food bases (Cooke & Rayner, 1984; Boddy, 1993). Mycelial strands are capable of translocating nutrients and water in both directions (Boddy, 1993; Jennings, 1995). This property is important not only for decomposer fungi, but also for species forming mycorrhizal symbioses with the roots of plants, many of which produce hyphal strands (Read, 1991).

1.3.2 Rhizomorphs

In contrast to mycelial strands or cords which consist of relatively undifferentiated aggregations of hyphae and are produced by a great variety of fungi, rhizomorphs are found in only relatively few species and contain highly differentiated tissues. Well-known examples of rhizomorph-forming fungi are provided by *Armillaria* spp. (Figs. 1.14 and 18.13b), which are serious parasites of trees and shrubs. In *Armillaria*, a central core of larger, thin-walled, elongated cells embedded in mucilage is surrounded by a rind of small, thicker-walled cells which are darkly pigmented due to melanin deposition in their walls. These root-like aggregations are a means for *Armillaria* to spread underground from one tree root system to another. In nature, two kinds are found – a dark, cylindrical type and a paler, flatter type. The latter is particularly common beneath the bark of infected trees (see p. 546). Rhizomorphs on dead trees measure up to 4 mm in diameter. It has been estimated that a rhizomorph only 1 mm in diameter must contain over 1000 hyphae aggregated together. The development of rhizomorphs in agar culture has been described by Garrett (1953, 1970) and Snider (1959). Initiation of rhizomorphs can first be

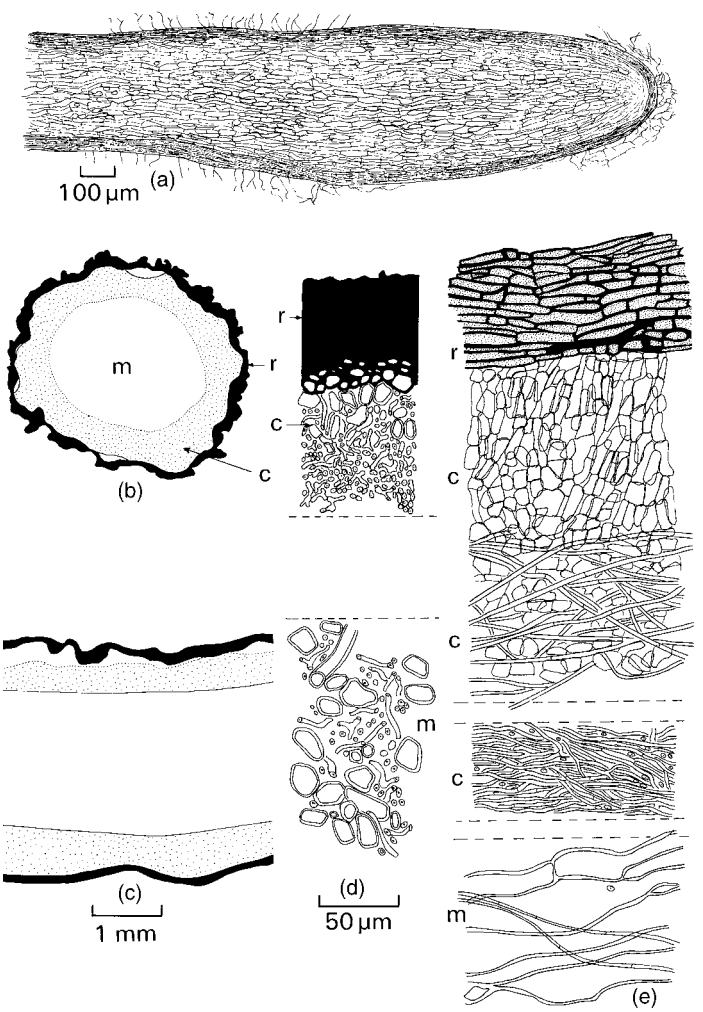

Fig 1.14 Rhizomorph structure of *Armillaria mellea* (Basidiomycota). (a) Longitudinal section. (b) Transverse section, diagrammatic. (c) L.S. diagrammatic. (d) T.S. showing details of cells in the rind (r), cortex (c) and medulla (m). (e) L.S. showing details of cells.

observed after about 7 days' mycelial growth on the agar surface as a compact mass of darkly pigmented hypertrophied cells. These pigmented structures have been termed **microsclerotia**. From white, non-pigmented points on their surface, the rhizomorphs develop. The growth of rhizomorphs can be several times faster than that of unorganized hyphae (Rishbeth, 1968). The most striking feature of the development of rhizomorphs is their compact growing point at the apex, which consists of small isodiametric cells protected by an apical cap of intertwined hyphae immersed in mucilage which they produce. Because of its striking similarity with a growing plant root, the rhizomorph tip was initially interpreted as a meristematic zone (Motta, 1967), but its hyphal nature can be demonstrated by careful ultrastructural observations (Powell & Rayner, 1983; Rayner *et al*., 1985). Behind the apex there is a zone of elongation. The centre of the rhizomorph may be hollow or solid. Surrounding the central lumen or making up the central medulla is a zone of enlarged hyphae 4–5 times wider than the vegetative hyphae (Fig. 1.14e). Possibly these **vessel hyphae** serve in translocation (Cairney, 1992; Jennings, 1995). Towards the periphery of the rhizomorph, the cells become smaller, darker, and thicker walled. Extending outwards between the outer cells of the rhizomorph, there may be a growth of vegetative hyphae somewhat resembling the root-hair zone in a higher plant. Rhizomorphs may develop on monokaryotic mycelia derived from single basidiospores, or on dikaryotic

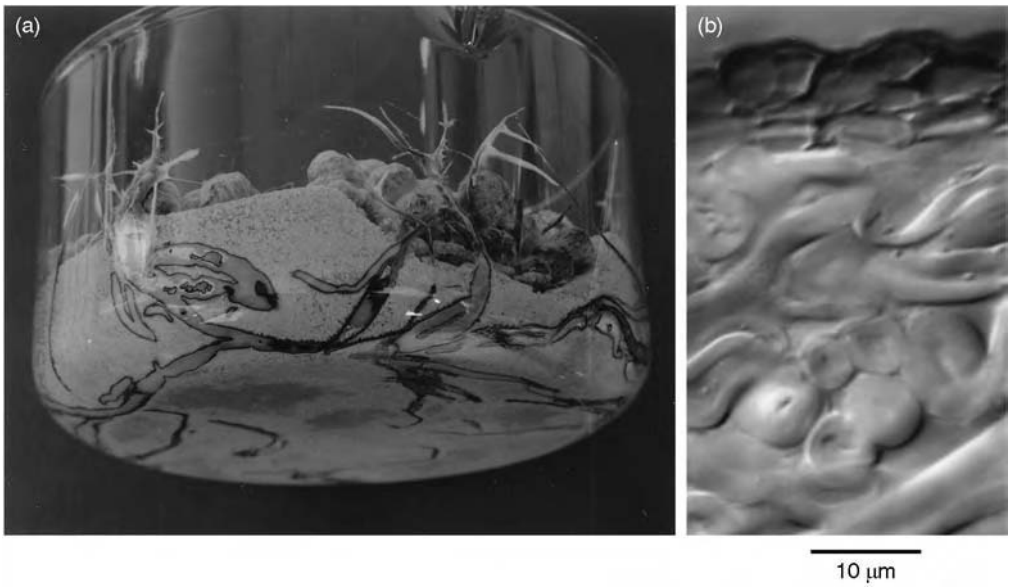

Fig 1.15 Rhizomorphs of *Podosordaria tulasnei* (Ascomycota). (a) Subterranean rhizomorphs by which the fungus spreads through the soil. (b) T.S. showing the dark rind (1–2 cells thick) and a cortex consisting of thick-walled hyaline cells.

mycelia following fusion of compatible monokaryotic hyphae. Dikaryotic rhizomorphs of *Armillaria* do not possess clamp connections (Hintikka, 1973).

Rhizomorphs are also produced by other Basidiomycota and a few Ascomycota (Fig. 1.15; Webster & Weber, 2000). They are mainly formed in soil. An interesting exception is presented by tropical *Marasmius* spp., which form a network of aerial rhizomorphs capable of intercepting falling leaves before they reach the ground (Hedger *et al*., 1993). Because these rhizomorphs have a rudimentary fruit body cap at their extending apex (Hedger *et al*., 1993), they have been interpreted as indefinitely extending fruit body stipes (Moore, 1994). Mycelial strands and rhizomorphs represent extremes in a range of hyphal aggregations, and several intergrading forms can be recognized (Rayner *et al*., 1985).

1.3.3 Sclerotia

Sclerotia are pseudoparenchymatous aggregations of hyphae embedded in an extracellular glucan matrix. A hard melanized rind may be present or absent. Sclerotia serve a survival function and contain intrahyphal storage reserves such as polyphosphate, glycogen, protein, and lipid (Willetts & Bullock, 1992). The glucan matrix, too, may be utilized as a carbohydrate source during sclerotium germination (Backhouse & Willetts, 1985). Sclerotia may also have a reproductive role and are the only known means of reproduction in certain species. They are produced by a relatively small number of Asco- and Basidiomycota, especially plant-pathogenic species such as *Rhizoctonia* spp. (p. 595), *Sclerotinia* spp. (p. 429) and *Claviceps purpurea* (p. 349). The form of sclerotia is very variable (Butler, 1966). The subterranean sclerotium of the Australian *Polyporus mylittae* (see Figs. 18.13c,d) can reach the size of a football and is known as native bread or blackfellow's bread. At the other extreme, they may be of microscopic dimensions consisting of a few cells only. Several kinds of development in sclerotia have been distinguished (Townsend & Willetts, 1954; Willetts, 1972).

The loose type

This is exemplified by *Rhizoctonia* spp., which are sclerotial forms of fungi belonging to the Basidiomycota. Sclerotia of the loose type are readily seen as the thin brownish-black scurfy scales so common on the surface of

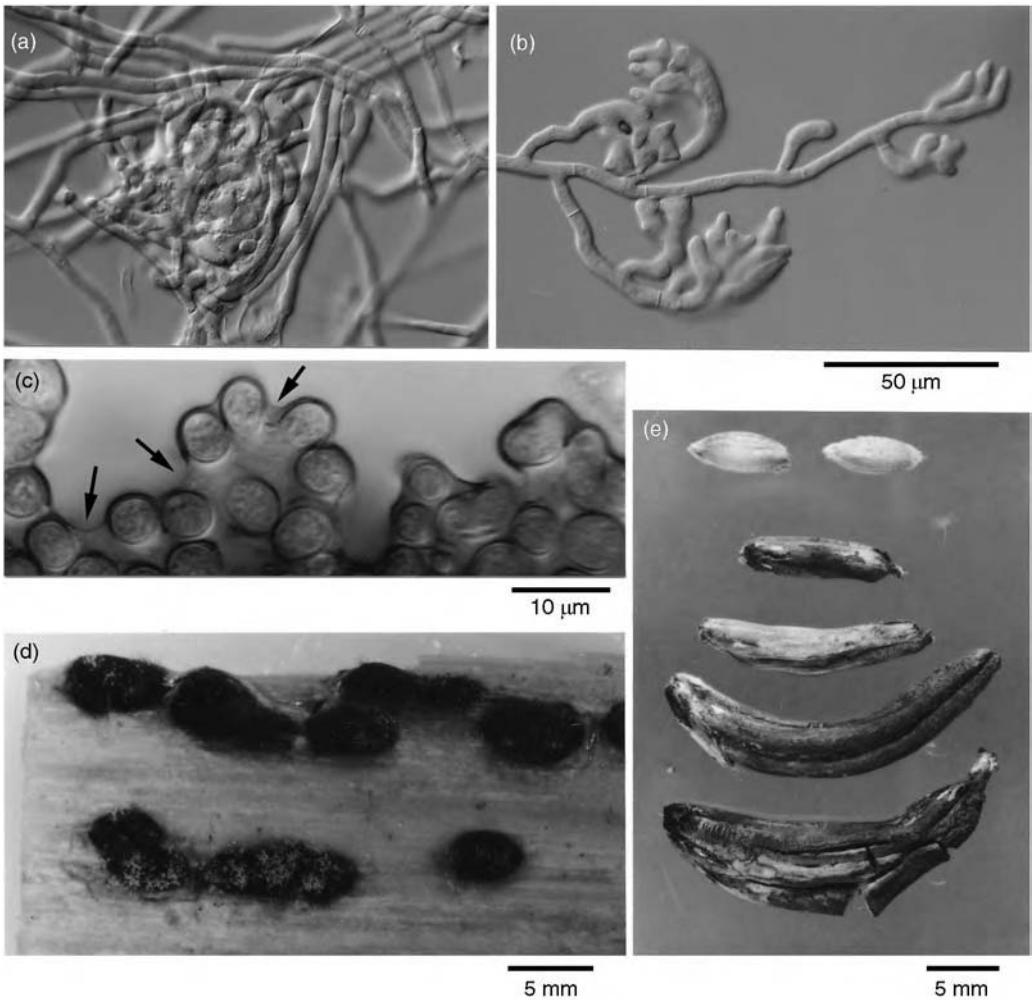

Fig 1.16 Development of sclerotia. (a) The loose type, as seen in *Rhizoctonia* (*Moniliopsis*) *solani*. (b) Hypha of *Botrytis cinerea* showing dichotomous branching on a glass coverslip to initiate the terminal type of sclerotium. (c) Later stage of sclerotium formation in *B. cinerea*. The hyphae have become melanized and are growing away from the glass surface. They are embedded in a glucan matrix (arrows). (d) Mature sclerotia of *B. cinerea* on a stem of *Conium*. Some sclerotia are germinating to produce tufts of conidiophores. (e) Sclerotia of *Claviceps purpurea* from an ear of rye (*Secale cereale*). Rye grains are shown for size comparison. (a) and (b) to same scale.

potato tubers. In pure culture, sclerotial initials arise by branching and septation of hyphae (Fig. 1.16a). These cells become filled with dense contents and numerous vacuoles, and darken to reddish-brown. The mature sclerotium does not show well-defined zones or 'tissues'. It is made up of a central part which is pseudoparenchymatous, although its hyphal nature can be seen. Towards the outside, the hyphae are more loosely arranged; a rind of thick-walled hyphae is absent (Willetts, 1969).

The terminal type

This form is characterized by a well-defined pattern of branching. It is produced, for example, by *Botrytis cinerea*, the cause of grey mould diseases on a wide range of plants, and by the saprotrophic *Pyronema domesticum* (see p. 415). Sclerotia of *B. cinerea* are found on overwintering stems of herbaceous plants, especially umbellifers such as *Angelica*, *Anthriscus*, *Conium* and *Heracleum*. They can also be induced to form in culture, especially on agar media with a high

carbon/nitrogen ratio. When growing on host tissue, the sclerotia of *Botrytis* may include host cells, a feature shared also by sclerotia of *Sclerotinia* spp. to which *Botrytis* is related (see p. 429). Sclerotia arise by repeated dichotomous branching of hyphae, accompanied by cross-wall formation (Fig. 1.16b). The hyphae then aggregate, melanize and produce mucilage, giving the appearance of a solid tissue (Fig. 1.16c). A mature sclerotium may be about 10 mm long and 3–5 mm wide, and is usually flattened, measuring 1–3 mm in thickness. It is often orientated parallel to the long axis of the host plant (Fig. 1.16d). It is differentiated into a rind composed of several layers of rounded, dark cells, a narrow cortex of thin-walled pseudoparenchymatous cells with dense contents, and a medulla made up of loosely arranged filaments. Nutrient reserves are stored in the cortical and medullary regions (Willetts & Bullock, 1992).

The strand type

Sclerotinia gladioli, the causal agent of dry rot of corms of *Gladiolus*, *Crocus* and other plants, forms sclerotia of this type. Sclerotial initials commence with the formation of numerous side branches which arise from one or more main hyphae. Where several hyphae are involved, they lie parallel. They are thicker than normal vegetative hyphae, and become divided by septa into chains of short cells. These cells may give rise to short branches, some of which lie parallel to the parent hypha, whilst others grow out at right angles and branch again before coalescing. The hyphae at the margin continue to branch, and the whole structure darkens. The mature sclerotium is about 0.1–0.3 mm in diameter, and is differentiated into a rind of small, thick-walled cells and a medulla of large, thin-walled hyphae. More complex sclerotia are found in *Sclerotium rolfsii*, the sclerotial state of *Pellicularia rolfsii* (Basidiomycota). Here the mature sclerotium is differentiated into four zones: a fairly thick skin or cuticle, a rind made up of 2–4 layers of tangentially flattened cells, a cortex of thin-walled cells with densely staining contents, and a medulla of loose filamentous hyphae with dense contents. Chet *et al*. (1969) have shown that the skin or cuticle is made up of the remnants of cell walls attached to the outside of the empty, melanized, thick-walled rind cells. All the cells of the strand-type sclerotium have thicker walls than those of vegetative hyphae. Cells of the outer cortex contain large storage bodies which consist of protein (Kohn & Grenville, 1989) and leave little room for cytoplasm or other organelles. The inner cortex is also densely packed with storage granules.

Other types

There is a great diversity of other types of sclerotia (Butler, 1966). The sclerotia of *Claviceps purpurea*, the 'ergots' of grasses and cereals (Fig. 1.16e; see also p. 349), develop from a pre-existing mass of mycelium which fills and replaces the cereal ovary, starting from the base and extending towards the apex. The outer layers form a violet, dark grey or black rind enclosing colourless, thick-walled cells. These contain abundant storage lipids which constitute 45% of the dry weight of a *C. purpurea* sclerotium (Kybal, 1964). *Cordyceps militaris*, an insect parasite, forms a dense mass of mycelium in the buried insect's body (p. 360). This mass of mycelium, from which fructifications develop, is enclosed by the exoskeleton of the host, not by a fungal rind. Many wood-rotting fungi enclose colonized woody tissue with a black zone-line of dark, thick-walled cells, and the whole structure may be regarded as a kind of sclerotium.

The giant sclerotium of *Polyporus mylittae* is marbled in structure, comprising white strata and translucent tissue. It has an outer, smooth, thin black rind. Three distinct types of hyphae make up the tissues: thin-walled, thick-walled and 'layered' hyphae. Thin- and thick-walled hyphae are abundant in the white strata but sparse in the translucent tissue, whereas the layered hyphae occur only in the translucent tissue. Detached sclerotia are capable of forming basidiocarps without wetting. It is believed that the translucent tissue functions as an extracellular nutrient and water store (Macfarlane *et al*., 1978). The structure of the sclerotium appears to be related to its ability to fruit in dry conditions, such as occur in Western Australia.

Germination of sclerotia

Sclerotia can survive for long periods, sometimes for several years (Coley-Smith & Cooke, 1971; Willetts, 1971). Germination may take place in three ways – by the development of mycelium, asexual spores (conidia) or sexual fruit bodies (ascocarps or basidiocarps). Mycelial germination occurs in *Sclerotium cepivorum*, the cause of white-rot of onion, and is stimulated by volatile exudates from onion roots (see p. 434). Conidial development occurs in *Botrytis cinerea* and can be demonstrated by placing overwintered sclerotia in moist warm conditions (Fig. 1.16d; Weber & Webster, 2003). The development of ascocarps (i.e. carpogenic germination) is seen in *Sclerotinia*, where stalked cups or apothecia, bearing asci, arise from sclerotia under suitable conditions (Fig. 15.2), and in *Claviceps purpurea*, where the overwintered sclerotia give rise to a perithecial stroma (Fig. 12.26c). Depending on environmental conditions, the sclerotia of some species may respond by germinating in different ways.

1.3.4 The mantle of ectomycorrhiza

The root tips of many coniferous and deciduous trees with ectomycorrhizal associations, especially those growing in relatively infertile soils, are covered by a **mantle**. This is a continuous sheet of fungal hyphae, several layers thick (see Fig. 19.10). The mycelium extends outwards into the litter layer of the soil, and inwards as single hyphae growing intercellularly, i.e. between the outer cortical cells of the root, to form the so-called **Hartig net**. Hyphae growing outwards from the mantle effectively replace the root hairs as a system for the absorption of minerals from the soil, and there is good evidence that, in most normal forest soils of low to moderate fertility, the performance and nutrient status of mycorrhizal trees is superior to that of uninfected trees (Smith & Read, 1997). Most fungi causing ectomycorrhizal infections are Basidiomycota, especially members of the Homobasidiomycetes (pp. 526 and 581). Within the soil or in pure culture, mycelial strands may form, but the mycelium is not aggregated into the tissue-like structure of the mantle.

1.3.5 Fruit bodies of Ascomycota and imperfect fungi

In the higher fungi, hyphae may aggregate in a highly regulated fashion to form fruiting structures which are an important and often species-specific feature of identification. In the Ascomycota, the fruit bodies produce sexual spores (i.e. as the result of nuclear fusion and meiosis) which are termed **ascospores** and are contained in globose or cylindrical cells called **asci** (Lat. *ascus* = a sac, tube). In most cases, the asci can discharge their ascospores explosively. Asci, although occasionally naked, are usually enclosed in an aggregation of hyphae termed an **ascocarp** or **ascoma**. Ascocarps are very variable in form, and several types have been distinguished (see Fig. 8.16). Their features and development will be described more fully later. Forms in which the asci are totally enclosed, and in which the ascocarp has no special opening, are termed **cleistothecia**. In contrast, **gymnothecia** consist of a loose mesh of hyphae. Both are found in the Plectomycetes (Chapter 11). A modified cleistothecium is characteristic of the Erysiphales (Chapter 13). Cup fungi (Discomycetes, Chapters 14 and 15) possess saucer-shaped ascocarps termed **apothecia**, with a mass of non-fertile hyphae supporting a layer of asci lining the upper side of the fruit body. The non-fertile elements of the apothecium often show considerable differentiation of structure. The asci in apothecia are free to discharge their ascospores at the same time. In other Ascomycota, the asci are contained within ascocarps with a very narrow opening or **ostiole**, through which each ascus must discharge its spores separately. Ascocarps of this type are termed **perithecia** or **pseudothecia**. Perithecia are found in the Pyrenomycetes (Chapter 12) whilst pseudothecia occur in the Loculoascomycetes (Chapter 17). These two types of ascocarp develop in different ways. In many of the Pyrenomycetes, the perithecia are borne on or embedded in a mass of fungal tissue termed the **perithecial stroma**, and these are

well shown by the Xylariales (p. 332), and by *Cordyceps* (p. 360) and *Claviceps* (p. 349). In some cases, in addition to the perithecial stroma, a fungus may develop a stromatic tissue on or within which asexual spores (conidia) develop. *Nectria cinnabarina* (p. 341), the coral spot fungus so common on freshly dead deciduous twigs, is such an example. It initially forms pink conidial stromata which later, under suitable conditions of humidity, become converted into perithecial stromata.

Among the imperfect (asexual) fungi, mycelial aggregations bearing conidia are seen in various genera. In some, there are tufts of parallel conidiophores termed **coremia** or **synnemata**, exemplified by *Penicillium claviforme* (see Fig. 11.19). In some imperfect fungi formerly called Coelomycetes, the conidia develop in flask-shaped cavities termed **pycnidia** (see Figs. 17.3–17.5). Various other kinds of mycelial fruiting aggregates are also known.

1.3.6 Fruit bodies of Basidiomycota

The fruit bodies of mushrooms, toadstools, bracket fungi, etc., are all examples of **basidiocarps** or **basidiomata** which bear the sexually produced spores (basidiospores) on basidia. Basidiocarps are almost invariably constructed from dikaryotic hyphae, but how vegetative hyphae aggregate to form a mushroom fruit body is still a mystery (Moore, 1994). Wessels (1997) has suggested that hydrophobins coating the surface of hyphae may confer adhesive properties, leading to their aggregation to form a fruit body initial as the first step in morphogenesis. Once an initial has been formed, its glucan matrix may provide an environment for the exchange of signalling molecules between hyphae. Moore (1994) speculated that morphogenesis might ultimately be determined by induction hyphae exerting a control over surrounding hyphae, leading to the development of morphogenetic units. This morphogenetic commitment must happen at a very early stage. For instance, in the ink-cap (*Coprinus cinereus*) an initial measuring only 1% of the final fruit body size is already differentiated into stipe and cap (Moore *et al*., 1979). Therefore, when a mushroom fruit body expands, this is due mainly to the enlargement of existing hyphae, whereas new apical growth is restricted mainly to branches filling up the space generated during expansion (Moore, 1994). Hyphae making up the mature basidiocarp may show considerable differentiation in structure and function. This is perhaps most highly developed in polypore-type basidiocarps, where a number of morphologically distinct hyphal types have been recognized (p. 517).

1.4 Spores of fungi

The reproduction by means of small spores is a cornerstone in the ecology of fungi. Although a single spore may have a negligible chance of reaching a suitable substrate, spores may be produced in such quantities that even discrete substrates can be exploited by the species as a whole. Only a few fungi make do without spores, surviving solely by means of mycelium and sclerotia. Spores may be organs of sexual or asexual reproduction, and they are involved in dispersal and survival. Gregory (1966) distinguished between **xenospores** (Gr. *xenos* = a foreigner) for spores which are dispersed from their place of origin and **memnospores** (Gr. *mémnon* = steadfast, to persist), which stay where they were formed. Some spores are violently discharged from the organs which bear them, energy for dispersal being provided by the spore itself or the structure producing it (Ingold, 1971). However, many spores are dispersed passively by the action of gravity, air or water currents, rain splash, or by animals, especially insects. Dispersal may also occur by human traffic. Spores may be present in the outdoor air at such high concentrations (e.g. 100 *Cladosporium* spores l^{-1}) that they can cause allergic respiratory diseases when inhaled (Lacey, 1996). In freshwater, the asexually produced spores (conidia) of aquatic hyphomycetes, which colonize autumn-shed tree leaves, may reach concentrations of 10 000–20 000 spores l^{-1} (see p. 685). Long-range dispersal of air-borne spores

over thousands of kilometres is known to occur in nature. For instance, the urediniospores of the coffee rust fungus, *Hemileia vastatrix*, are thought to have travelled from Africa to South America by wind at high altitudes, and the urediniospores of black stem rust of wheat (*Puccinia graminis*) undergo an annual migration from states bordering the Gulf of Mexico to the prairies of North America and Canada (Fig. 22.11). These spores are protected from the deleterious effects of UV irradiation in the upper atmosphere by pigments in the spore wall.

Some spores are not dispersed but survive *in situ*, e.g. the oospores of many soil-inhabiting Oomycota (Chapter 5), the zygospores of Zygomycota (Chapter 7) and the chlamydospores of Glomales (see p. 217) and other fungi. Fungal spores may remain dormant for many years, especially under dry and cold conditions (Sussman & Halvorson, 1966; Sussman, 1968). An extreme example of spore survival is shown by the recovery of viable spores of several fungi from glacial ice cores, including those of *Cladosporium cladosporioides* from ice samples 4500 years old (Ma *et al*., 2000).

The morphology and structure of fungal spores show great variability, from unicellular to multicellular, branched or unbranched or sometimes spirally coiled, thin- or thick-walled with hyaline or pigmented walls, dry or sticky, smooth or ornamented by mucilaginous extensions, spines, folds or reticulations. A number of general descriptive terms have been applied to characterize spores in relation to the number of cells and septa which they contain. Single-celled spores are termed **amerospores** (Gr. *a* = not, *meros* = a part; i.e. not divided), two-celled spores are **didymospores** (Gr. *didymos* = double), spores with more than one transverse septum are **phragmospores** (Gr. *phragmos* = a hedge, barricade), and spores with transverse and longitudinal septa are **dictyospores** (Gr. *dictyon* = a net). These terms may be qualified by prefixes indicating spore pigmentation such as hyalo- for colourless (hyaline) spores and phaeo- for spores with dark-coloured (melanized) walls.

Special terms have also been used to refer to spore shape. **Scolecospores** (Gr. *skolex* = a worm) are worm-shaped, **helicospores** (Gr. *helix* = twisted or wound) are spores with a two- or three-dimensional spiral shape, whilst **staurospores** (Gr. *stauros* = a cross) have arms radiating from a central point or axis. Spore septation, colour and shape, along with other criteria such as the arrangement of structures which bear the spores, have been used in classification and identification, especially in conidial fungi which do not show sexual reproduction. These criteria rarely lead to natural systems of classification, but to '**form genera**' or 'anamorph genera' made up of species unified by having similar spore forms.

Some of the more common spore types are described below. There are numerous other, less-common kinds of spore found in fungi, and they are described later, in relation to the particular fungal groups in which they occur.

1.4.1 Zoospores

These are spores which are self-propelled by means of flagella. Propulsion is often coupled with chemotactic movement, zoospores having the ability to sense chemicals diffusing from suitable substrata and to move towards them, or gametes detecting and following extremely low concentrations of hormones. In some cases oxygen or light are also stimuli for tactic movement. The fungal groups which possess flagella are mostly aquatic or, if terrestrial, rely on water for dispersal or infection. Their zoospores are of four kinds (see Fig. 1.17):

1. Posteriorly flagellate zoospores with flagella of the whiplash type are characteristic of the Chytridiomycota (Chapter 6). Each whiplash flagellum has 11 microtubules arranged in the 9 + 2 pattern typical of eukaryotes. The microtubules are enclosed in a smooth, membranous axoneme sheath continuous with the plasma membrane. In most members of the Chytridiomycota there is a single posterior flagellum (Fig. 1.17a), but in the rumen-inhabiting Neocallimastigales there may be up to 16 flagella (Fig. 1.17b). Such spores are driven forward by sinusoidal rhythmic beating of the flagellum. This type of zoospore

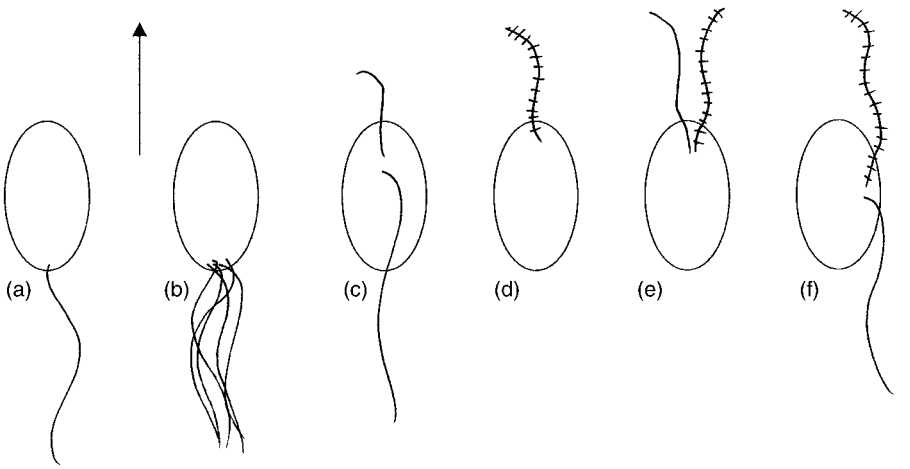

Fig 1.17 Zoospore types found in fungi, diagrammatic and not to scale. The arrow indicates the direction of movement of the zoospore. (a) Posteriorly uniflagellate (opisthokont) zoospore with a flagellum of the whiplash type found in many Chytridiomycota. (b) Posteriorly multiflagellate zoospore with numerous (up to 16) whiplash flagella which occur in certain anaerobic rumen-inhabiting Chytridiomycota (Neocallimastigales). (c) Zoospore with unequal (anisokont) whiplash flagella characteristic of the Myxomycota and the Plasmodiophoromycota. (d) Anteriorly uniflagellate zoospore with a flagellum of the tinsel type, the axoneme being clothed with rows of mastigonemes, typical of the Hyphochytriomycota. (e,f) Biflagellate zoospores with heterokont flagella, one of the whiplash and the other of the tinsel type, which are found in different groups of the Oomycota. For more details turn to the different fungal groups.

flagellation is termed **opisthokont** (Gr. *opisthen* = behind, at the back; *kontos* = a pole). Detailed descriptions of the fine structure of chytridiomycete zoospores are given on p. 129.

2. Biflagellate zoospores with two whiplash flagella of unequal length are called **anisokont** (Fig. 1.17c) and are found in some Myxomycota and the Plasmodiophoromycota, both now classified among the Protozoa (see Chapters 2 and 3).

3. Anteriorly uniflagellate zoospores with a flagellum of the tinsel type are characteristic of the Hyphochytriomycota (Chapter 4). The axoneme sheath of the tinsel or **straminipilous** flagellum (Lat. *stramen* = straw; *pilus* = hair) is adorned by two rows of fine hairs (Fig. 1.17d). These are called **tripartite tubular hairs** or **mastigonemes** (Gr. *mastigion* = a small whip; *nema* = a thread). Rhythmic sinusoidal beating of the tinsel type flagellum pulls the zoospore along, in contrast to the pushing action of whiplash flagellum. Details of the fine structure of this type of zoospore are given in Fig. 4.5.

4. Biflagellate zoospores with anteriorly or laterally attached flagella, one of which is of the whiplash type and the other of the tinsel type (Figs. 1.17e,f), are characteristic of the Oomycota (Chapter 5). Zoospores with the two different kinds of flagellum are **heterokont**. Where the two types of flagellum are attached anteriorly, as in the first-released zoospores of *Saprolegnia*, their propulsive actions tend to work against each other and the zoospore is a very poor swimmer (Fig. 1.17e). However, the secondary zoospore (termed the principal zoospore) in *Saprolegnia* and in many other Oomycota has laterally attached flagella, with the tinsel-type (pulling action) flagellum pointing forwards and the whiplash-type (pushing action) flagellum directed backwards and possibly acting as a rudder, jointly providing much more effective propulsion (Fig. 1.17f).

1.4.2 Sporangiospores

In the Zygomycota, and especially in the Mucorales (see p. 180), the asexual spores are contained in globose sporangia (Fig. 1.18) or cylindrical merosporangia. Because they are non-motile, the spores are sometimes termed **aplanospores** (Gr. *a* = not, *planos* = roaming).

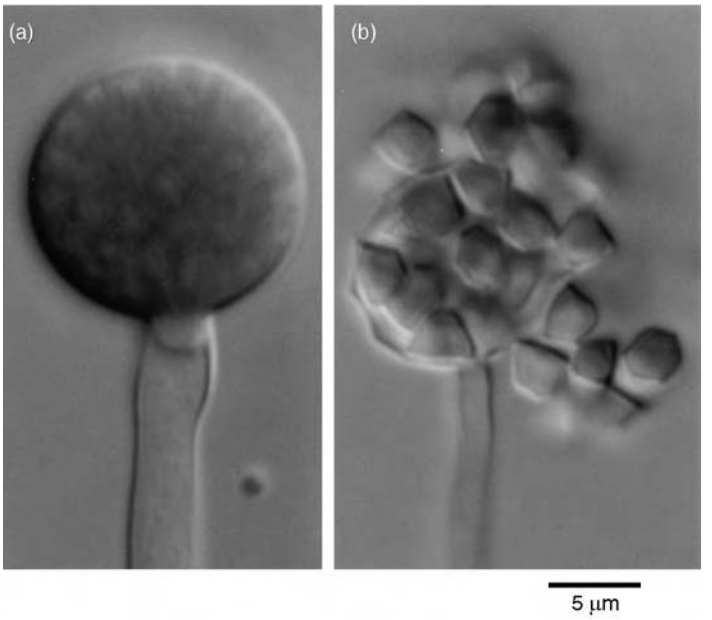

Fig 1.18 Sporangia in *Mortierella* (*Umbelopsis*) *vinacea*. (a) Maturing sporangium in which the cytoplasm is being cleaved into numerous sporangiospores. (b) Release of sporangiospores by breakdown of the sporangial wall. Unusually, in *M. vinacea* the sporangiospores are angular in shape.

The spores may be uni- or multinucleate and are unicellular. They generally have thin, smooth walls and are almost always globose or ellipsoid in shape. They are formed by cleavage of the sporangial cytoplasm. They vary in colour from hyaline (colourless) to yellow, due to carotenoid pigments in the cytoplasm. When mature, they may be surrounded by mucilage, in which case they are usually dispersed by rain splash or insects, or they may be dry and dispersed by wind currents. In some genera, e.g. *Pilobolus*, entire sporangia become detached. The number of sporangiospores per sporangium may vary from several thousand to only one. The detachment and dispersal of intact sporangia containing a few sporangiospores or a single one is indicative of the way in which conidia may have evolved from one-spored sporangia.

1.4.3 Ascospores

Ascospores are the characteristic spores of the largest group of fungi, the Ascomycota or ascomycetes. They are meiospores and are formed in the developing ascus as a result of nuclear fusion immediately followed by meiosis. The four haploid daughter nuclei then divide mitotically to give eight haploid nuclei around which the ascospores are cut out. Details of ascospore development are described in Fig. 8.11. In most ascomycetes, the eight ascospores are contained within a cylindrical ascus, from which they are squirted out together with the ascus sap when the tip of the turgid ascus breaks down and the elastic ascus walls contract. The distance of discharge may be 1 cm or more. In some cases, for example, the Plectomycetes (Chapter 11) and in ascomycetes with subterranean fruit bodies, such as the false truffles (*Elaphomyces* spp.; Fig. 11.21) and truffles (*Tuber* spp. and their allies; p. 423), ascospore release is non-violent and their asci are not cylindrical but globose. Ascospores vary greatly in size, shape and colour. In size, the range is from about $4-5 \times 1\,\mu m$ in small-spored forms such as the minute cup fungus *Dasyscyphus*, to $130 \times 45\,\mu m$ in the lichen *Pertusaria pertusa*. The shape of ascospores varies from globose to oval, elliptical, lemon-shaped, sausage-shaped, cylindrical, or needle-shaped. Ascospores are often asymmetric in form with a wider, blunter, anterior part and a narrower, more tapering posterior. This shape increases their acceleration as they are squeezed out through the opening of the ascus. Ascospores may be uninucleate or multinucleate, unicellular or multicellular, divided up by transverse or by transverse and longitudinal septa. In some

genera, e.g. *Hypocrea* (Fig. 12.15) or *Cordyceps* (Fig. 12.33), the multicellular ascospores may break up into part-spores within the ascus prior to discharge. The ascospore wall may be thin or thick, hyaline or coloured, smooth or rough, sometimes cast into reticulate folds or ornamented by ridges, and it may have a mucilaginous outer layer which is sometimes extended to form simple or branched appendages, especially in marine ascomycetes where they aid buoyancy and attachment. In many cases, ascospores are resting structures which survive adverse conditions. They may have extensive food reserves in the form of lipids and sugars such as trehalose. Because the formation of ascospores involves meiosis, they are important not only as a means of dispersal and survival but also in genetic recombination.

It is obvious that there is no such thing as a typical ascospore. *Neurospora tetrasperma* will serve as an example of an ascospore whose structure has been extensively studied (Lowry & Sussman, 1958, 1968). This fungus is somewhat unusual in that it has four-spored asci and the ascospores are binucleate. The spores are black, thick-walled and shaped rather like a rugby football, but with flattened ends. The name *Neurospora* refers to the ribbed spores, because the dark outer wall is made up of longitudinal raised ribs, separated by interrupted grooves. The structure of a spore in section is shown in Fig. 1.19. Within the cytoplasm of the spore are the two nuclei, fragments of endoplasmic reticulum (not illustrated), swollen mitochondria and vacuoles, bounded by single unit membranes. The wall surrounding the protoplast is composed of several layers. The innermost layer is the **endospore**, outside of which is the **epispore**. The ribbed layer is termed the **perispore**. Between the ribs are lighter intercostal veins containing a material which is chemically distinct from the ribs. This material is continuous over the whole surface of the spore, giving it a relatively smooth surface. The spore germinates by the extrusion of germ tubes from a preexisting **germ pore**, a thin area in the epispore at either end of the spore. In many ascomycetes a trigger is required for germination, e.g. heat shock in *Neurospora* or a chemical stimulus, for example in ascomycetes which grow and fruit on the dung of herbivorous mammals and whose spores are subjected to digestive treatment.

1.4.4 Basidiospores

Basidiospores are the sexual spores which characterize a large group of fungi, the Basidiomycota or basidiomycetes. In comparison with the morphological diversity of ascospores, basidiospores are more uniform. They also show a smaller size range, from about 3 to 20 μm, which is possibly related to their unique method of discharge. They are normally found in groups of four attached by tapering sterigmata to the cell which bears them, the **basidium**. At the time of their discharge all basidiospores

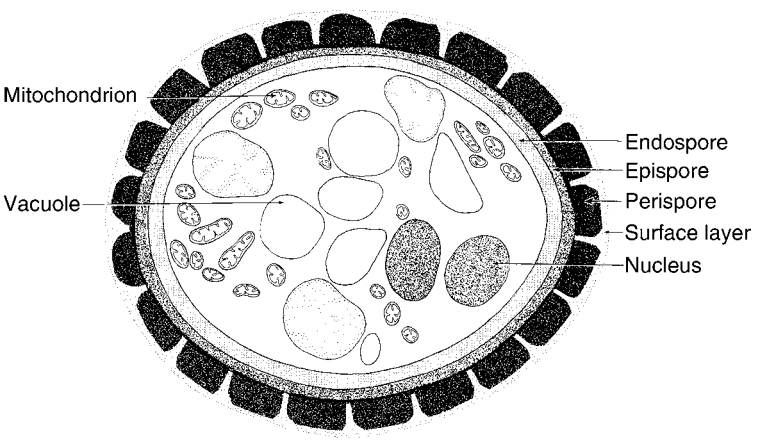

Fig 1.19 *Neurospora tetrasperma*. T.S. ascospore. Simplified diagram based on an electron micrograph by Lowry in Sussman and Halvorson (1966).

are unicellular, but they may become septate after release in some members of the Heterobasidiomycetes (Chapter 21). In shape, basidiospores are asymmetric and vary from sub-globose, sausage-shaped, fusoid, to almond-shaped (i.e. flattened), and the wall may be smooth or ornamented with spines, ridges or folds. The colour of basidiospores is important for identification. They may be colourless, white, cream, yellowish, brown, pink, purple or black. The spore colour may be due to pigments in the spore cytoplasm or in the spore wall. The appearance of pigments in the wall occurs relatively late in spore development. This explains the change of colour of the gill of a domestic mushroom (*Agaricus*) from pink, due to cytoplasmic spore pigments, to dark purplish-brown when mature, due to wall pigments.

The generalized structure of a basidiospore is illustrated in Fig. 1.20. Most basidiospores have a flatter adaxial face and a more curved abaxial face. The point of attachment of the spore to the sterigma is the **hilum**, which persists as a scar at the base of a discharged spore. Close to the hilum is a small projection, the **hilar appendix**. This is involved in the unique mechanism of basidiospore discharge, in which a drop of liquid perched on the hilar appendix coalesces with a second blob of liquid on the spore surface,

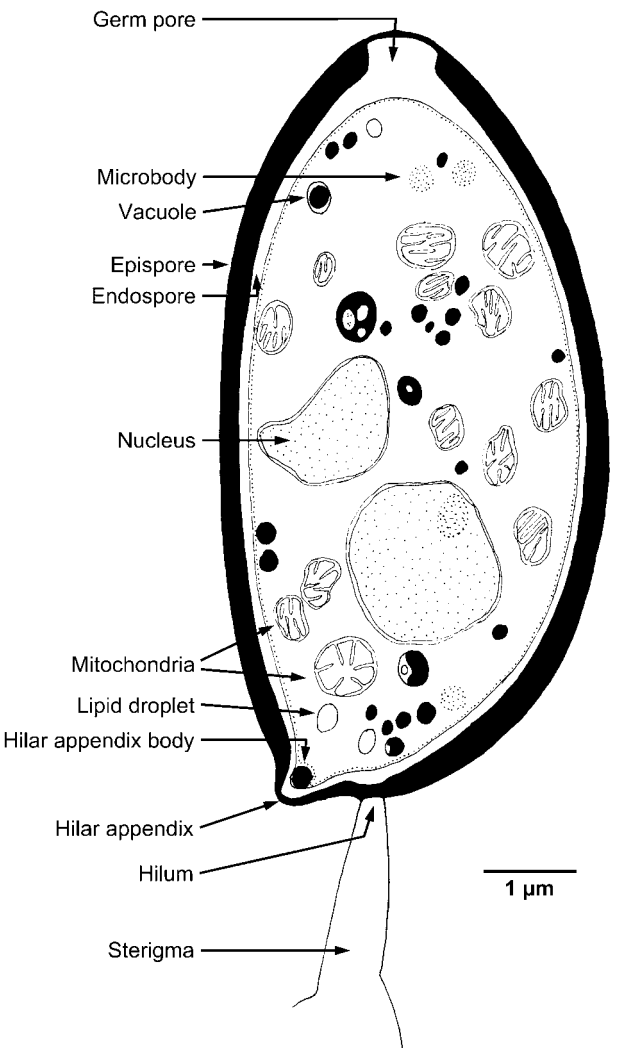

Fig 1.20 Generalized view of a median vertical section through a basidiospore as seen by transmission electron microscopy. For clarity, structures such as endoplasmic reticulum and ribosomes are not illustrated. Diagram based on *Agrocybe acericola*, after Ruch and Nurtjahja (1996).

creating a momentum which leads to acceleration of the spore (Money, 1998; see p. 493). The spore is projected for a short distance (usually less than 2 mm) from the basidium. Violently projected spores are termed **ballistospores** (Lat. *ballista* = a military engine for throwing large stones), but whilst most basidiospores are ballistospores, some are not. For example, in the Gasteromycetes (Chapter 20), which include puffballs, stinkhorns and their allies, violent spore projection has been lost in the course of evolution from ancestors which possessed it. Likewise, the basidiospores of smut fungi (Ustilaginales, Chapter 23) are not violently discharged. The term **statismospore** (Lat. *statio* = standing still) is sometimes used for a spore which is not forcibly discharged.

The cytoplasm of basidiospores usually contains a single haploid nucleus resulting from meiotic division in the basidium; sometimes a post-meiotic division gives rise to two genetically identical nuclei. The structure of the wall is complex. In *Agrocybe acericola* there are two layers, a thicker, dark-pigmented, electron-dense outer layer or epispore, and a thinner, electron-transparent inner layer, the endospore (Ruch & Nurtjahja, 1996; see Fig. 1.20). The cultivated mushroom, *Agaricus bisporus*, has a three-layered wall making up some 35% of the dry weight of the spore (Rast & Hollenstein, 1977), whereas the wall of the *Coprinus cinereus* basidiospore comprises six distinct layers (McLaughlin, 1977). A histochemical feature of the walls of some basidiospores is that they are **amyloid**, i.e. they include starch-like material which stains bluish-purple with iodine-containing stains such as Melzer's reagent. This reaction is used as a taxonomic character. The amyloid reaction is due to the presence of unbranched, short-chain amylose molecules. It has been suggested that this 'fungal starch' may aid dormancy by creating a permeability barrier to oxygen in dry spores. When the amyloid material is dissolved as water becomes available, dormancy is lost and spore germination can proceed (Dodd & McCracken, 1972). In some basidiospores, e.g. those of *Coprinus cinereus* and *Agrocybe acericola*, the basidiospore has a distinct germ pore at the end opposite to the hilum (see Fig. 1.20). In other basidiomycetes, e.g. *Oudemansiella mucida*, *Schizophyllum commune* and *Flammulina velutipes*, the basidiospores have no specialized pore.

The reserve contents of the spore may vary. In some species, lipid is the major storage product, and there is an apparent lack of insoluble polysaccharides such as glycogen (Ruch & Motta, 1987). In other spores, glycogen predominates. Where lipid is present, germination may be fuelled by its breakdown and utilization, but where it is absent spores are dependent on external nutrient supplies before germination and further development is possible. In addition to the usual organelle complement, microbodies are also prominent in basidiospores. These are single membrane-bound organelles often associated with mitochondria and lipid globules; they may function as glyoxisomes containing enzymes involved in the oxidation of lipids (Ruch & Nurtjahja, 1996).

1.4.5 Zygospores

Zygospores are sexually produced resting structures formed as a result of plasmogamy between gametangia which are usually equal in size (Fig. 1.21a). Nuclear fusion may occur early, or may be delayed until shortly before meiosis and zygospore germination. Zygospores are typical of Zygomycota (Chapter 7). They are often large, thick-walled, warty structures with abundant lipid reserves and are unsuitable for long-distance dispersal, usually remaining in the position in which they were formed and awaiting suitable conditions for further development. The gametangia which fuse to form the zygospore may be uninucleate or multinucleate, and correspondingly the zygospore may have one, two or many nuclei within it. Zygospore germination may be by a germ tube or by the formation of a germ sporangium.

1.4.6 Oospores

An oospore is a sexually produced spore which develops from unequal gametangial copulation or markedly unequal (oogamous) gametic fusion (Fig. 1.21b). It is the characteristic sexually

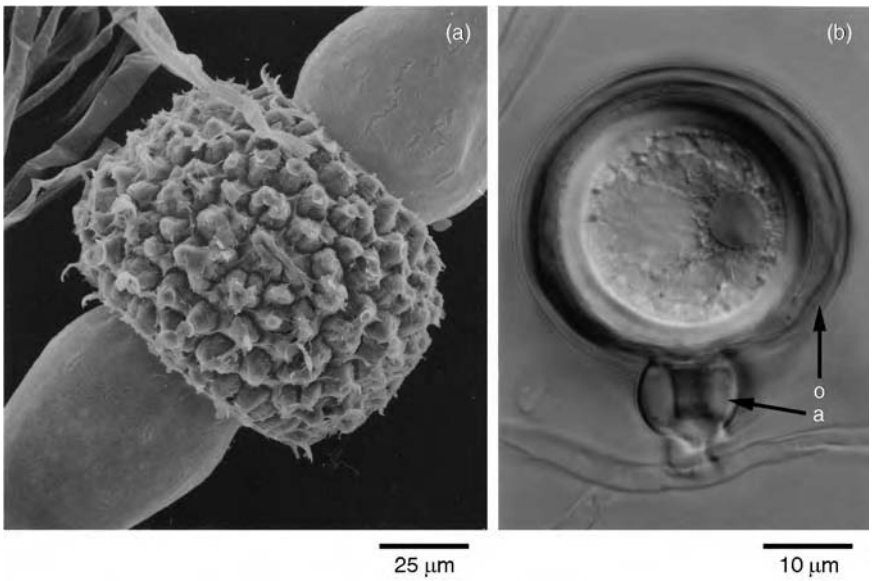

Fig 1.21 Sexual resting structures. (a) Zygospore of *Rhizopus sexualis*. The zygote has been produced by fusion of two gametangia and has laid down a thick wall with warty ornamentations. (b) Oospore of *Phytophthora erythroseptica*. The oogonium (o) has grown through the antheridium (a), and the oosphere has picked up a fertilization nucleus in the process. a kindly provided by H.-M. Ho; reprinted from Ho and Chen (1998) with permission of *Botanical Bulletin of Academia Sinica*.

produced spore of the Oomycota (Chapter 5), although oospores are also found in the Monoblepharidales (Chytridiomycota; Fig. 6.25). In the Oomycota, oospore development begins with the formation of one or more oospheres within the larger gametangium, the oogonium. After fertilization, i.e. the receipt of an antheridial nucleus by the oosphere, this lays down a thick wall and becomes the oospore. The number of oospores per oogonium may vary, and this is an important taxonomic criterion. Meiotic nuclear divisions precede oosphere and antheridial maturation in the Oomycota and nuclear fusion follows fertilization, so that the oospore is diploid. The oospore develops a thick outer wall and lays down food reserves, usually in the form of lipids. In the Peronosporales the outer wall of the oospore is surrounded by periplasm, the residual cytoplasm left in the oogonium after the oospheres have been cleaved out. Oospores are sedentary (memnospores) and are important in survival rather than dispersal. They often require a period of maturation before germination can occur and may remain dormant for long periods.

1.4.7 Chlamydospores

In most groups of fungi, terminal or intercalary segments of the mycelium may become packed with lipid reserves and develop thick walls within the original hyphal wall (Fig. 1.22). The new walls may be colourless or pigmented, and are often hydrophobic. Structures of this type have been termed chlamydospores (Gr. *chlamydos* = a thick cloak). They are formed asexually. Generally there is no mechanism for detachment and dispersal of chlamydospores, but they may become separated from each other by the collapse of the hyphae producing them. They are therefore typical memnospores, forming important organs of asexual survival, especially in soil fungi. Chlamydospores may develop within the sporangiophores of some species of the Mucorales, e.g. in *Mucor racemosus* (see Fig 7.14). The Glomales, which are fungal partners in symbiotic mycorrhizal associations with many vascular plants, reproduce primarily by large, thick-walled chlamydospores. These develop singly or in clusters (sporocarps) on coarse hyphae attached to their host plants. They are sedentary in soil but may be dispersed

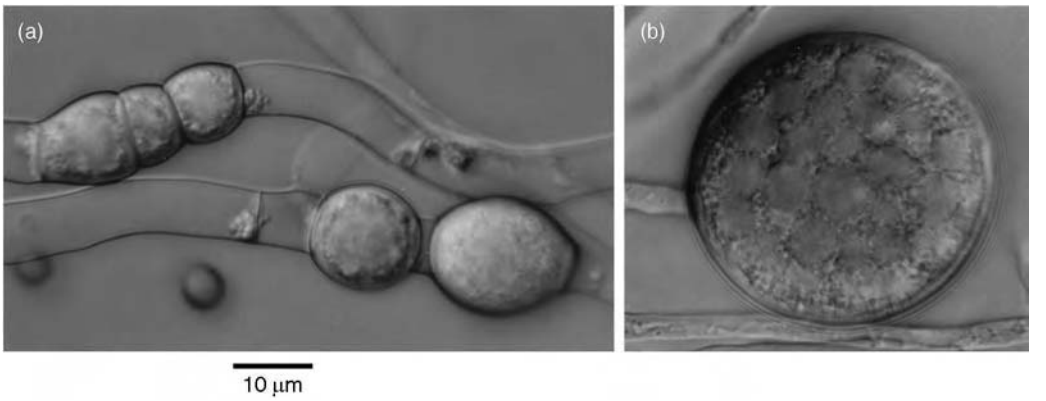

Fig 1.22 Chlamydospores formed by soil-borne fungi. (a) Intercalary hyphal chlamydospores in *Mucor plumbeus* (Zygomycota). (b) Terminal chlamydospore in *Pythium undulatum* (Oomycota). Both images to same scale.

by wind or by burrowing rodents which eat the spores. Chlamydospores may also develop within the multicellular macroconidia of *Fusarium* spp. and may survive when other, thin-walled cells making up the spore are degraded by soil micro-organisms. Similar structures are found in old hyphae of the aquatic fungus *Saprolegnia* (see Fig. 5.6g), either singly or in chains. In this genus, the chlamydospores may break free from the mycelium and be dispersed in water currents. Chlamydospores which are dispersed in this way are termed **gemmae** (Lat. *gemma* = a jewel).

The term chlamydospore is also sometimes used to describe the thick-walled dikaryotic spore characteristic of smut fungi (Ustilaginales; Chapter 23) but the term teliospore is preferable in this context. Hughes (1985) has discussed the use of the term chlamydospore.

1.4.8 Conidia (conidiospores)

Conidiospores, commonly known as conidia, are asexual reproductive structures. The word is derived from the Greek *konidion*, a diminutive of *konis*, meaning dust (Sutton, 1986). Conidia are found in many different groups of fungi, but especially within Ascomycota and Basidiomycota. The term conidium has, unfortunately, been used in a number of different ways, so that it no longer has any precise meaning. It has been defined by Kirk *et al.* (2001) as 'a specialized non-motile (cf. zoospore) asexual spore, usually caducous (i.e. detached), not developed by cytoplasmic cleavage (cf. sporangiospore) or free cell formation (cf. ascospore); in certain *Oomycota* produced through the incomplete development of zoosporangia which fall off and germinate to produce a germination tube'. In many fungi conidia represent a means of rapid spread and colonization from an initial focus of infection.

In general, conidia are dispersed passively, but in a few cases discharge is violent. For instance, in *Nigrospora* the conidia are discharged by a squirt mechanism (Webster, 1952), and in *Epicoccum* (Fig. 17.8) discharge is brought about by the rounding-off of a two-ply septum separating the conidium from its conidiogenous cell (Webster, 1966; Meredith, 1966). In the *Helminthosporium* conidial state of *Trichometasphaeria turcica*, drying and shrinkage of the conidiophore is associated with the sudden development of a gas phase, causing a jolt sufficient to project the conidium (Meredith, 1965; Leach, 1976).

There is great variation in conidial ontogeny. This topic will be dealt with more fully later when considering the conidial states of Ascomycota, and at this stage it is sufficient to distinguish between the major types of conidial development, which may be either **thallic** or **blastic**. Cells which produce conidia are conidiogenous cells. The term thallic is used to describe development where there is no enlargement of the conidium initial (Fig. 1.23a), i.e. the conidium arises by conversion of a pre-existing segment of the fungal thallus. An example of this kind is *Galactomyces candidus*, in which the conidia are

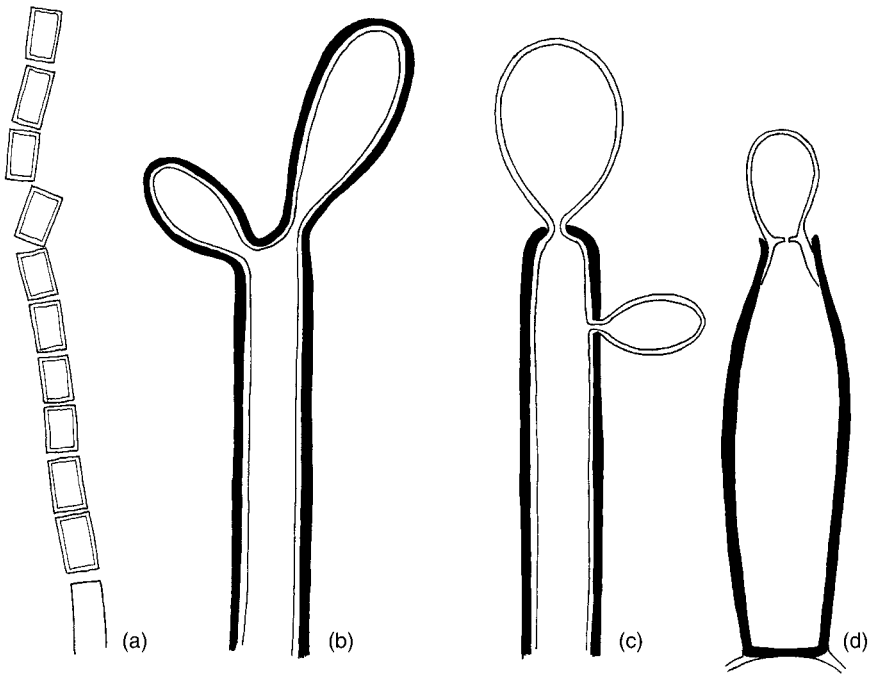

Fig 1.23 Diagrams to illustrate different kinds of conidial development. (a) Thallic development. There is no enlargement of the conidium initial. (b) Holoblastic development. All the wall layers of the conidiogenous cell balloon out to form a conidium initial recognizably larger than the conidiogenous cell. (c) Enteroblastic tretic development: only the inner wall layers of the conidiogenous cell are involved in conidium formation. The inner wall layers balloon out through a narrow channel in the outer wall. (d) Phialidic development: the conidiogenous cell is a phialide. The wall of the phialide is not continuous with the wall surrounding the conidium. The conidial wall arises de novo from newly synthesized material in the neck of the phialide. Diagrams based on Ellis (1971a).

formed by dissolution of septa along a hypha (Fig. 10.10). In most conidia, development is blastic, i.e. there is enlargement of the conidium initial before it is delimited by a septum. Two main kinds of blastic development have been distinguished:

1. **Holoblastic**, in which both the inner and outer wall layers of the conidiogenous cell contribute to conidium formation (Fig. 1.23b). An example of this kind of development is shown by the conidia of *Sclerotinia fructigena* (Fig. 15.3).

2. **Enteroblastic**, in which only the inner wall layers of the conidiogenous cell are involved in conidium formation. Where the inner wall layer balloons out through a narrow pore or channel in the outer wall layer, development is described as **tretic** (Fig. 1.23c). Examples of enteroblastic tretic development are found in *Helminthosporium velutinum* (Fig. 17.12) and *Pleospora herbarum* (Fig. 17.9d). Another important method of enteroblastic development is termed **phialidic** development. Here the conidiogenous cell is a specialized cell termed the **phialide**. During the expansion of the first-formed conidium, the tip of the phialide is ruptured. Further conidia develop by the extension of cytoplasm enclosed by a new wall layer which is laid down in the neck of the phialide and is distinct from the phialide wall. The protoplast of the conidium is pinched off by the formation of an inwardly growing flange which closes to form a septum (Fig. 1.23d). New conidia develop beneath the earlier ones, so that a chain may develop with the oldest conidium at its apex and the youngest at its base. Details of phialidic development are discussed more fully in relation to *Aspergillus* and *Penicillium* (p. 299), which reproduce by means of chains of dry phialoconidia dispersed by wind. Sticky phialospores which accumulate in slimy droplets at the tips of the phialides are common in many genera; they are usually dispersed by insects, rain splash or other agencies.

As mentioned on p. 24, the term conidium is sometimes used for structures which are probably homologous to sporangia. A series can be erected in the Peronosporales in which there are forms with deciduous sporangia which release zoospores when in contact with water (e.g. *Phytophthora*), and other forms which germinate directly, i.e. by the formation of a germ tube (e.g. *Peronospora*). A similar series can be erected in the Mucorales where in some forms the number of sporangiospores per sporangium is reduced to several or even one (see Figs. 7.24, 7.26, 7.30). One-spored sporangia may be distinguished from conidia by being surrounded by two walls, i.e. that of the sporangium and that of the spore itself.

There are numerous other kinds of spore found in fungi, and they are described later in this book in relation to the particular groups in which they occur.

1.4.9 Anamorphs and teleomorphs

Fungi may exist in a range of forms or morphs, i.e. they may be **pleomorphic**. The morph which includes the sexually produced spore form, e.g. the ascocarp of an ascomycete or the basidiocarp of a basidiomycete, is termed the **teleomorph** (Gr. *teleios*, *teleos* = perfect, entire; *morphe* = shape, form) (Hennebert & Weresub, 1977). Many fungi also have a morph bearing asexually produced spores, e.g. conidiomata. These asexual morphs are termed **anamorphs** (Gr. *ana* = throughout, again, similar to). In the older literature, the term **perfect state** was used for the teleomorph and **imperfect state** for the anamorph. This is the origin of the name of the artificial group Fungi Imperfecti or Deuteromycetes, which included fungi believed to reproduce only by asexual means. The term **mitosporic fungi** is sometimes used alternatively for such fungi. The complete range of morphs belonging to any one fungus is termed the **holomorph** (Gr. *holos* = whole, entire) (see Sugiyama, 1987; Reynolds & Taylor, 1993; Seifert & Samuels, 2000). Some fungi have more than one anamorph as in the microconidia and macroconidia of some *Neurospora*, *Fusarium* and *Botrytis* species. These distinctive states are **synanamorphs** and may play different roles in the biology of the fungus. The morph may have a purely sexual role as a fertilizing agent, e.g. in the case of spermatia of many ascomycetes and rust fungi. Such states have been termed **andromorphs** (Gr. *andros* = a man, male) (Parbery, 1996a).

The existence of different states in the life cycle of a fungus has nomenclatural consequences, because they had often been described separately and given different names before the genetic connection between them was established. Further, even after the proof of an anamorph–teleomorph relationship, usually achieved by pure-culture studies, the anamorphic name may still be in wide use, especially where it is the more common state encountered in nature or culture. For example, most fungal geneticists refer to *Aspergillus nidulans* (the name of the conidial state) instead of *Emericella nidulans* (the name for the ascosporic state; p. 308). Similarly, most plant pathologists use *Botrytis cinerea*, the name for the conidial state of the fungus causing the common grey mould disease of many plants, in preference to the rarely encountered *Sclerotinia* (*Botryotinia*) *fuckeliana*, the name given to the apothecial (ascus-bearing) state (see p. 434).

1.5 Taxonomy of fungi

Taxonomy is the science of classification, i.e. the 'assigning of objects to defined categories' (Kirk *et al*., 2001). Classification has three main functions: it provides a framework of recognizable features by which an organism under examination can be identified; it is an attempt to group together organisms that are related to each other; and it assists in the retrieval of information about the identified organism in the form of a list or catalogue.

All taxonomic concepts are man-made and therefore to a certain extent arbitrary. This is especially true of classical approaches relying on macroscopic or microscopic observations because it is a matter of opinion whether the difference in a particular character – say, a spore

or the way in which it is formed – is significant to distinguish two fungi and, if so, at which taxonomic level. The great fungal taxonomist R. W. G. Dennis (1960) described taxonomy as 'the art of classifying organisms: not a science but an art, for its triumphs result not from experiment but from disciplined imagination guided by intuition'.

Recently, great efforts have been made at introducing a seemingly more objective set of criteria based directly on comparisons of selected DNA sequences encoding genes with a conserved biological function, instead of or in addition to phenotypic characters. The results of such comparisons are usually displayed as **phylogenetic trees** (see Fig. 1.26), which imply a common ancestry to all organisms situated above a given branch. Such a grouping is ideally 'monophyletic'. However, as we shall see later, quite different phylogenies may result if different genes are chosen for comparison. Further, a decision on the degree of sequence divergence required for a taxonomic distinction is based mainly on numerical parameters generated by elaborate computerized statistical treatments, occasionally at the expense of sound judgement. An excessive emphasis on such purely descriptive studies in the recent literature has led an eminent mycologist to characterize phylogenetic trees as 'the most noxious of all weeds'. Despite their limitations, these methods have led to a revolution in the taxonomy of fungi. At present, a new, more 'natural' classification is beginning to take shape, in which DNA sequence data are integrated with microscopic, ultrastructural and biochemical characters. However, many groups of fungi are still poorly defined, and many more trees will grow and fall before a comprehensive taxonomic framework can be expected to be in place. One of the core problems in fungal taxonomy is the seemingly seamless transition between the features of two taxa, and the question as to where to apply the cut-off point. To quote Dennis (1960) again, 'a taxonomic species cannot exist independently of the human race; for its constituent individuals can neither taxonomise themselves into a species, nor be taxonomised into a species by science in the abstract; they can only be grouped into species by individual taxonomisers'.

1.5.1 Traditional taxonomic methods

Early philosophers classified matter into three Kingdoms: Animal, Vegetable, and Mineral. Fungi were placed in the Vegetable Kingdom because of certain similarities to plants such as their lack of mobility, absorptive nutrition, and reproduction by spores. Indeed, it was at one time thought that fungi had evolved from algae by loss of photosynthetic pigmentation. This was indicated by the use of such taxonomic groups as Phycomycetes, literally meaning 'algal fungi'. This grouping, approximately synonymous with the loose term 'lower fungi', is no longer used because it includes taxa not now thought to be related to each other (chiefly Oomycota, Chytridiomycota, Zygomycota). Early systems of classification were based on morphological (macroscopic) similarity, but the invention of the light microscope revealed that structures such as fruit bodies which looked alike could be anatomically distinct and reproduce in fundamentally different ways, leading them to be classified apart.

Until the 1980s, the taxonomy of fungi was based mainly on light microscopic examination of typical morphological features, giving rise to classification schemes which are now known to be unnatural. Several examples of unnatural groups may be found by comparing the present edition with the previous edition of this textbook (Webster, 1980). Examples of traditional taxonomic features include the presence or absence of septa in hyphae, fine details of the type, formation and release mechanisms of spores (e.g. Kendrick, 1971), or aspects of the biology and ecology of fungi. Useful ultrastructural details, provided by transmission electron microscopy, concern the appearance of mitochondria, properties of the septal pore, details of the cell wall during spore formation or germination, or the arrangement of secretory vesicles in the apex of growing hyphae (Fig. 1.4). Biochemical methods have also made valuable contributions, especially in characterizing higher taxonomic levels. Examples include the chemical composition of

the cell wall (Table 1.1), alternative pathways of lysine biosynthesis (see p. 67), the occurrence of pigments (Gill & Steglich, 1987) and the types and amounts of sugars or polyols (Pfyffer *et al*., 1986; Rast & Pfyffer, 1989).

Microscopic features are still important today for recognizing fungi and making an initial identification which can then, if necessary, be backed up by molecular methods. Indeed, the comparison of DNA sequences obtained from fungi is meaningful only if these fungi have previously been characterized and named by conventional methods. It is therefore just as necessary today as it ever was to teach mycology students the art of examining and identifying fungi.

1.5.2 Molecular methods of fungal taxonomy

A detailed description of modern taxonomic methods is beyond the scope of this book, and the reader is referred to several in-depth reviews of the topic (e.g. Kohn, 1992; Clutterbuck, 1995). A particularly readable introduction to this subject has been written by Berbee and Taylor (1999). Only the most important molecular methods are outlined here. They are based either directly on the DNA sequences or on the properties of their protein products, especially enzymes.

Proteins extracted from the cultures of fungi can be separated by their differential migration in the electric field of an electrophoresis gel. The speed of migration is based on the charge and size of each molecule, resulting in a characteristic banding pattern. Numerous bands will be obtained if the electrophoresis gel is stained with a general protein dye such as Coomassie Blue. More selective information can be obtained by **isozyme analysis**, in which the gel is incubated in a solution containing a particular substrate which is converted into a coloured insoluble product by the appropriate enzyme, or in which an insoluble substrate such as starch is digested. In this way, the number and electrophoretic migration patterns of isoenzymes can be compared between different fungal isolates. Protein analysis is useful mainly for distinguishing different strains of the same species or members of the same genus (Brasier, 1991a).

Gel electrophoresis can also be used for the separation of DNA fragments generated by various methods. One such method is called **RFLP** (restriction fragment length polymorphisms) and involves the digestion of a total DNA extract or a previously amplified target sequence with one or more restriction endonucleases, i.e. enzymes which cut DNA only at a particular target site defined by a specific oligonucleotide sequence. Fragments from this digest can be blotted from the gel onto a membrane; fragments belonging to a known gene can be visualized by hybridizing with a fluorescent or radioactively labelled DNA probe of the same gene. In this way, a banding pattern is obtained and can be compared with that of other fungal isolates prepared under identical experimental conditions.

A similar method, **RAPD** (random amplified polymorphic DNA), produces DNA bands not by digestion, but by the amplification of DNA sequences. For this purpose, a DNA extract is incubated with a DNA polymerase, deoxynucleoside triphosphates and one or more short oligonucleotides which act as primers for the polymerase by binding to complementary DNA sequences which should be scattered throughout the genome. Amplification is achieved by means of the **PCR** (polymerase chain reaction), in which the mixture is subjected to repeated cycles of different temperatures suitable for annealing of DNA and primer, polymerization, and dissociation of double-stranded DNA. The largest possible size of the amplification product depends on the polymerization time; bands visible on a gel will be produced only if two primer binding sites happen to be in close proximity to each other, so that the intervening stretch of DNA sequence can be amplified from both ends within the chosen polymerization time. The number and size of RAPD bands on electrophoresis gels can be compared between different fungi, provided that all samples have been produced under identical conditions.

Isozyme, RFLP and RAPD analyses all generate data which are useful mainly for comparing

closely related isolates. Since the results strongly depend on the experimental conditions employed, there are no universal databases for these types of analysis. Further, they are unsuitable for comparisons of distantly related or unrelated organisms. A breakthrough in the taxonomy of fungi as well as other organisms was achieved when primers were developed which guided the PCR amplification of specific stretches of DNA universally present and fulfilling a homologous function in all life forms. Once amplified, the sequence of bases can be determined easily. Such methods were first applied to bacterial systematics with spectacular results (Woese, 1987). In eukaryotes, the most widely used target sequences are those encoding the 18S or 28S ribosomal RNA (rRNA) molecules, which fulfil a structural role in the small or large ribosomal subunits (respectively), or the non-coding DNA stretches (**ITS**, internal transcribed spacers), which physically separate these genes from each other and from the 5.8S rRNA sequence in the nuclear genome (Fig. 1.24; White *et al*., 1990). The structural role which rRNA molecules play in the assembly of ribosomes requires them to take up a particular configuration which is stable because of intramolecular base-pairing. Since certain regions of each rRNA molecule hybridize with complementary regions within the same molecule or with other rRNA molecules, mutations in the DNA encoding these regions are rare because they would impair hybridization and thus the functioning of the rRNA molecule unless accompanied by a mutation at the complementary binding site. The non-pairing loop regions of the rRNA gene and the ITS sequences are not subjected to such a strong selective pressure and thus tend to show a higher rate of mutation. Nucleotide sequences therefore permit the comparison of closely related species or even strains of the same species (ITS sequences), as well as that of distantly related taxa or even members of different kingdoms (18S or 28S rRNA). Further, because extensive databases are now available, the sequence analysis of a single fungus can provide meaningful taxonomic information when compared with existing sequences. In addition to ribosomal DNA sequences, genes encoding cytochrome oxidase (*COX*), tubulins or other proteins with conserved functions are now used extensively for phylogenetic purposes.

Once comparative data have been obtained either by banding patterns or gene sequencing, they need to be evaluated. This is usually done by converting the data into a matrix, e.g. by scoring the absence or presence of a particular band. With comparisons of aligned DNA sequences, only informative positions are selected for the matrix, i.e. where variations in the nucleotides between different fungi under investigation are observed. When the matrix has been completed, it can be subjected to statistical treatments, and phylogenetic trees are drawn by a range of algorithms. In some, the degree of relatedness of taxa is indicated by the length of the branch separating them (see Figs. 1.25, 1.26). Such information is thought to be of evolutionary significance; the greater the number of differences between two organisms, the earlier the separation of their evolutionary lines should have occurred.

1.5.3 How old are fungi?

Several lines of evidence indicate that fungi are a very ancient group of organisms. Berbee and Taylor (2001) have attempted to add a timescale to phylogenetic trees by applying the concept of a 'molecular clock', i.e. the assumption that the rate of mutations leading to phylogenetic diversity is constant over time and in various groups of organisms. By calibrating their molecular clock against fossil evidence, Berbee and Taylor (2001) estimated that fungi may have separated from animals some 900 million years ago, i.e. long before the evolution of terrestrial organisms. This estimate is consistent with the discovery of fossilized anastomozing hypha-like structures in sediments about 1 billion years old (Butterfield, 2005). Fungi recognizable as Chytridiomycota, Zygomycota and Ascomycota have been discovered among fossils of early terrestrial plants from the Lower Devonian Rhynie chert, formed some 400 million years ago (Taylor *et al*., 1992, 1999, 2005). It is apparent that these early terrestrial plants already entertained mycorrhizal symbiotic associations

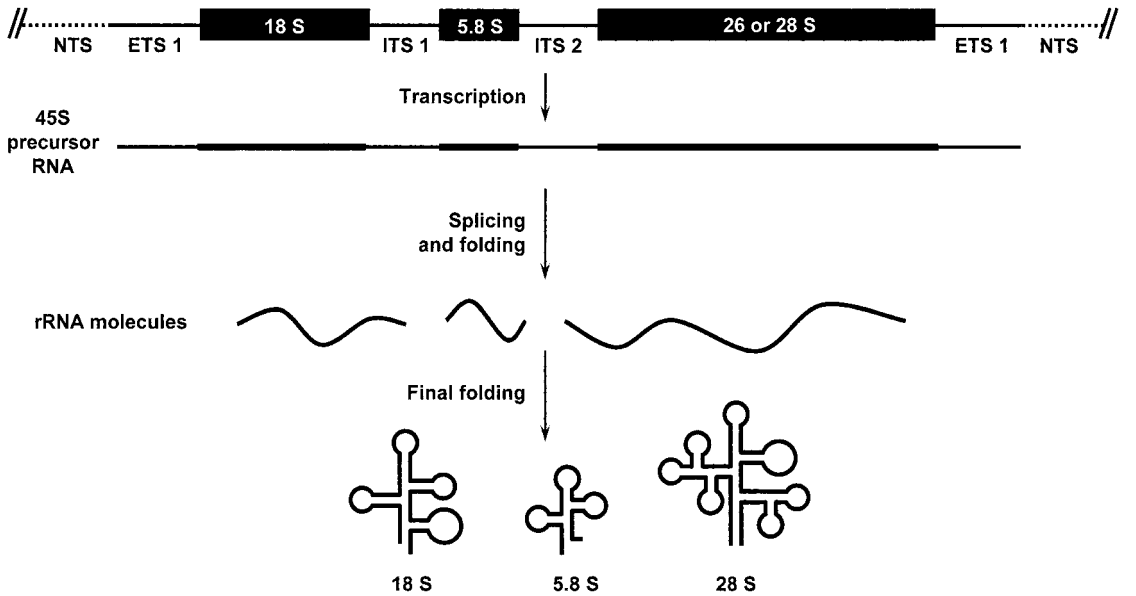

Fig 1.24 The spatial arrangement of a nuclear rRNA gene repeat unit. Each haploid fungal genome contains about 50–250 copies of this repeat, depending on the species (Vilgalys & Gonzalez, 1990). The three structural rRNA genes encoded by one repeat unit, i.e. 18S, 5.8S and 28S, are separated by internal and external transcribed spacers (ITS and ETS, respectively). Adjacent copies of the repeat unit are separated by a short non-transcribed spacer (NTS). The whole unit is transcribed into a 45S precursor RNA in one piece, followed by excision of the three structural RNA molecules from the spacers which are not used. The 5S rRNA gene is encoded at a separate locus. The 18S rRNA molecule is part of the small ribosomal subunit, whereas the other three contribute to the large subunit.

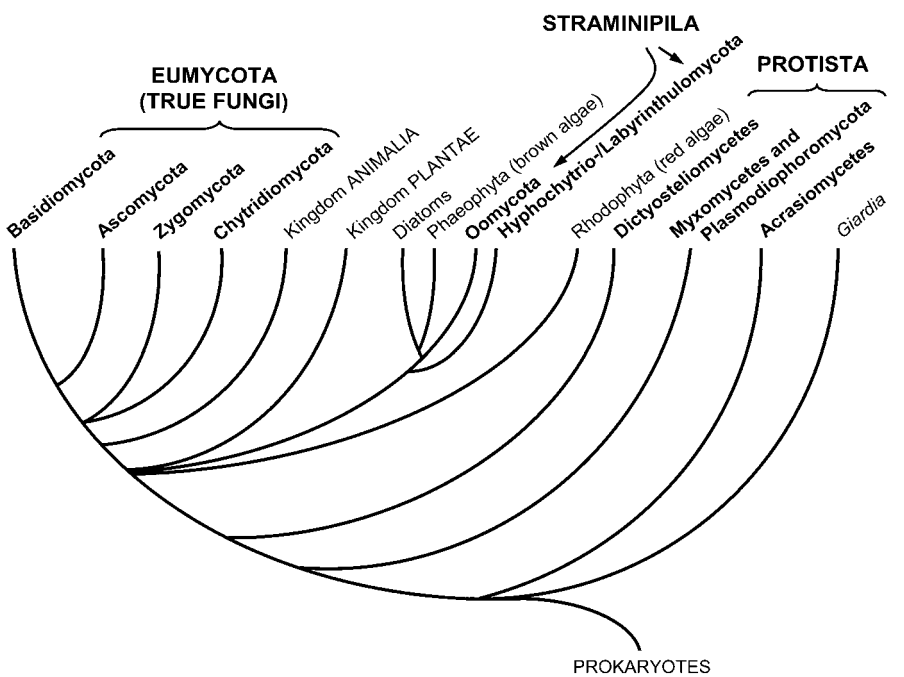

Fig 1.25 The phylogenetic relationships of Fungi and fungus-like organisms studied by mycologists (printed in bold), with other groups of Eukaryota. The analysis is based on comparisons of 18S rDNA sequences. Modified and redrawn from Bruns *et al*. (1991) and Berbee and Taylor (1999).

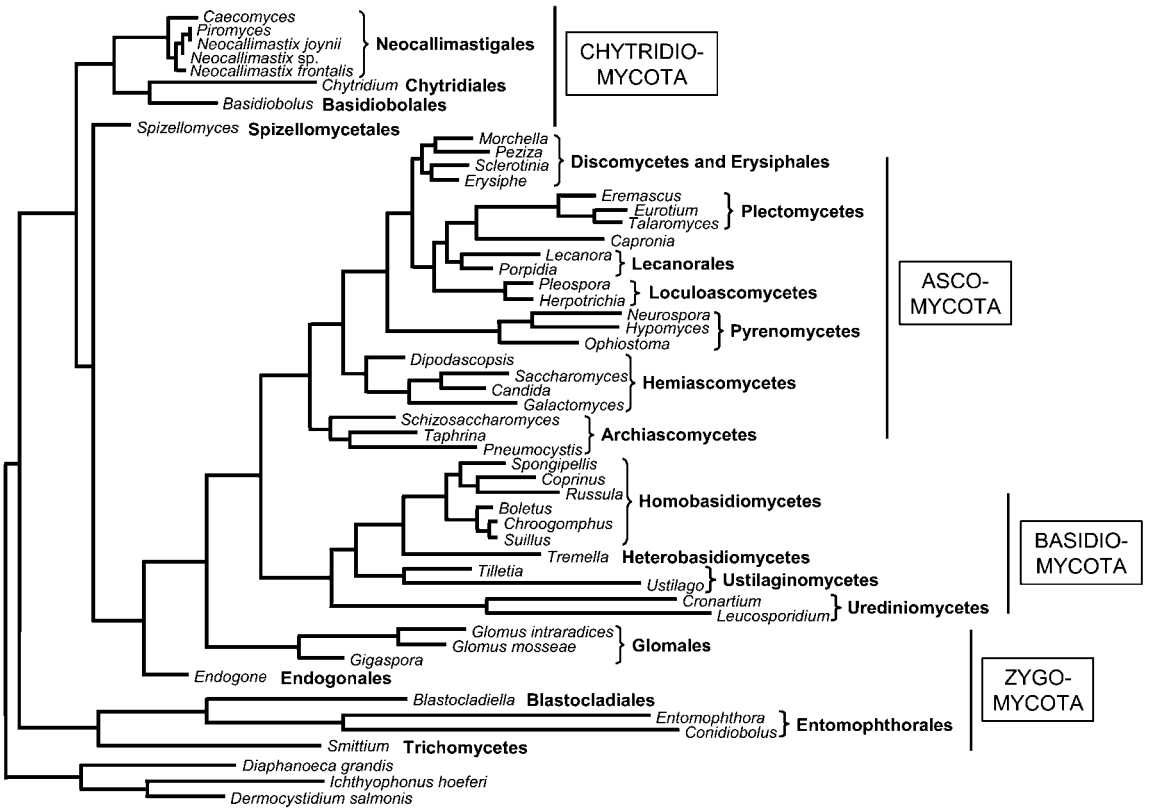

Fig 1.26 Phylogenetic relationships within the Eumycota, based on 18S rDNA comparisons. This tree illustrates the analytical power of molecular phylogenetic analyses; all four phyla of Eumycota are resolved. However, it also highlights problems in that *Basidiobolus* groups with the Chytridiomycota, although sharing essential biological features with the Zygomycota, and that conversely *Blastocladiella* groups with the Zygomycota instead of the Chytridiomycota. Modified and redrawn from Berbee and Taylor (2001), with kind permission of Springer Science and Business media.

with glomalean members of the Zygomycota (see p. 218).

1.5.4 The taxonomic system adopted in this book

The discipline of fungal taxonomy is evolving at an unprecedented speed at present due mainly to the contributions of molecular phylogeny. Numerous taxonomic systems exist, but this is not the place to discuss their relative merits (see Whittaker, 1969; Margulis *et al*., 1990; Alexopoulos *et al*., 1996; Cavalier-Smith, 2001; Kirk *et al*., 2001). In this book we have tried to follow the classification proposed in *The Mycota* Volumes VIIA and VIIB (McLaughlin *et al*., 2001), but even in these volumes the authors of different chapters have used their own favoured systems of classification rather than adopting an imposed one. In cases of doubt, we have attempted to let clarity prevail over pedantry.

Fungi in the widest sense, as organisms traditionally studied by mycologists, currently fall into three kingdoms of Eukaryota, i.e. the Eumycota which contain only fungi, and the Protozoa and Chromista (= Straminipila), both of which contain mainly organisms not studied by mycologists and were formerly lumped together under the name Protoctista (Beakes, 1998; Kirk *et al*., 2001). The Protozoa are notoriously difficult to resolve by phylogenetic means, and the only firm statement which can be made at present is that they are a diverse and ancient group somewhere between the higher Eukaryota ('crown eukaryotes') and the

Table 1.2. The classification scheme adopted in this book, showing mainly those groups treated in some detail.

KINGDOM PROTOZOA
Myxomycota (Chapter 2)
- Acrasiomycetes
- Dictyosteliomycetes
- Protosteliomycetes
- Myxomycetes

Plasmodiophoromycota (Chapter 3)
- Plasmodiophorales
- Haptoglossales (Oomycota?)

KINGDOM STRAMINIPILA
Hyphochytriomycota (Chapter 4)
Labyrinthulomycota (Chapter 4)
- Labyrinthulomycetes
- Thraustochytriomycetes

Oomycota (Chapter 5)
- Saprolegniales
- Pythiales
- Peronosporales

KINGDOM FUNGI (EUMYCOTA)
Chytridiomycota (Chapter 6)
- Chytridiomycetes

Zygomycota (Chapter 7)
- Zygomycetes
- Trichomycetes

Ascomycota (Chapter 8)
- Archiascomycetes (Chapter 9)
- Hemiascomycetes (Chapter 10)
- Plectomycetes (Chapter 11)
- Hymenoascomycetes
  - Pyrenomycetes (Chapter 12)
  - Erysiphales (Chapter 13)
  - Pezizales (Chapter 14)
  - Helotiales (Chapter 15)
  - Lecanorales/lichens (Chapter 16)
- Loculoascomycetes (Chapter 17)

Basidiomycota (Chapter 18)
- Homobasidiomycetes (Chapter 19)
- Homobasidiomycetes: gasteromycetes (Chapter 20)
- Heterobasidiomycetes (Chapter 21)
- Urediniomycetes (Chapter 22)
- Ustilaginomycetes (Chapter 23)

prokaryotes (Kumar & Rzhetsky, 1996). An overview of eukaryotic organisms, in which those groups treated in this book are highlighted, is given in Fig. 1.25. Among the Protozoa, the Plasmodiophoromycota are given extensive treatment because of their role as pathogens of plants (Chapter 3), whereas the various forms of slime moulds are considered only briefly (Chapter 2). Similarly brief overviews will be given of most groups of Straminipila studied by mycologists (Chapter 4), except for the Oomycota which, despite their separate evolutionary origin, represent a major area of mycology (Chapter 5). All remaining chapters deal with members of the Eumycota (= Kingdom Fungi). The scheme is summarized in Table 1.2 and illustrated in Fig. 1.26. An overview of the nomenclature used for describing taxa within the Eumycota is given in Table 1.3.

In the past, fungi which solely or mainly reproduce asexually (Fungi Imperfecti, Deuteromycota, mitosporic fungi, anamorphic fungi) were considered separately from their sexually reproducing relatives the teleomorphs, and separate anamorph and teleomorph genera were erected. However, information from pure-culture studies and molecular phylogenetic approaches has linked many anamorphs with their teleomorphs. For instance, the conidial (imperfect) state of the common brown-rot fungus of apples and other fruits is called *Monilia fructigena*, whereas the sexual (perfect)

Table 1.3. Example of the hierarchy of taxonomic terms. The wheat stem rust fungus, *Puccinia graminis*, is used as an example.

Kingdom Fungi
Subkingdom Eumycota
Phylum Basidio**mycota**
Class Uredinio**mycetes**
Order Uredin**ales**
Family Puccini**aceae**
Genus *Puccinia*
Species *Puccinia graminis*
Race *Puccinia graminis*
f. sp. *tritici*

state is apothecial, being called *Sclerotinia* (*Monilinia*) *fructigena*. As far as is possible, we shall consider anamorphic states of fungi in the context of their known sexual state. Thus, an account of the brown-rot of fruits, although encountered predominantly as the conidial state, will be given in the chapter dealing with apothecial fungi (Helotiales, Chapter 15). Where practical, we have given the teleomorph name priority over the anamorph. As a long-term future goal, Seifert and Samuels (2000) and Seifert and Gams (2001) have outlined a unified taxonomy which might ultimately lead to the abolition of the names of anamorphic genera.

However, with certain ecological groups such as the Ingoldian aquatic fungi (Section 25.2) and nematophagous fungi (Section 25.1), which have diverse relationships, we have deliberately chosen to consider them in their ecological context rather than along with their varied taxonomic relatives.