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CHAPTER 21

Viral and Prion Infections

Biotechnology

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VIRAL INFECTIONS AND ANTIVIRAL AGENTS

Many human diseases are due to viruses. These agents consist of genomes of either DNA or RNA inside a protein shell. Despite this deceptive simplicity, virus infections are less well understood than bacterial diseases, largely because viruses cannot be grown alone in culture but depend on a host cell. Until recently, protection against virus diseases relied on public health measures and vaccination. Only since the late 1980s have a significant number of specific antiviral agents become available.

Pathogenic bacteria contain many unique components not found in eukaryotic cells, which can be targeted by antibiotics. In contrast, because viruses rely on the host cell for almost all of their metabolic reactions, they usually have few unique components apart from the structural proteins of the virus particle. Consequently, most chemical agents that prevent virus metabolism are also toxic to the host cells. Another problem is that viruses mutate rapidly and so develop resistance to antiviral agents relatively quickly. This is especially serious for RNA viruses, such as influenza or HIV, which have extremely high mutation rates.

Like pathogenic bacteria, viruses must also attach to and invade host cells. Recognition proteins on the surface of the virus capsid bind to specific receptors on the surface of the host cell. After entry, viral replication occurs at the expense of the host cell, which supplies not only raw material and energy, but also the ribosomes needed for synthesis of viral proteins and often many of the enzymes required for synthesis of viral nucleic acids as well. Finally, new virus particles are assembled and exit the cell. These stages, and some corresponding antiviral agents are shown in Figure 21.1 and listed in Table 21.1. Antiviral agents that combat HIV are discussed in the later section on AIDS. In addition to HIV, we have chosen to focus on influenza, as it is one of the most widespread human viruses and illustrates many facets of virus biology and of the use of biotechnology for virus control.

Finally, we discuss prions. These infectious agents were originally believed to be anomalous viruses, hence their inclusion here. However, they consist solely of protein, with no enclosed nucleic acid. Thus, they are definitely not viruses despite sharing the superficial properties of size and infectiousness. Indeed, recent work suggests prion disease is related to other neurological disorders, not normally regarded as infectious.

Relatively few antiviral agents are available compared to the number of antibiotics for treating bacterial infections. Moreover, most antivirals have harmful side effects.

INTERFERONS COORDINATE THE ANTIVIRAL RESPONSE

Interferons are a class of proteins induced in animal cells in response to virus infection. Clinical treatment with interferons is used to treat viral infections in a few cases (e.g., against hepatitis B and hepatitis C infections). **Interferons α** and **β (INF α** and **INF β)** block the spread of viruses by interfering with virus replication. (**Interferon** γ is quite distinct and is not induced directly by virus infection. It responds to intracellular pathogens.) Double-stranded RNA, which is symptomatic of the replication of most RNA viruses, activates secretion of interferons α and β. They bind to the interferon receptors of both the infected cell itself and its neighbors. Locally, this triggers a phosphorelay signal pathway that activates several genes that combat virus infection (Fig. 21.2). Interferons also help activate immune system cells, such as NK cells, which selectively destroy virus-infected cells.

Antiviral proteins induced by interferon include oligoadenylate synthetase, which converts ATP into 2′-5′-linked poly(A). This removes the ATP required as an energy source for viral

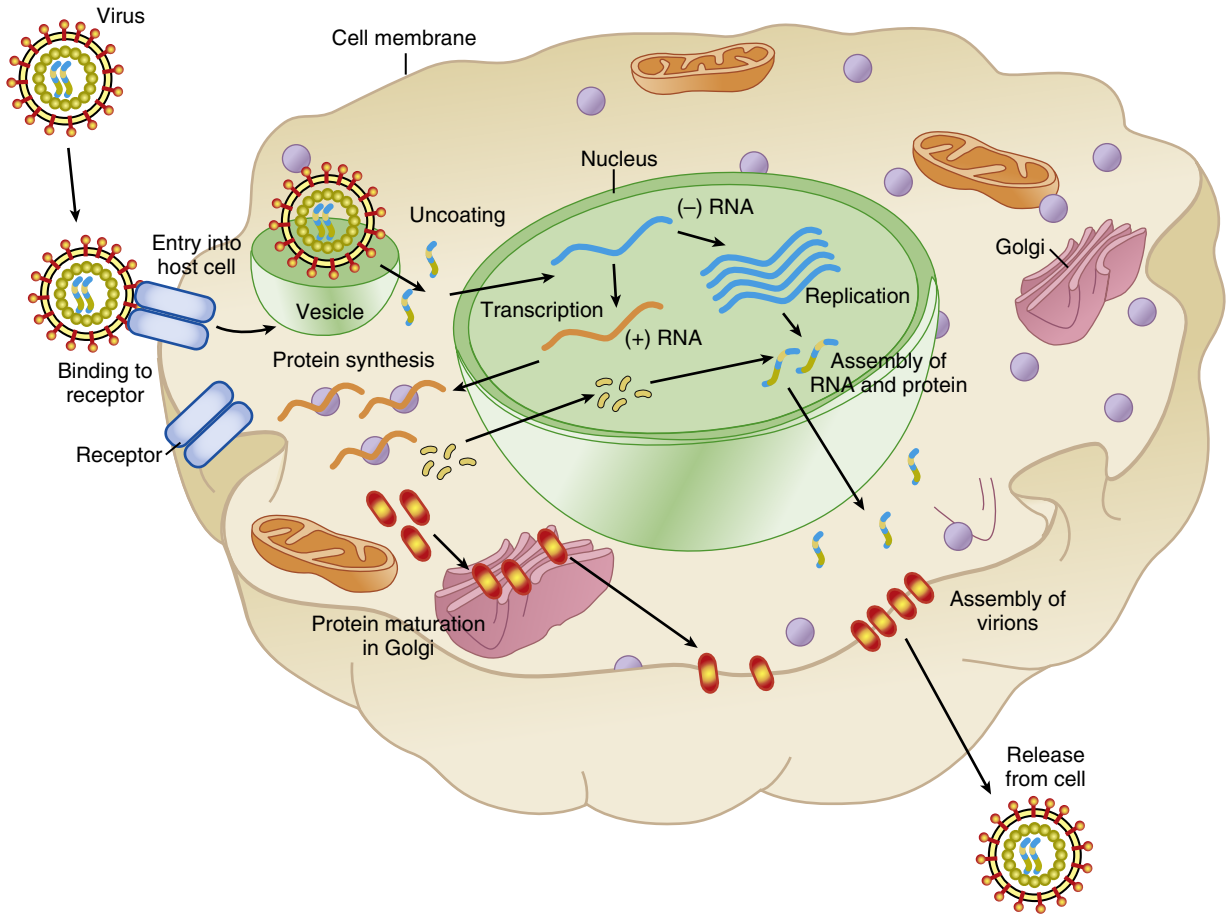


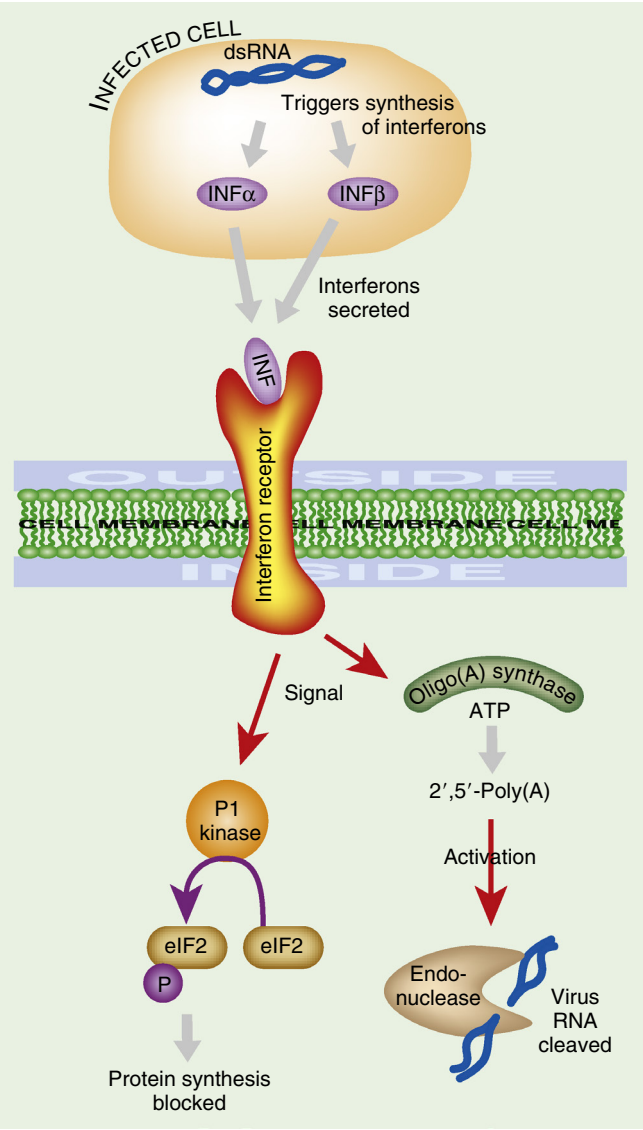
FIGURE 21.1 Virus Life Cycle with Antiviral Targets
The stages of virus life cycle provide several possible targets for antiviral agents. The virus shown here contains negative sense RNA (red). This is transcribed in the nucleus to give positive RNA (orange) that is translated by the ribosomes in the host cell cytoplasm to give viral proteins. Some proteins associate with the viral nucleic acid, whereas others move to the host cell membrane, via the Golgi apparatus, before virus assembly.

Table 21.1 Virus Life Cycle and Antagonists

Stage in Life Cycle	Possible Antiviral Agents
Binding to receptor	WIN compounds (picornavirus), zinc (rhinovirus)
Entry into host cell	Amantadine (influenza)
Gene expression	Interferons α and β
Reverse transcription (retroviruses only)	Reverse transcriptase inhibitors
Replication	Nucleoside analogs
Assembly of virions	Protease inhibitors
Release from cell	Neuraminidase inhibitors (influenza)

replication. In addition, 2′-5′-poly(A) activates an endonuclease that cleaves viral RNA. P1 kinase is also activated and phosphorylates initiation factor eIF2, halting protein synthesis. The **Mx proteins** are GTPases that interfere with the assembly of the RNA polymerase of negative-strand RNA viruses (e.g., influenza, parainfluenza). The Mx proteins form a ring that surrounds the viral RNA (Fig. 21.3), thus preventing the RNA polymerase from moving along and replicating the genome.

FIGURE 21.2
Interferons and Antiviral Proteins
The presence of dsRNA inside an infected cell triggers production of INFα and INFβ. These are secreted to neighboring cells, bind to the interferon receptor, and activate various antiviral proteins. P1 kinase blocks protein synthesis by phosphorylating eIF2 (an elongation factor). Oligo(A) synthetase converts ATP to 2′,5′-poly(A), which activates an endonuclease to digest dsRNA and depletes the ATP supply. Without ATP and protein synthesis, the virus cannot survive in the host cell.



Different strains of influenza virus differ in their susceptibility to Mx proteins, and conversely, different animals have slightly different Mx proteins. These variants play a major role in determining both virulence and the transmission of virus between different animals.

Interferon alpha was one of the first mammalian proteins to be manufactured via genetic engineering. However, its clinical effects have been disappointing except in a few cases, such as treatment of hepatitis C. Recent attempts at antiviral therapy have moved away from interferons and focused on using the RNA interference system.

Interferons are animal proteins that promote the antiviral response by inducing synthesis of a range of enzymes with specific antiviral activities.

ANTIVIRAL THERAPY USING RNA INTERFERENCE

The basics of RNA technology were discussed in Chapter 5. Antisense RNA and ribozyme therapy have been proposed for antiviral therapy, but neither has proven effective so far. However, using RNA interference (RNAi) to treat virus infection looks promising.

RNA interference is a natural defense system used by cells to protect themselves against invasion by RNA viruses. RNAi targets double-stranded RNA (dsRNA) derived from RNA virus replication and destroys both the dsRNA and corresponding single-stranded RNA (in practice, this will usually be viral mRNA). RNAi is triggered by short dsRNA molecules of just over 20 nucleotides, known as short-interfering RNA (siRNA).

Not surprisingly, many viruses have evolved mechanisms to avoid destruction by RNAi. However, in mammals, administration of artificially synthesized siRNA around 17–21 nucleotides long provokes a strong RNAi response even against viruses with protection mechanisms. The sequence of the siRNA is designed to represent conserved regions of the RNA virus genome.

RNAi therapy is especially useful for viruses infecting the respiratory tract. The reason is that the siRNA can be administered easily by inhalation. RNAi is effective against respiratory syncytial virus, influenza, parainfluenza, measles, and several coronaviruses. The siRNA sequences can be screened for effectiveness in cell culture before being used on whole organisms. Phase II clinical trials using siRNA against respiratory syncytial virus are underway, and so far the results are promising.

RNAi can also protect plants against RNA viruses. To achieve this resistance, scientists engineer constructs that generate siRNA internally into transgenic plants (see Chapter 15 for details of plant genetic engineering). One common approach is to express RNA that folds into hairpin structures and hence includes a length of dsRNA. This triggers the synthesis of siRNA and RNAi, and the plants become resistant to the virus as a result (Fig. 21.4). RNAi is now being investigated for protecting crop plants, especially rice, against RNA viruses. Several RNA viruses of rice that are spread by insects may cause major crop losses.

RNAi is a natural form of defense against RNA viruses. It can be stimulated by administration of siRNA. Respiratory infections are especially easy to treat with RNAi because the siRNA can be inhaled as a spray.

INFLUENZA IS A NEGATIVE-STRAND RNA VIRUS

Influenza virus, an **orthomyxovirus**, is an example of a negative-strand single-stranded RNA virus. In other words, the virus genome is present in the virus particle as noncoding (= antisense = negative-strand) RNA. The flu virus particle contains a segmented genome consisting of eight separate pieces of single-stranded RNA ranging from 890 to 2341 nucleotides long. These pieces are each packed into an inner **nucleocapsid** and are surrounded by an outer envelope (Fig. 21.5). Although the outer membrane is derived from host-cell material, it contains virus-encoded proteins such as neuraminidase, hemagglutinin, and ion channels. These viral proteins are made on the ribosomes of the infected host cell and are involved in virus recognition and entry into successive host cells. The hemagglutinin (H) and neuraminidase (N) of influenza differ slightly but significantly between strains of flu. These variants are designated by H and N numbers. Thus, the Spanish flu of 1918 was H_1N_1, and the avian flu presently spreading worldwide is H_5N_1. The virulent outbreak of novel avian flu in China in 2013 was H_7N_9. This virus contains segments from several different avian flu strains. Genome analysis confirms increased virulence and implies resistance to amantadine (see following discussion).

When a flu virus comes in contact with an appropriate host cell, it is engulfed and ends up inside a vesicle. Both the vesicle and the outer coat of the virus particle are dissolved, releasing the nucleocapsids, which enter the nucleus. The nucleocapsids disassemble inside the nucleus, releasing the RNA molecules (Fig. 21.6).

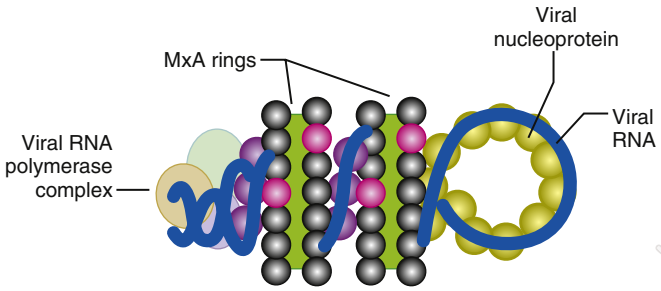


FIGURE 21.3
Mechanism of Action of Mx Protein
The viral RNA polymerase moves along the viral genomic RNA to replicate it. The Mx protein assembles into ring structures that surround the viral RNA. This blocks the movement of the RNA polymerase and consequently prevents replication. From Gao S et al. (2011). Structure of myxovirus resistance protein a reveals intra- and intermolecular domain interactions required for the antiviral function. *Immunity* **35**, 514–525.

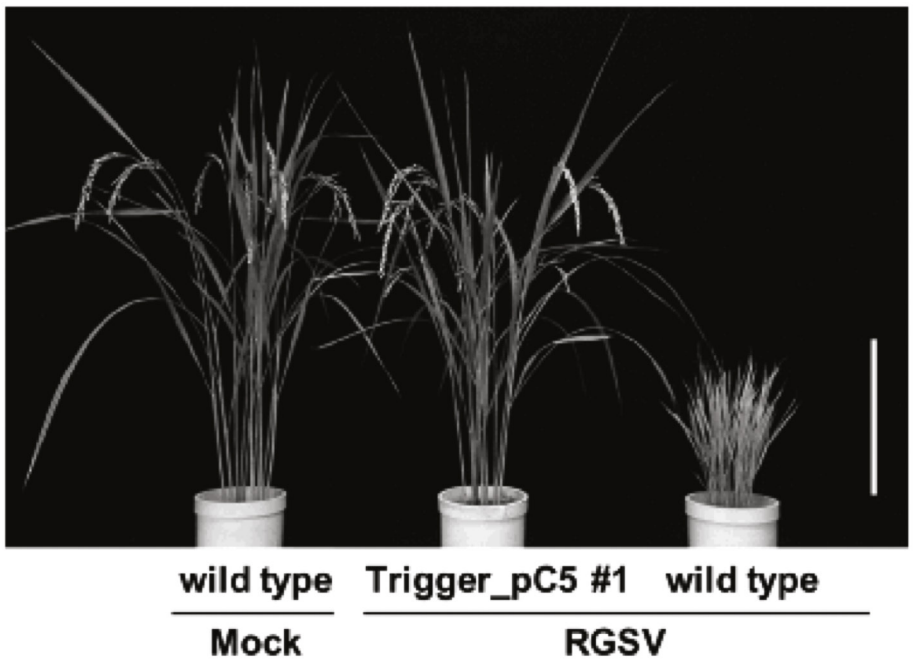


FIGURE 21.4 **RNAi versus Plum Pox Virus**
Transgenic *Nicotiana benthamiana* plants were constructed that expressed a hairpin RNA that triggers RNAi versus plum pox virus (the agent of "sharka disease"). *N. benthamiana* is a close relative of *N. tabacum*, the tobacco plant that grows in Australia. Wild-type and transgenic *Nicotiana benthamiana* plants were then tested against infection with plum pox virus. After 7 days, severe wilting was seen in the wild-type but not the transgenic plants. From Pandolfini T, et al. (2003). Expression of self-complementary hairpin RNA under the control of the *rolC* promoter confers systemic disease resistance to plum pox virus without preventing local infection. *BMC Biotechnol* **3**, 7.

FIGURE 21.5
Structure of the Influenza Virus
The influenza virus has an outer envelope containing neuraminidase, hemagglutinin, and ion channels. Several individual negative-strand ssRNA molecules are packaged within the outer membrane. Each strand is coated with nucleocapsid proteins. An RNA replicase molecule is also included with each ssRNA strand to ensure expression.

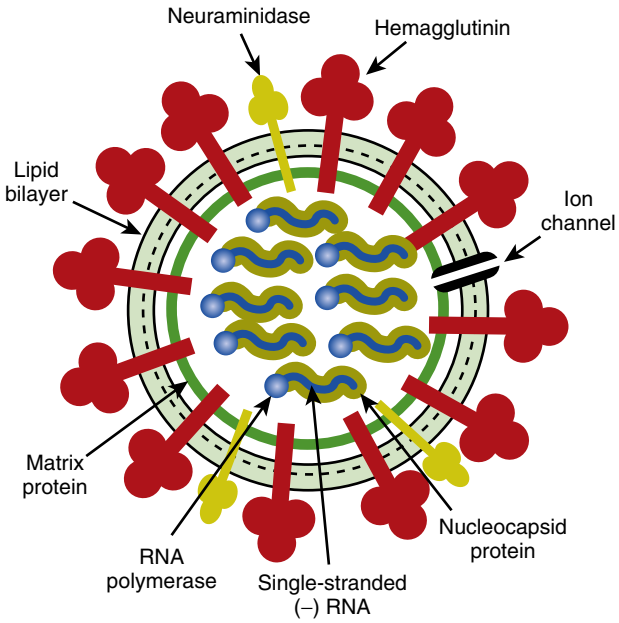
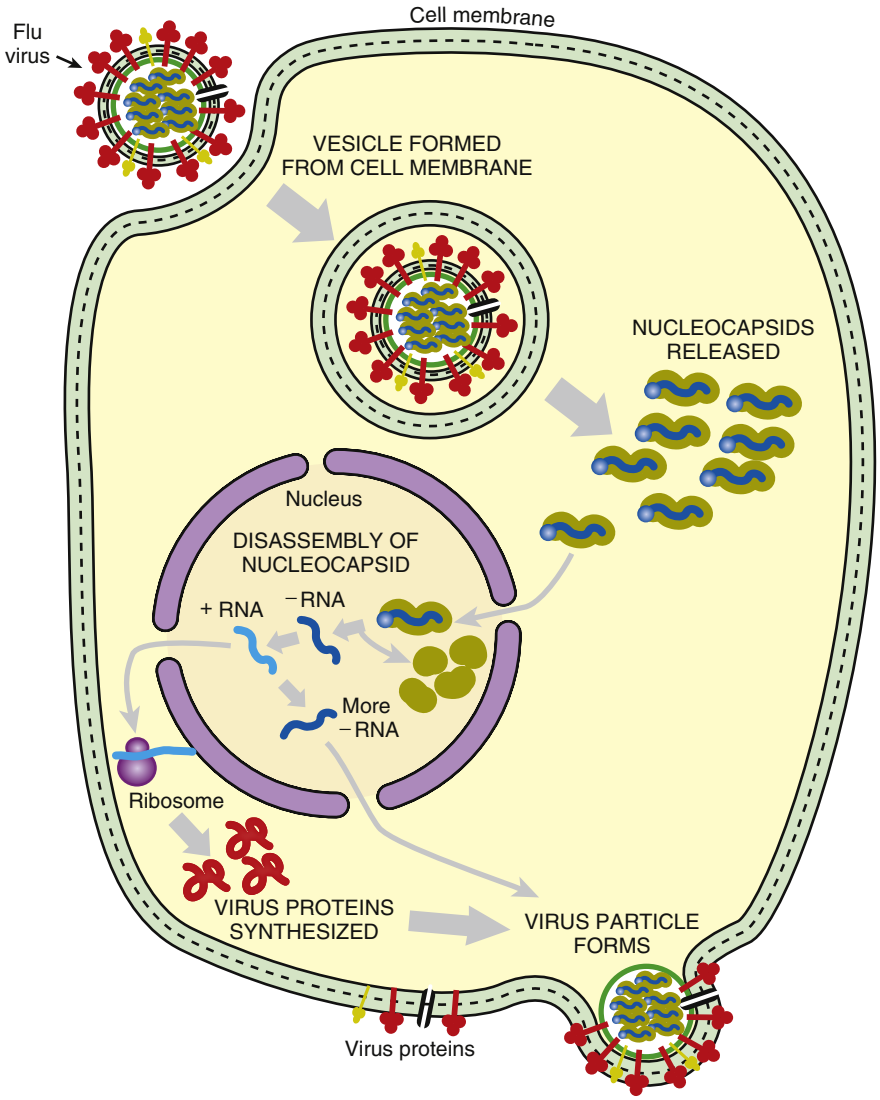


FIGURE 21.6
Life Cycle of the Influenza Virus
After entry into the host cell, the nucleocapsids enter the nucleus before disassembly. There the viral replicase makes positive RNA strands and more negative strands. The (+) RNA strands are exported to the ribosomes, where they act as mRNA and are translated. The resulting viral proteins are assembled into more virus particles, together with the (–) RNA strands.



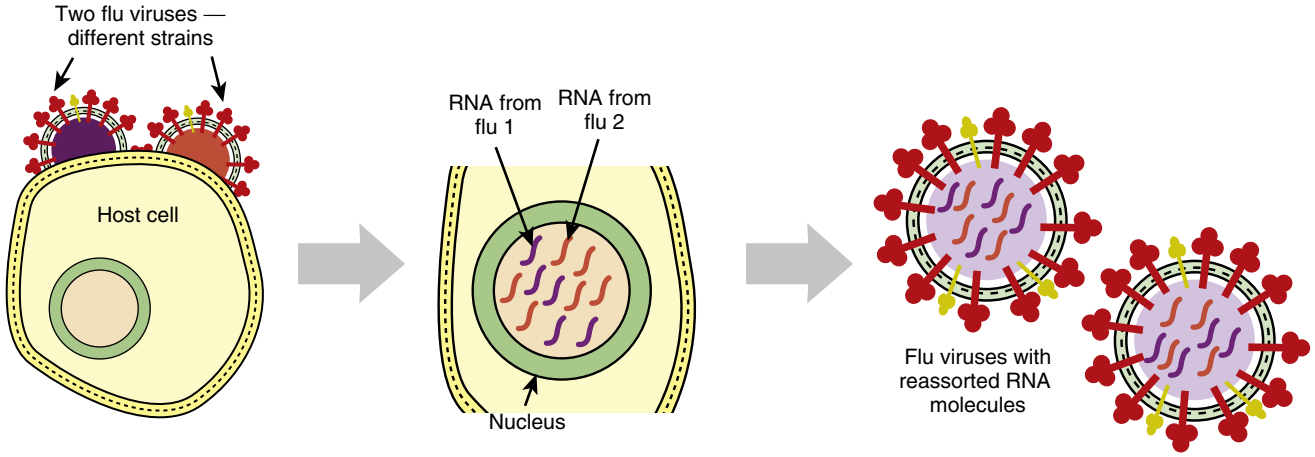


FIGURE 21.7 Influenza Viral Genomes Can Switch RNA Segments
If two different influenza strains infect the same host cell, the genomes of both will enter the nucleus. When new virus particles are formed, some nucleocapsids from strain 1 may be packaged with strain 2, and vice versa. Thus, complete ssRNA molecules from different influenza strains may be reshuffled to generate new assortments. Such reshuffling more often happens in pigs and birds than in human hosts.

Replication of the influenza RNA occurs in the nucleus. The viral mRNA exits the nucleus just like normal cellular mRNA and travels to the ribosomes in the cytoplasm. Here, the proteins for the new virus particles are made.

Because influenza virus has its genes scattered over eight separate molecules of RNA, different strains of flu can trade segments of RNA and form new genetic combinations (Fig. 21.7). In addition, mutations occur at a higher rate during RNA replication than in DNA. These two mechanisms result in a lot of genetic diversity. Consequently, different strains of flu emerge every couple of years. The changing surface antigens of the virus allow it to avoid immune recognition. These different flu strains vary greatly in their apparent virulence. However, this depends as much on the immune history of the human population as on genetic changes in the virus.

Influenza viruses fall into two major groups: influenza A and B. Mutation of both A and B causes annual epidemics due to slow antigenic drift. Influenza B is largely restricted to humans and has less genetic variation. Influenza A has a wider host range ("people, pigs, and poultry"). As a result, influenza A gives rise to severe but less common epidemics due to reassortment of viruses from different hosts during mixed infections.

The Spanish flu of 1918–1919 was the worst influenza A pandemic so far and is estimated to have killed around 50 million people (more than World War I). Will there be another major flu pandemic soon? The major threat seems to be the successive versions of avian flu emerging in Asia. Relatively few humans catch these viruses by direct transmission from birds. The real danger is that these avian viruses will mutate to become transmissible from person to person.

Amantadine is a tricyclic amine that binds to the M2 protein, one of the transmembrane ion channels found in the outer envelope of influenza A virus. M2 is not expressed by influenza B, and consequently, amantadine works only against type A influenza. Amantadine blocks the M2 ion channel, and this stops entry of protons, which prevents uncoating of the virus particle (Fig. 21.8). Thus, entry of the virus is prevented. Amantadine must be given very early in infection. Amantadine was the first specific antiviral agent to be discovered, although its mode of action was only elucidated later.

Influenza (both A and B) may also be treated with neuraminidase inhibitors, such as oseltamivir (=Tamiflu) or zanamivir. These inhibitors are analogs of *N*-acetylneuraminic acid. Neuraminidase normally cleaves this from the virus receptor, allowing progeny virus particles to be released. If neuraminidase is inhibited, progeny virus is trapped in infected cells. Resistance can arise due to mutations in the N protein; for example, H247Y (changing His247 to Tyr) results in resistance to oseltamivir but not to zanamivir.

Influenza is an extremely common viral infection of humans and some other animals. Its genome consists of eight pieces of RNA of negative complementarity. As a result, it shows a high rate of both mutation and recombination. Very few drugs are available to treat influenza.

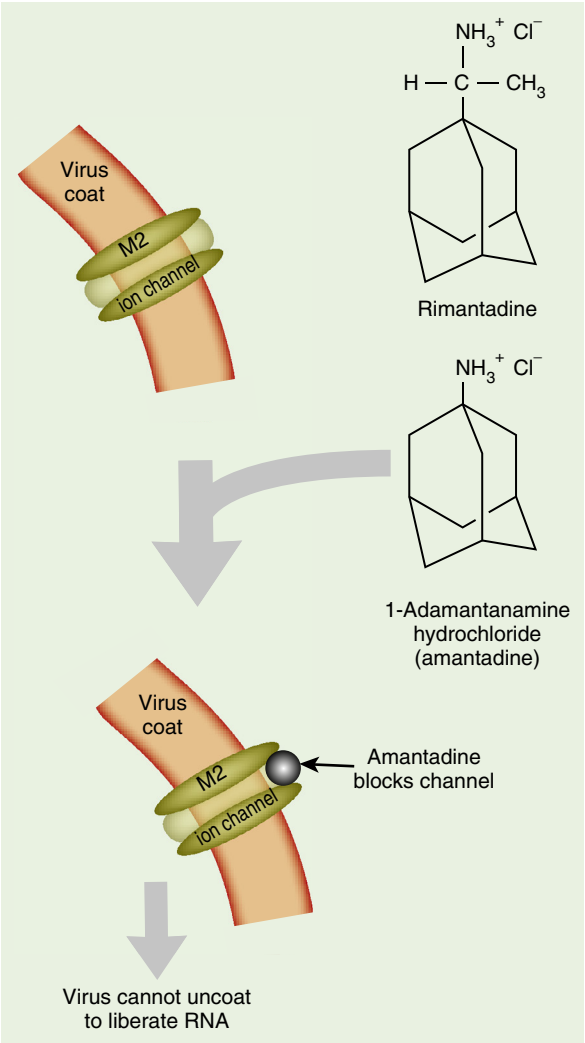


FIGURE 21.8
Amantadine Blocks M2 Ion Channel
The amantadine molecule blocks ions from passing though the M2 channel in the virus coat, thus preventing uncoating and RNA molecule release.

THE AIDS RETROVIRUS

Acquired immunodeficiency syndrome (AIDS) is caused by human immunodeficiency virus (HIV), which damages the immune system. Most AIDS patients die of **opportunistic infections**. These infections are seen only in patients with defective immune systems and are caused by assorted viruses, bacteria, protozoans, and fungi that are normally relatively harmless but may attack if host defenses are down. In addition, without immune surveillance, cancers caused by other viruses or somatic mutations often grow out of control.

HIV infects white blood cells belonging to the immune system, the **T cells**. The **CD4 protein** is found on the surface of many T cells, where it acts as an important receptor during the immune response (see Chapter 6). HIV also uses the CD4 protein as a receptor (Fig. 21.9). The **gp120** protein in the outer envelope of HIV is a glycoprotein with a molecular weight of 120 kDa. It recognizes and binds to CD4, which is needed for entry of the virus.

The CD4 protein is also found on the surface of some other immune system cells—the monocytes and macrophages. HIV does not seriously harm these two cell types, but the cells become reservoirs to spread the virus to more T cells. The damage to the T cells is most critical to immune function. Once HIV has entered the T cell, the DNA form of the retrovirus genome integrates into the host chromosome and begins to express virus genes. Viral proteins are manufactured on host ribosomes. In particular, the HIV envelope protein, gp120, is made in large amounts and inserts into the T-cell membrane. The gp120 on the surface of infected T cells binds to the CD4 protein on other T cells. Consequently, several T cells clump together and fuse (Fig. 21.10). The giant, multiple cell soon dies. About 70% of the body's T cells carry the CD4 receptor. As they gradually die off, the immune response fades away over a 5- to 10-year period.

AIDS is caused by a retrovirus that uses the CD4 protein on the surface of T cells as a receptor. Damage to T cells cripples the immune response, leaving the body open to other infections.

CHEMOKINE RECEPTORS ACT AS CO-RECEPTORS FOR HIV

The entry of HIV into T cells requires binding of virus to both the CD4 protein and one of several chemokine receptors, which act as **co-receptors**. The **chemokine receptors** are

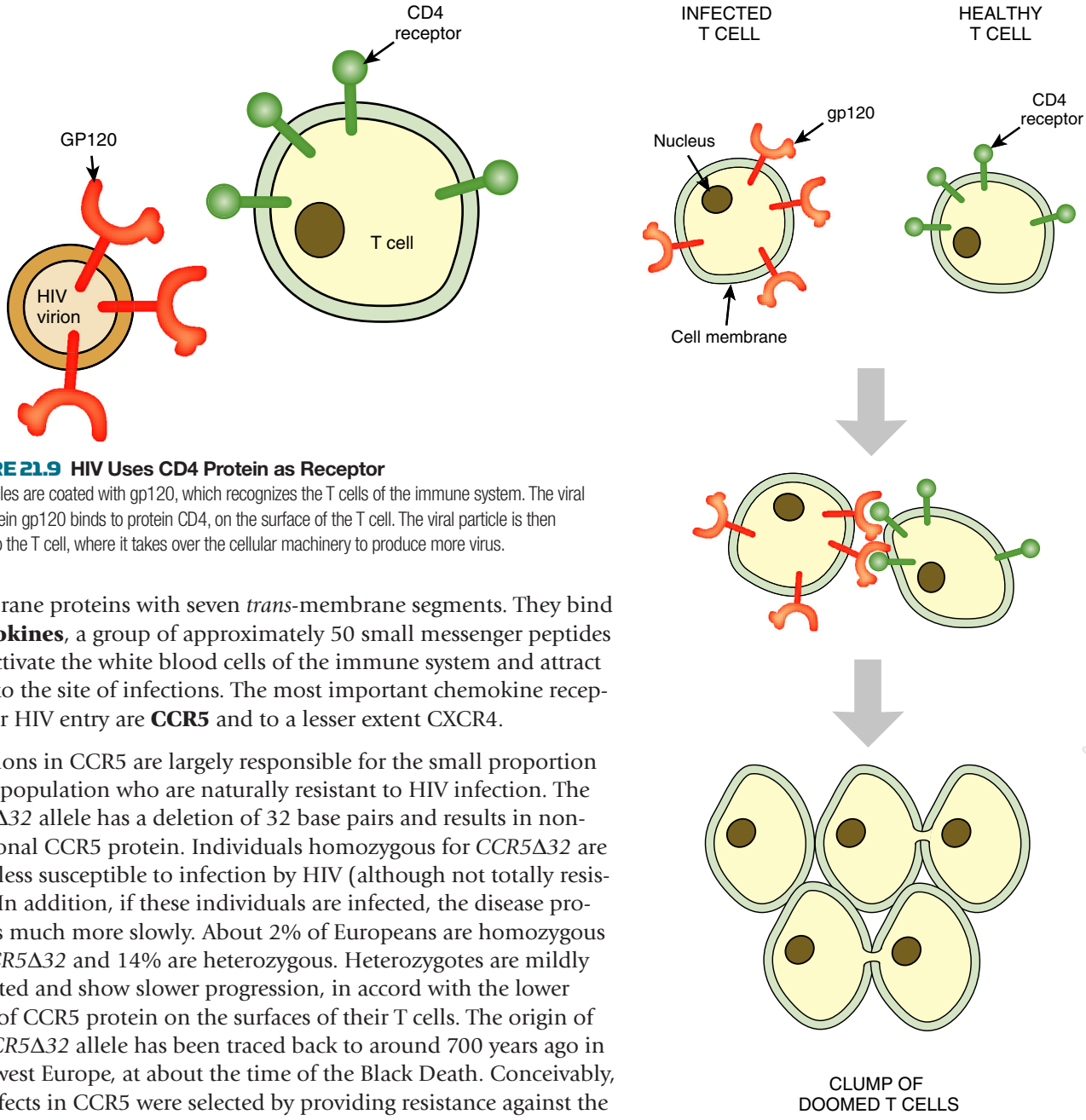


FIGURE 21.9 HIV Uses CD4 Protein as Receptor
HIV particles are coated with gp120, which recognizes the T cells of the immune system. The viral glycoprotein gp120 binds to protein CD4, on the surface of the T cell. The viral particle is then taken into the T cell, where it takes over the cellular machinery to produce more virus.

membrane proteins with seven *trans*-membrane segments. They bind **chemokines**, a group of approximately 50 small messenger peptides that activate the white blood cells of the immune system and attract them to the site of infections. The most important chemokine receptors for HIV entry are **CCR5** and to a lesser extent CXCR4.

Mutations in CCR5 are largely responsible for the small proportion of the population who are naturally resistant to HIV infection. The *CCR5Δ32* allele has a deletion of 32 base pairs and results in nonfunctional CCR5 protein. Individuals homozygous for *CCR5Δ32* are vastly less susceptible to infection by HIV (although not totally resistant). In addition, if these individuals are infected, the disease progresses much more slowly. About 2% of Europeans are homozygous for *CCR5Δ32* and 14% are heterozygous. Heterozygotes are mildly protected and show slower progression, in accord with the lower levels of CCR5 protein on the surfaces of their T cells. The origin of the *CCR5Δ32* allele has been traced back to around 700 years ago in northwest Europe, at about the time of the Black Death. Conceivably, the defects in CCR5 were selected by providing resistance against the bubonic plague. Variations in susceptibility to AIDS also result from alterations in the DNA sequence of the promoter for the *CCR5* gene. Presumably, these alterations cause variations in the level of CCR5 protein expressed.

Receptors that take up important molecules into animal cells are often the targets for viruses. It is quite possible for the same host cell protein to be used as a receptor by unrelated infectious agents, including both viruses and bacteria. Thus, the myxoma poxvirus, which causes immune deficiency in rabbits, also uses the CCR5 and CXCR4 chemokine receptors. Which receptors are used by smallpox or other poxviruses is still unknown. Other pathogens, including the malaria parasite, also target chemokine receptors, although not CCR5 and CXCR4. Scientists are presently trying to identify the functions of the various receptors on immune cells in the hope of understanding how viruses exploit them for their own use.

FIGURE 21.10
Fusion of Infected T Cells
Once HIV has entered the T cell, gp120 is made in large amounts and is inserted into the host cell membrane. T cells with gp120 in their membranes bind to other T cells via the CD4 receptor, which causes the cells to fuse. The process continues until large clumps of T cells form. These cells soon die, crippling the immune system.

Entry of HIV into target cells requires co-receptors. Natural resistance to AIDS results from defects in co-receptors, especially the CCR5 chemokine receptor.

TREATMENT OF THE AIDS RETROVIRUS

No complete cure or effective vaccine yet exists for AIDS, although several treatments are available that significantly extend patients' lives. About 50% of the antiviral drugs in clinical use are for AIDS. The fundamental problem with all anti-AIDS drugs is that HIV is an RNA virus and so has a relatively high mutation rate. HIV mutates at a rate of approximately one base per genome per cycle of replication. Even within a single patient, HIV exists as a swarm of closely related variants known as a **quasi-species**. Consequently, strains of HIV resistant to individual drugs appear at a relatively high frequency. Attempts to control AIDS (Fig. 21.11; Table 21.2), whether by using vaccines, protein processing inhibitors, or antisense

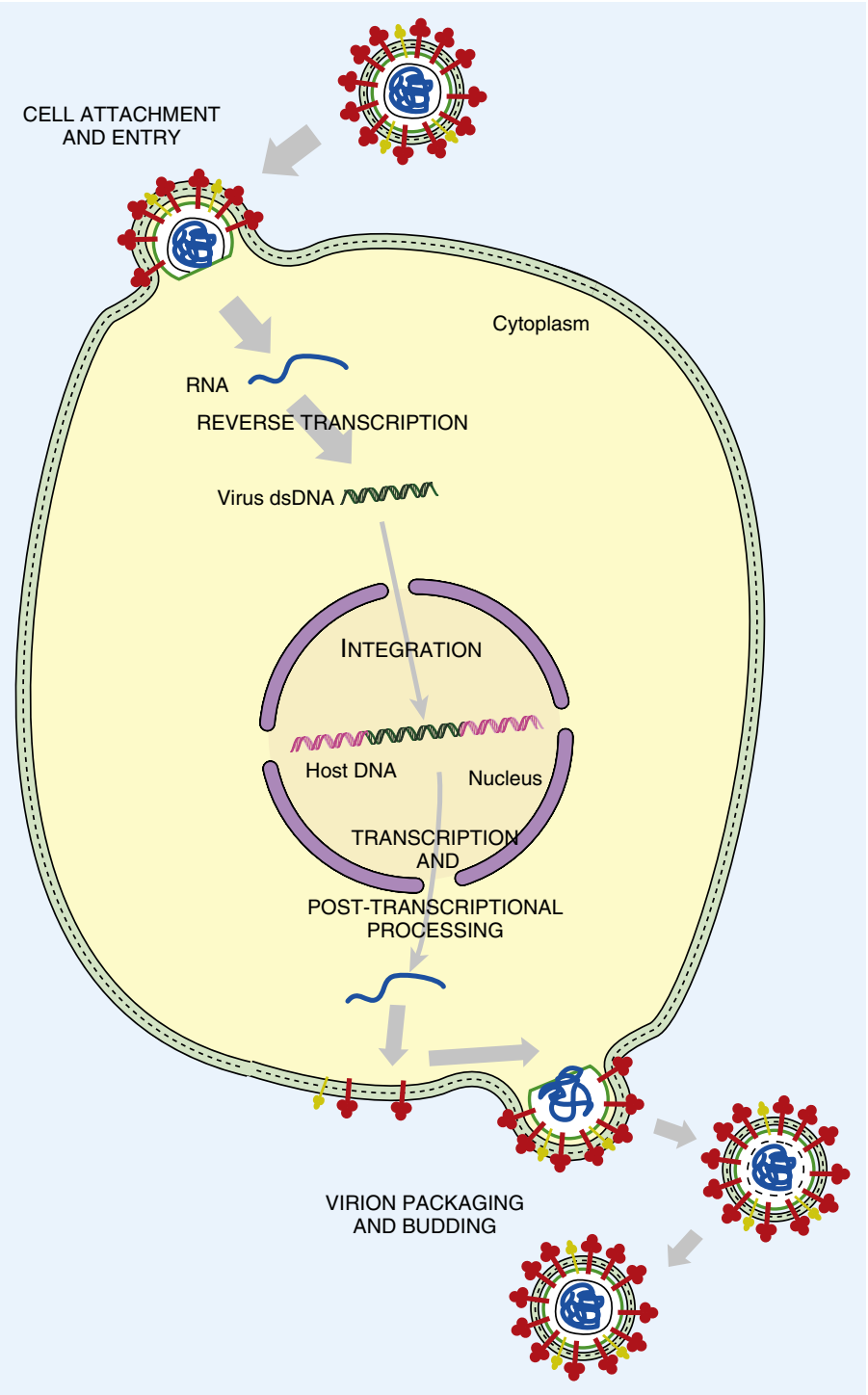


FIGURE 21.11
Possible Steps for HIV Inhibition
HIV infections could be stopped at the following steps: (1) at the cell surface, competing molecules could prevent virus attachment; (2) enzyme inhibitors may block the action of reverse transcriptase; (3) integration of the viral genome could be prevented; (4) transcription and translation could be blocked; (5) finally, blocking virion packaging and budding would protect other cells from becoming infected.

Table 21.2 HIV Antagonists

Stage in Life Cycle	Possible Antiviral Agents
Binding to receptor	CCR5 co-receptor inhibitor; Maraviroc
Membrane fusion	Fusion inhibitors
Reverse transcription	(a) Nucleoside analogs (chain terminators) (b) Non-nucleoside reverse transcriptase inhibitors (NNRTI)
Integration	(a) Integrase strand transfer inhibitor (INSTI) (b) Integrase LEDG inhibitors (LEDGIN)
Assembly of virions	Protease inhibitors

RNA, all face the same problem: HIV will mutate to produce resistant variants. In practice, this problem may be partially overcome by simultaneous treatment with several drugs that hit different targets.

Azidothymidine (**AZT**, or zidovudine) was one of the first drugs used against AIDS. It is an analog of thymidine that lacks the 3′-hydroxyl group. Various other **nucleoside analogs** that lack the 3′-hydroxyl group are also in use. AZT and other 3′-deoxy base analogs are converted to the 5′-triphosphate by the cell and then incorporated into the growing DNA chain during reverse transcription (Fig. 21.12). Because AZT lacks a 3′-hydroxyl group, the DNA chain cannot be extended. AZT is thus a DNA **chain terminator**. Although AZT is incorporated more readily by the viral reverse transcriptase than by most host-cell DNA polymerases, it is not completely specific. Thus, one major drawback is that AZT partially inhibits host DNA synthesis in uninfected cells of the body. In particular, it is toxic to bone marrow cells (B cells), which are another part of the immune system. Mutations in the HIV reverse transcriptase may cause resistance to base analogs. For example, Met41Leu (i.e., replacement of methionine at position 41 with leucine) increases resistance to AZT by 4-fold, and a second mutation of Thr215Tyr gives an overall 70-fold resistance.

Certain drugs that do not bind at the active site can also inhibit reverse transcriptase. They are referred to as **non-nucleoside reverse transcriptase inhibitors** (**NNRTI**; Fig. 21.13). They bind to the enzyme at a separate site, relatively close to the active site. This distorts the structure of reverse transcriptase and inhibits its activity. Unfortunately, mutations that alter the NNRTI binding site occur quite frequently, and they give rise to resistant reverse transcriptase enzyme. These drugs are therefore generally used in combination with nucleoside analogs.

Most individual HIV proteins are joined together as polyproteins when first made and must therefore be cut apart by HIV protease. For example, the *env* gene is transcribed and translated to give gp160, which is cleaved to gp41 and gp120. The *gag* gene encodes a polyprotein that includes the proteins of the virus core. Consequently, inhibition of polyprotein cleavage will prevent the assembly of the virus particle. The HIV protease recognizes and binds a stretch of seven amino acids around the cleavage site. This step may be blocked with **protease**

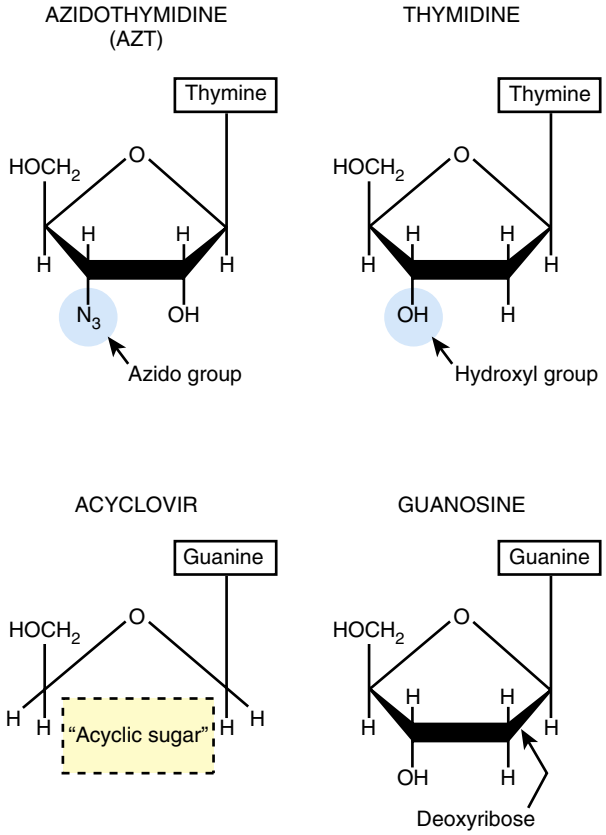


FIGURE 21.12
Nucleoside Analogs Act as Chain Terminators
Two examples of chain terminators are azidothymidine (AZT) and acyclovir, which replace thymine and guanine, respectively. AZT has an azido group on the 3′ position of the deoxyribose ring rather than a hydroxyl. The entire deoxyribose ring is altered in acyclovir. In both cases, the analogs are incorporated into DNA during the reverse transcriptase reaction. Once the analog has been inserted, reverse transcriptase cannot elongate the DNA chain any further because the analogs lack the 3′-OH group to which the next nucleotide would be added.

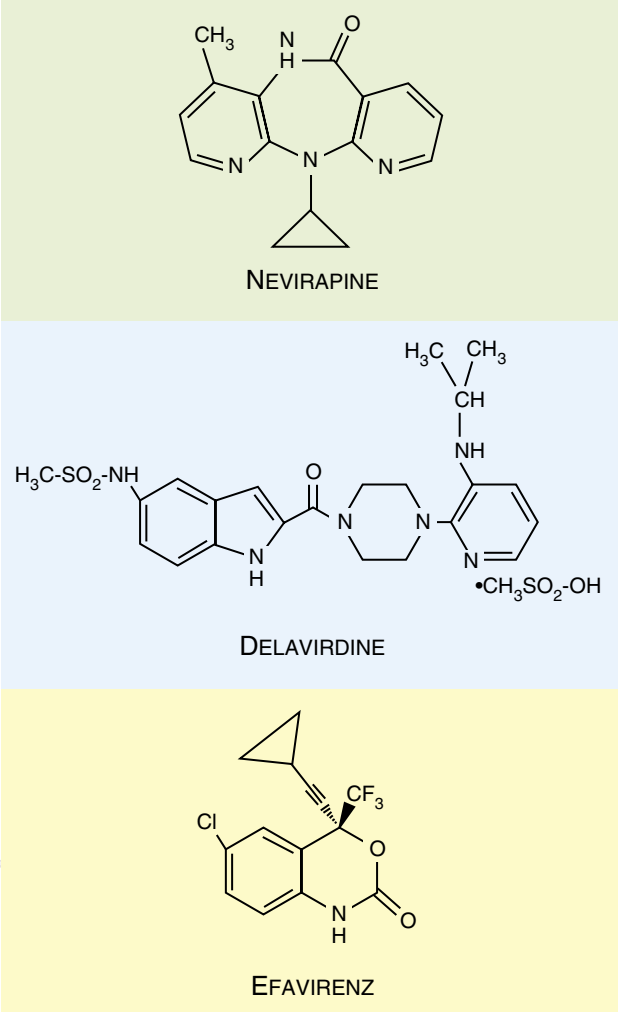


FIGURE 21.13
Non-Nucleoside Reverse Transcriptase Inhibitors
Chemical structures of three NNRTIs that are presently in use: nevirapine, delavirdine, and efavirenz. They are specific for HIV-1 and have no effect on HIV-2.

inhibitors that are analogs of several amino acid residues around the cut site (Fig. 21.14). For example, saquinavir is an analog of Asn-Tyr-Pro.

At present, the favored approach in AIDS therapy, referred to as Highly Active Anti Retroviral Therapy (HAART), is to use three or four drugs with different mechanisms in combination. Different drugs should *not* be used one after the other because this allows resistance to develop to each drug in turn. If several drugs are used simultaneously, emerging virus mutants that are resistant to one drug will be killed by the others. A typical cocktail consists of two chain termination inhibitors plus a non-nucleoside reverse transcriptase inhibitor or a protease inhibitor. Since the mid 1990s, deaths from AIDS have dropped 60% to 80% in those nations whose citizens can afford expensive long-term treatment with costly pharmaceuticals. In the United States, treatment with such a cocktail may cost from $800 to $1500 per month, although the cost keeps dropping.

Integrase inhibitors and fusion inhibitors are newer additions to the AIDS arsenal. Integrase inhibitors prevent integration of the HIV DNA into the host genome. Fusion inhibitors prevent the fusion of host and viral membranes that occurs during the uptake process, after receptor binding. CCR5 co-receptor inhibitors block binding to those HIV strains that use CCR5. Before use, the patient must be checked for the co-receptor specificity of the virus, an expensive process. These drugs are generally used when others fail due to resistance or harmful side effects.

Hydroxyurea was once used in cocktails to treat AIDS. Hydroxyurea inhibits enzymes of the human host cell that are needed for the AIDS virus to replicate. Because human genes encode these proteins, the virus cannot mutate to produce hydroxyurea-resistant enzymes. The advantage and the problem with hydroxyurea are that it also inhibits human cell DNA replication. Hydroxyurea is no longer used due to its toxicity.

Most recently developed antiviral agents were designed to treat AIDS. They include nucleoside analogs (chain terminators), non-nucleoside reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors and fusion inhibitors.

INFECTIOUS PRION DISEASE

Prions are proteins with unique properties that are capable of causing inherited, spontaneous, or infectious disease. The **prion protein (PrP)** exists in two conformations, the normal harmless or "cellular" form (**PrP^{c}**) and the pathogenic (**PrP^{Sc}**) form, named after **scrapie**, a disease of sheep (Fig. 21.15). Rogue prion proteins bind to their normal relatives and induce them to refold into the disease-causing conformation. Thus, a small number of misfolded prions will eventually subvert the population of normal proteins. Over time, this leads to neural degeneration and eventually death.

Mutations within the ***Prnp* gene** that encodes the prion protein may result in prions with a greatly increased likelihood of misfolding. This causes hereditary prion disease. Several clinically different variants are known, depending on the precise location of the mutation within the prion protein and the nature of the amino acid alteration. The most common is

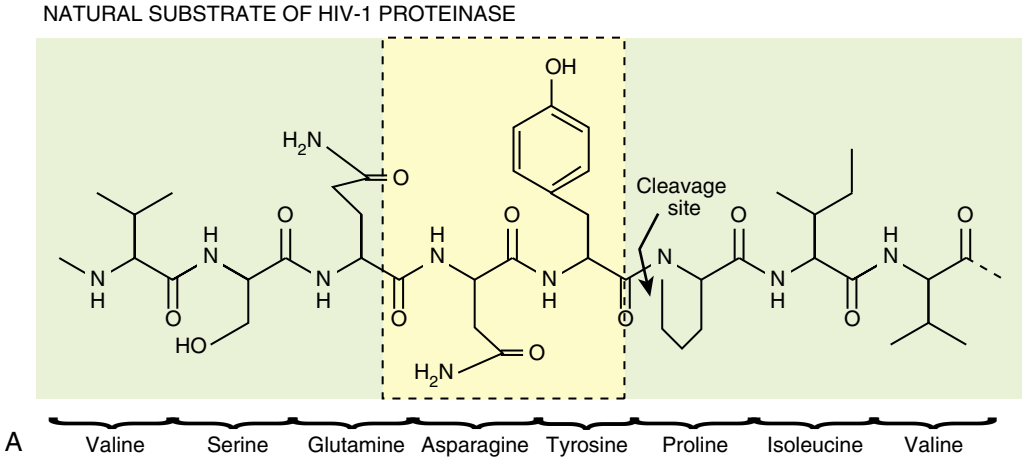


FIGURE 21.14 Protease Inhibitors
(A) HIV-1 protease recognizes Asn-Tyr-Pro, cleaving the protein between the tyrosine and proline. (B) Saquinavir has a structure that mimics these three amino acids. HIV-1 proteinase binds to saquinavir but cannot cleave or release it because the cleavage site is missing.

Creutzfeldt–Jakob disease (CJD). Even normal prions occasionally misfold. The result is spontaneous prion disease, which occurs at a rate of about one per million of the human population.

The pathogenic misfolded prions form insoluble aggregates known as **amyloids**. These are fibrils consisting of protein with a high beta-sheet content. The beta-sheets are short and form stacks that run sideways relative to the long axis of the fibers (Fig. 21.16).

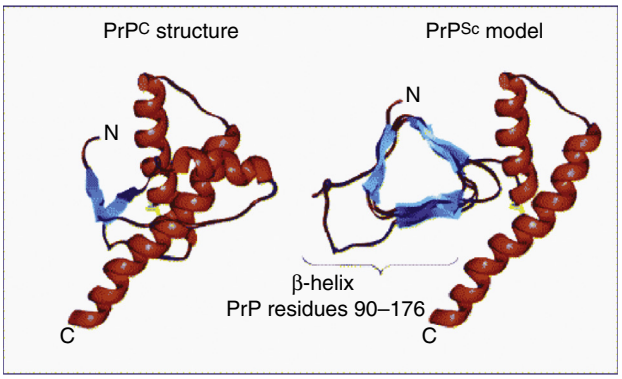


FIGURE 21.15 Normal and Pathogenic Forms of the Prion Protein
The PrP^{C} structure is on the left, and the PrP^{Sc} structure is on the right. Note the greatly increased proportion of beta-sheet in the PrP^{Sc} structure. From Eghiaian F (2005). Structuring the puzzle of prion propagation. *Curr Opin Struct Biol* **15,** 724–730. Reprinted with permission.

If misfolded prions are transmitted to another susceptible host, the result is infectious prion disease, also known as **transmissible spongiform encephalopathy (TSE)**. Such an infection can be passed from one cell to another and one animal to another by entry of the PrP^{Sc} form of the prion. The two individuals may be of the same or different species. Infection of a new victim by prions is relatively difficult. It requires uptake of rogue prion proteins from infected nervous tissue, especially brain, but the details of infection remain obscure. The best-known infectious prion diseases are

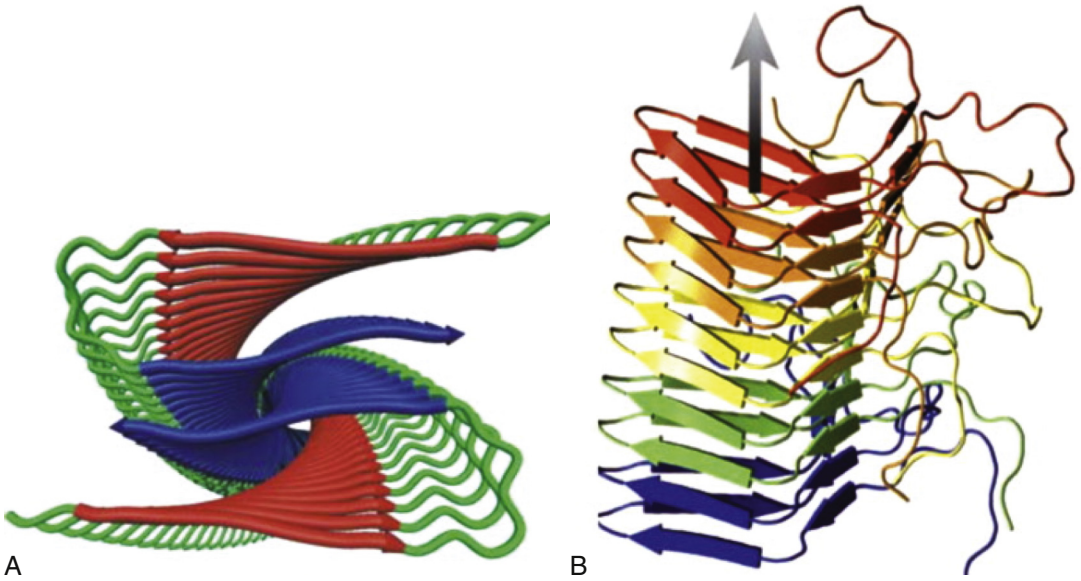


FIGURE 21.16 Amyloid Structure
Models of a typical amyloid structure found in prion proteins. (A) The $A\beta^{1-40}$ structure is parallel in-register with each peptide in a hairpin configuration and monomers stacked. Two stacks are aligned as shown. (B) The yeast HET-s prion domain (residues 218–289) forms a two-turn β-helix with partial directly repeated sequences in the peptide aligned. (From Wickner RB et al. (2011). Prion diseases of yeast: amyloid structure and biology. *Semin Cell Dev Biol* **22**, 469–475.)

1. Scrapie, a disease of sheep and goats
2. **Kuru**, a disease of cannibals
3. **Mad cow disease**, officially known as **bovine spongiform encephalopathy (BSE)**
4. **Chronic wasting disease (CWD)** of deer and elk, which can be transmitted via saliva, unlike the other TSEs

Scrapie is a disease of sheep and related animals that has been recorded going back several hundred years in Europe. The name comes from the behavior of infected sheep that constantly scrape themselves against fences, trees, or walls and often seriously injure themselves (Fig. 21.17). Only certain breeds of sheep are susceptible because of the slight differences in prion sequence between breeds. Dead and decomposing sheep may contaminate the grass of their fields with prion proteins. These proteins are unusually stable and long-lived and may be eaten by healthy sheep.

Kuru was transmitted by ritual cannibalism and used to be endemic among the Fore tribe of New Guinea. The women had the honor of preparing the brains of dead relatives and participating in their ritual consumption. As a result, 90% of the victims were women, together with younger children who accompanied them. Developing symptoms took from 10 to 20 years, but once they did, the progression from headaches to difficulty walking to death from neural degeneration took from 1 to 2 years. No one born since 1959, when cannibalism stopped, has developed kuru.

Brain degeneration, or spongiform encephalopathy, due to misfolded prions is possible in any mammalian species. In addition to scrapie, BSE, and CWD, a variety of less well-characterized prion diseases are known in other animals. In a way, they are really all the same disease because there is a single prion gene encoding a single prion protein that is found in the brain of all mammals. Symptoms vary slightly from species to species, but after a long incubation period, the result is degeneration and death of cells of the central nervous system. As the popular name *mad cow disease* indicates, progressive degeneration of the brain and nervous system causes the infected animals to behave bizarrely during later stages of the disease.

Mad cow disease was spread by overly intensive farming practices. Animal remains, including the brains, were ground up and incorporated into animal feed. Because sheep remains were included in feed for cows, the epidemic of mad cow disease, which began in England in 1986, was originally blamed on sheep with scrapie. However, people in England and other European countries

have eaten sheep with scrapie since the 1700s without any noticeable ill effects. Nor have any other domestic animals, including cows, ever caught scrapie, despite sharing the same fields. Moreover, sheep prions are not infectious for cows. It is now thought that a random flip-flop event converted a normal prion into the rogue form inside a cow's brain somewhere in England in the late 1970s or early 1980s. The rogue cow prions were recycled in animal feed and spread, eventually causing an epidemic. After mad cow disease broke out in England, the recycling of animal remains in feed was prohibited and infected herds were destroyed.

FIGURE 21.17
Sheep with Scrapie
This sheep with scrapie is from the Caine Veterinary Teaching Center, Caldwell, ID. Photograph provided by Sharon Sorenson.

Mad cow disease can be transmitted to humans, but the rate of infection is extremely low. The first human cases were confirmed in 1996 and were named **variant CJD** in an attempt to obscure their origin. However, when the rogue cow prion infects humans, the misfolded prions are characteristic of mad cow disease, not genuine CJD. In humans with CJD or kuru, the precise conformation of the misfolded prions is different. The human victims of mad cow disease are scattered randomly throughout the population, suggesting that relatively few humans are actually susceptible to infection. As of 2013, about 225 people, mostly in England, have come down with BSE. Calculations based on the history and age distribution of BSE in humans since the outbreak started suggest an average incubation period of about 15 years and that the total number of cases will be under 300. These estimates reflect the extremely low infectivity of prions when crossing from one species to another.

Prion diseases (scrapie, Creutzfeldt–Jakob disease, mad cow disease) are due to misfolding of the prion protein that is expressed at high levels in nervous tissue, especially brain. Inherited, infectious, and spontaneous variants of prion disease are found.

DETECTION OF PATHOGENIC PRIONS

The emergence of mad cow disease (BSE) has created the need to screen cows and their products for the presence of the pathogenic form of the prion protein (PrP^{Sc}). This screening is presently done by immunological detection. Early assays lacked separate antibodies specific to the normal (PrP^{c}) and pathogenic (PrP^{Sc}) isoforms. Consequently, because the pathogenic form of the prion is protease resistant, samples were first treated with protease to destroy the normal (PrP^{c}) form and then subjected to immunological testing by Western blotting (see Chapter 6). The overall procedure is tedious and only moderately sensitive. The development of isoform specific antibodies led to the **conformation-dependent immunoassay (CDI)** and was a major improvement.

Further improvements relied on amplification schemes. The first of these was the **protein misfolding cyclic amplification (PMCA)** procedure, which amplifies the levels of misfolded prion in a manner analogous to the use of PCR for amplifying DNA (Fig. 21.18). This allows greatly increased sensitivity of detection of PrP^{Sc} in clinical samples. Small samples suspected of containing PrP^{Sc} are mixed with normal brain homogenate containing a surplus of the normal PrP^{c}. The PrP^{c} is converted to PrP^{Sc} and incorporated into the growing PrP^{Sc} aggregates. The sample is then sonicated to break up the aggregates. This procedure is repeated for several cycles. Increases of around 60-fold over five cycles are typical.

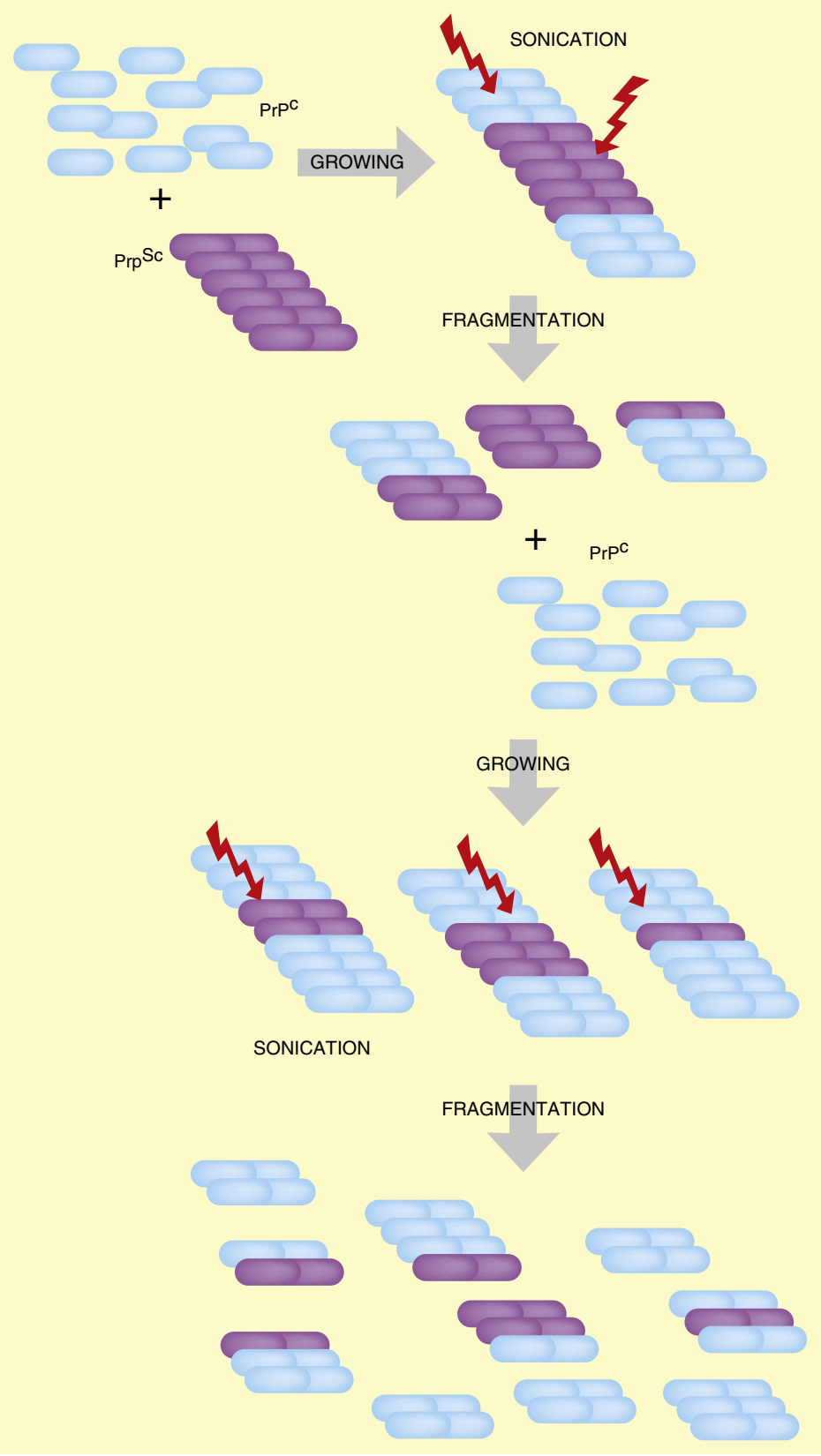


FIGURE 21.18 Protein Misfolding Cyclic Amplification (PMCA)
Amplification involves multiple cycles of incubating PrP^{Sc} in the presence of excess PrP^{c} followed by sonication. During the incubation periods, the size of PrP^{Sc} aggregates (purple) increases because of incorporation of normal prion protein (blue). During sonication, the aggregates are disrupted, producing more pathogenic conversion units.

Various modifications are increasing the sensitivity of the current methods for detecting PrP^{Sc}. Improvements include using recombinant prion protein (expressed by bacteria) and replacing sonication with shaking to give the quaking-induced conversion (QuIC) assay. This allows detection of subfemtogram amounts of PrP^{Sc} within 24 hours. Another subtle but effective improvement is based on the principle of real-time PCR, which uses fluorescence for increased speed and sensitivity (see Chapter 4). A similar real-time version of the quaking-induced conversion assay has been developed using the dye thioflavin T, which fluoresces upon binding to aggregated prion protein. A novel method to detect prions using nanopores is in development (see Box 21.1).

Detection of prions is technically difficult. Cyclic amplification of prions has greatly increased the sensitivity of detection.

APPROACHES TO TREATING PRION DISEASE

At present, there is no effective treatment for any of the prion diseases, although a variety of agents are being tested. Relatively few drugs cross the blood–brain barrier effectively. Nonetheless, random screening of those known to do so revealed that both quinacrine and chlorpromazine eliminate prions from infected animal brain cells in culture (Fig. 21.19). (Quinacrine is a rarely used antimalarial drug, and chlorpromazine is

Box 21.1 Nanopore Detection of Single Prion Protein Molecules

Nanopores may be used to capture and identify single protein molecules. (For background information on nanotechnology, see Chapter 7.) Both glass and protein nanopores have been shown to detect the prion protein. The nanopore is immersed in a conducting solution, and the current through the nanopore is monitored. When large molecules enter the nanopore, they partially block it, and consequently, there is a drop in the ionic current through the nanopore.

In the experiment featured here, a natural protein, α-hemolysin, is used as the nanopore. It is inserted in a lipid bilayer, as shown in Figure A. The prion protein is attracted into the nanopore by a transmembrane voltage that attracts the positively charged N-terminus of the prion protein. Both the normal and pathogenic forms of prion protein can be detected. More importantly, single molecules of the two prion forms can be distinguished by their ion-current signatures.

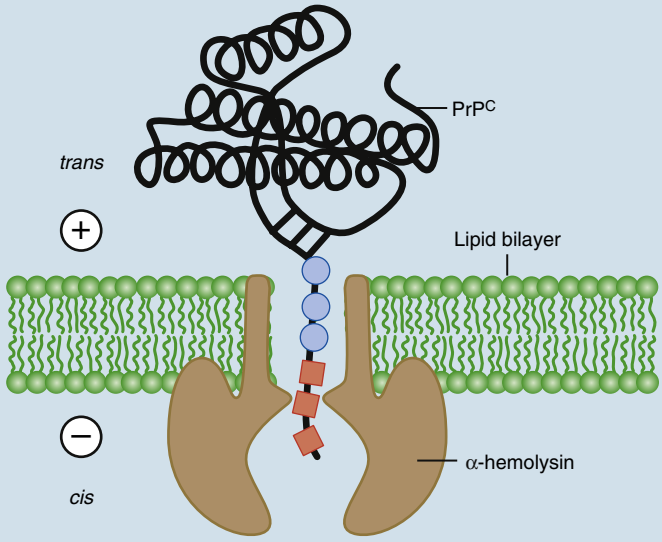


FIGURE A Nanopore Detection of Prion Protein
Capture of a single prion molecule by an alpha-hemolysin nanopore. From Jetha NN et al. (2013). Nanopore analysis of wild-type and mutant prion protein (PrP(C)): single molecule discrimination and PrP(C) kinetics. *PLoS One* **8**(2), e54982.

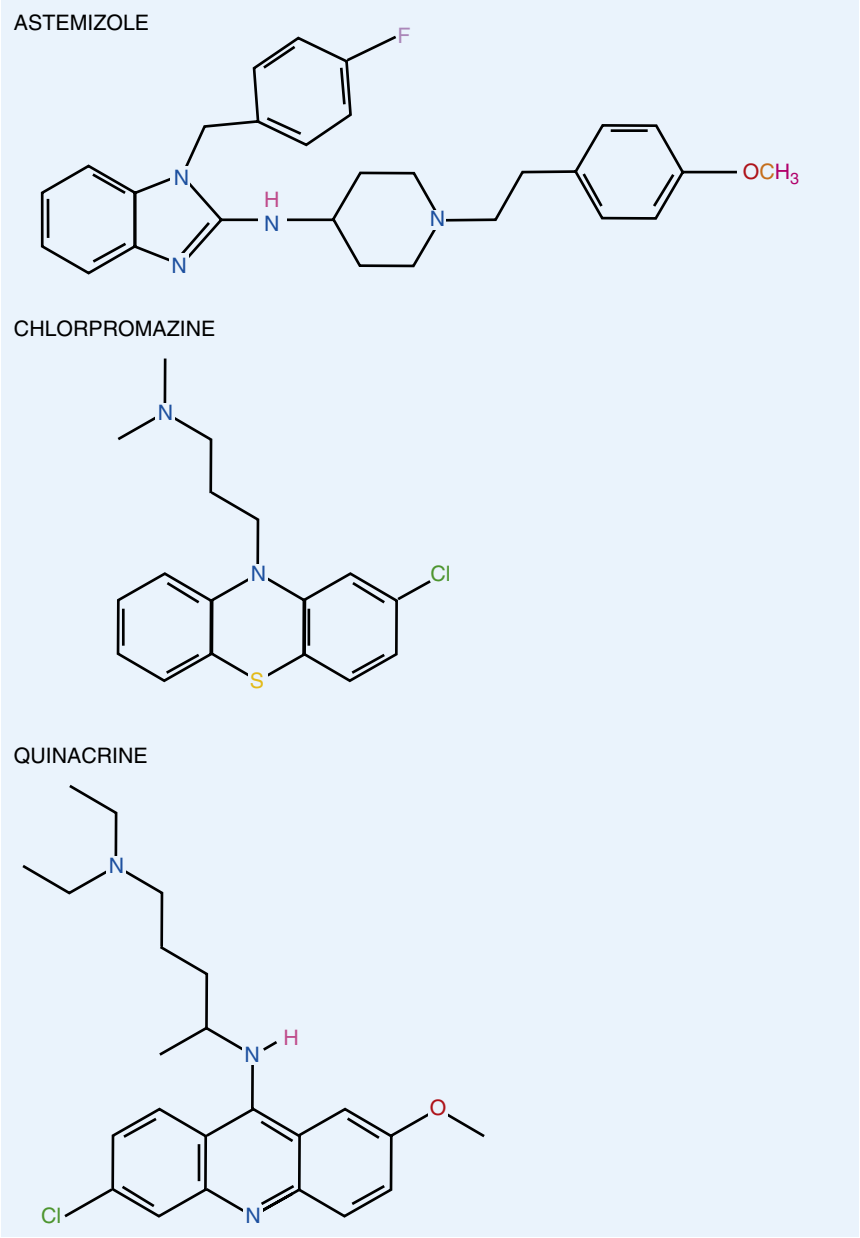


FIGURE 21.19 Antiprion Agents
Structures of chlorpromazine, quinacrine, and astemizole.

widely used to treat schizophrenia.) Quinacrine binds to PrP^{c} and probably helps to stabilize it against conformational change induced by PrP^{Sc}. Unfortunately, these compounds do not cure the disease in whole animals. A variety of structurally similar compounds are being screened. Some have antiprion activity, but none has yet progressed to clinical testing. One strong candidate is Astemizole, which has been previously used as an antihistamine.

Removal of prions from infective material is also important. In particular, blood transfusion using contaminated blood has been a source of prion infection. Filters have recently been developed that remove prions. Combinatorial libraries (see Chapter 11) were screened for ligands that bound prion protein. The ligands were then attached to resins and placed in columns for filtration of blood or other liquids that might contain active prions. When scrapie-infected hamster blood was filtered in this manner and then injected into hamsters, prion infection was prevented.

RNA interference (see Chapter 5) is widely used to suppress gene expression in laboratory studies. It is possible to generate siRNA that will suppress expression of the *Prnp* gene in mice. To generate the siRNA in prion-infected cells, scientists used a retrovirus vector that expresses short hairpin RNAs. These are processed in the target cells by Dicer to give the siRNA. This in turn triggers RNA interference directed against *Prnp* mRNA, which is degraded. Retroviral vectors were chosen because they can infect nongrowing cells, such as those of the nervous system. At least in mice, intracranial injection of the vector-siRNA construct reduced prion levels and prolonged survival.

Knocking out the prion gene in livestock is another approach to eliminating prion disease. Transgenic mice lacking both copies of the *Prnp* gene were engineered several years ago. They grow and develop normally; however, they are unable to make prion protein and are resistant to infection by pathogenic prions. This confirmed that the host cell is responsible for making new prion proteins. During infectious prion disease, these proteins change conformation. Although the prion gene is not needed for survival and its role is still unclear, it does appear to be involved in long-term memory and spatial learning.

Recently, cattle lacking both copies of the *Prnp* gene have been engineered and after 2 years are normal in growth and development. Brain cells from such animals are resistant to prion infection. *Prnp* knockout livestock could be used to provide prion-free products, if transgenic animals are approved as a source of human food.

There is presently no treatment for prion disease, although several lead compounds have been found with partial activity in cell culture.

PRIONS IN YEAST

For a long time, the strange behavior of the mammalian prion protein was thought to be unique. However, other proteins whose misfolded forms catalyze conversion of the normal protein into the misfolded version exist. Furthermore, the misfolded versions form insoluble amyloid aggregates. The first of these to be discovered were the **yeast prions**. More recently, it seems that prion-like behavior may be involved in certain neurological conditions, such as Alzheimer's, Huntington's, and Parkinson's diseases (see later discussion).

Yeast prions were discovered to be the cause behind some weird genetic behavior. The yeast prions are nonlethal and are soluble cytoplasmic proteins rather than membrane proteins. Nonetheless, yeast prions show nucleic acid-free inheritance, and their misfolded forms catalyze conversion of the normal protein into the misfolded version. Furthermore, the misfolded versions of yeast prions form insoluble amyloid aggregates, like those of mammals. However, although they display similar structural domains, the mammalian and yeast prion proteins show no sequence homology.

The two best-known yeast prions are [URE3] and [PSI+], which are the misfolded forms of the Ure2p and Sup35p proteins. Ure2p takes part in nitrogen regulation, and Sup35p is a translation termination factor. Yeast with the prion version of Sup35p show different colony morphology and can be monitored by using a variety of reporter constructs (Fig. 21.20). Surveys of yeast strains have revealed quite a few more prions, several with unknown roles. Around a third of several hundred wild strains of *Saccharomyces* contain prions.

As with mammalian prions, a given yeast prion may exist as a variety of strains with slightly different structures. Although yeast prions are generally nonlethal, some variants of the [PSI+] prion are severely detrimental or even lethal. Even the relatively harmless common forms of the well-known yeast prions convey a small growth disadvantage under most natural conditions. However, yeasts carrying prions have an advantage under specific conditions. The [URE3] prion allows yeast to better use poor nitrogen sources, while the [PSI+] prion acts as a nonsense suppressor and promotes better growth in strains with nonsense mutations.

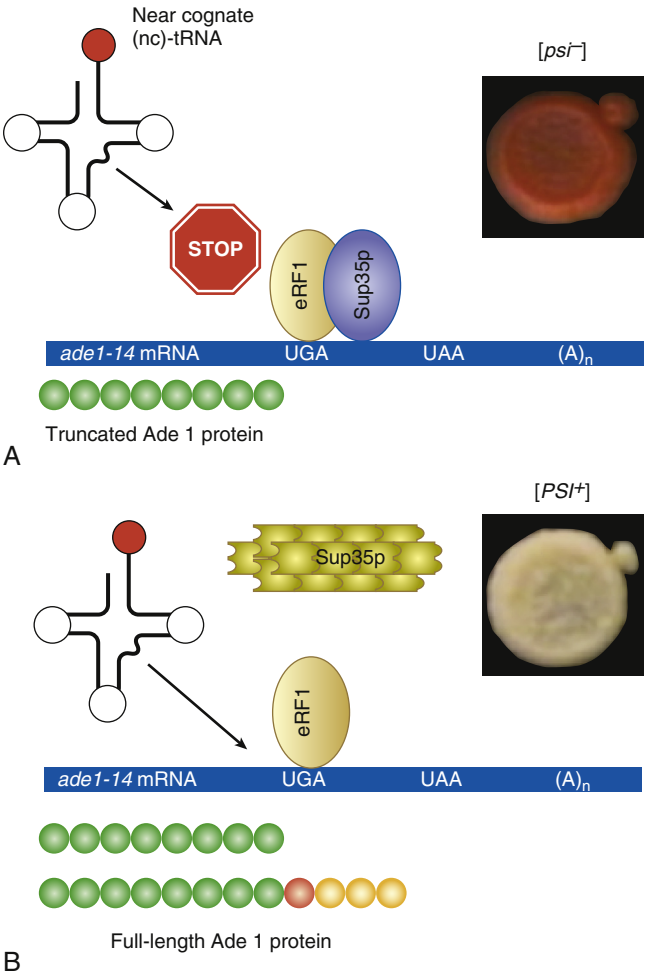


FIGURE 21.20
Yeast Prion Phenotype
The [*PSI*⁺] prion of *S. cerevisiae* is an alternative form of the Sup35 protein that causes defective termination of translation. This may be monitored by using the *ade1–14* allele as reporter gene. This allele contains a premature UGA stop codon that blocks adenine synthesis and causes accumulation of a red pigment. (A) In prion-free [*psi*⁻] cells, the Sup35 protein binds to eRF1, termination occurs, and red colonies are observed. (B) In [*PSI*⁺] cells, most of the Sup35 takes part in the prion aggregates. Defective termination allows near-cognate tRNAs to translate the UGA codon. Consequently, functional *ADE1* gene product is made, and white colonies are observed. Modified from Tuite MF (2013). The natural history of yeast prions. *Adv Appl Microbiol* **84**, 85–137.

USING YEAST PRIONS AS MODELS

A new approach to screening compounds for use in prion therapy is based on the use of yeast prions. As mentioned previously, Sup35p is a translation termination factor. Its prion form, [PSI+], is insoluble and inactive. Conversion of Sup35p to [PSI+] causes increased read-through of stop codons. This forms the basis for a clever and quick genetic screening system for possible antiprion drugs.

Yeast mutants defective in adenine biosynthesis turn red because of accumulation of metabolic by-products. A yeast mutant with a nonsense mutation (i.e., a premature stop codon) in the ADE1 gene thus forms red colonies. If the Sup35p protein is in its prion form, read-through of stop codons occurs, and enough full-length protein is made to allow adenine synthesis; that is, the mutation is suppressed. Consequently, prion-positive strains form white colonies. If the prions are lost, the yeast goes back to forming red colonies. This allows rapid color-based screening of chemical compounds simply by adding them to the medium and looking for those causing a white-to-red color shift of the yeast colonies. Although a variety of compounds have been found that block prion replication, none have yet been clinically effective.

Prions have been discovered in yeast. This has allowed systematic screening for antiprion agents.

AMYLOID PROTEINS IN NEUROLOGICAL DISEASES

Several degenerative diseases of the nervous system are characterized by the buildup of insoluble amyloid protein aggregates in cells of the brain. They include Alzheimer's, Huntington's, and Parkinson's diseases. There is also some evidence that amyloid fibrils may be involved in non-neurological conditions, including atherosclerosis and arthritis.

The amyloid-beta protein of Alzheimer's and the alpha-synuclein protein of Parkinson's disease both demonstrate prion-like behavior. Both form insoluble aggregates that can self-propagate due to seeding by misfolded protein. In both cases, mutations are known that increase the formation of amyloid. Although most cases of Parkinson's disease are sporadic, around 10% of cases are inherited. Both point mutations and duplications in the gene for alpha-synuclein are associated with the familial forms of Parkinson's disease.

At least in animal models, both amyloid-beta and alpha-synuclein can be transmitted between cells. However, there is no evidence that either Alzheimer's or Parkinson's diseases can be transmitted from person to person.

Summary

Viruses rely on a host cell for growth and replication. As a consequence, it is often difficult to stop viral replication without damaging the host. The recent increased development of antiviral agents has largely been driven by the AIDS epidemic and, more recently, by the threat of pandemic flu. Agents have been developed that target most of the important steps in the virus life cycle. Recently, RNA interference has been used against viral infections, first in plants and then in people.

Prions are infectious proteins that cause neurodegenerative diseases in mammals. Fungi, including yeasts, also have prions, but they act as regulatory mechanisms for adjusting to changes of environment. The amyloid state that is typical of the aggregated pathogenic form of the prion protein is also found in proteins involved in other neurodegenerative diseases. So far, no effective therapy has been found to combat prion infection or other amyloid-related conditions.

End-of-Chapter Questions

1. Which of the following is a possible antiviral agent?
   - **a.** interferon β
   - **b.** amantadine
   - **c.** nucleoside analogs
   - **d.** protease inhibitors
   - **e.** all of the above

2. All of the following are potential outcomes of the presence of dsRNA inside a human cell except
   - **a.** activation of NK cells
   - **b.** interferon production
   - **c.** blocking of protein synthesis by P1 kinase
   - **d.** activation of an endonuclease
   - **e.** increase ATP supply

3. What prompts interferon β to be secreted?
   - **a.** ssDNA
   - **b.** dsRNA
   - **c.** ssRNA
   - **d.** dsDNA
   - **e.** mtDNA

4. Which of the following is not a component of influenza virus?
   - **a.** nucleocapsid
   - **b.** neuraminidase
   - **c.** negative ssRNA
   - **d.** outer envelope
   - **e.** caspase

5. Which influenza group causes the most severe outbreaks?
   - **a.** influenza A
   - **b.** avian influenza
   - **c.** influenza B
   - **d.** influenza AB
   - **e.** all of the above

(*Continued*)

6. What is the mode of action for the drug Tamiflu?
- **a.** hemagglutinin protease
- **b.** ion channel blocker
- **c.** neuraminidase inhibitor
- **d.** reverse transcriptase blocker
- **e.** none of the above

7. Which HIV protein binds to CD4?
- **a.** ganglioside GM_1
- **b.** neuraminidase
- **c.** hemagglutinin
- **d.** gp120
- **e.** chemokines

8. A mutation in which gene is responsible for natural resistance to HIV infection?
- **a.** CD4
- **b.** CCR6
- **c.** CCR5
- **d.** CXCR4
- **e.** gp120

9. What is the main problem with treatment of HIV/AIDS?
- **a.** availability of antiviral drugs
- **b.** the high mutation rate of the virus
- **c.** socioeconomic issues within populations
- **d.** adequate testing facilities for the disease
- **e.** adequate education about HIV/AIDS prevention

10. What is the mode of action for AZT?
- **a.** a DNA chain terminator
- **b.** reverse transcriptase inhibitor
- **c.** gp120 analog
- **d.** CCR5 analog
- **e.** none of the above

11. What is the favored method for HIV/AIDS therapy?
- **a.** treatment with a two-drug cocktail
- **b.** treatment with a three-drug cocktail
- **c.** treatment with a reverse transcriptase inhibitor only
- **d.** treatment with a DNA chain terminator
- **e.** treatment with a protease inhibitor only

12. How do prions cause disease?
- **a.** Prion proteins bind to cells and induce apoptosis.
- **b.** Prion proteins bind to DNA polymerase and prevent replication.
- **c.** Prion proteins induce normal cellular proteins to refold into the prion form.
- **d.** Prion proteins induce an immune response against the “self.”
- **e.** none of the above

13. Which of the following diseases is not caused by a prion?
- **a.** kuru
- **b.** BSE
- **c.** scrapie

d. HIV
e. CJD

14. Which method is used to identify prion infections?
a. PMCA
b. PCR
c. RT-PCR
d. brain biopsy
e. none of the above

15. How might prion diseases be treated?
a. RNAi
b. *Prnp* knockouts
c. removal of prions using a filter
d. quinacrine or chlorpromazine treatment
e. all of the above

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