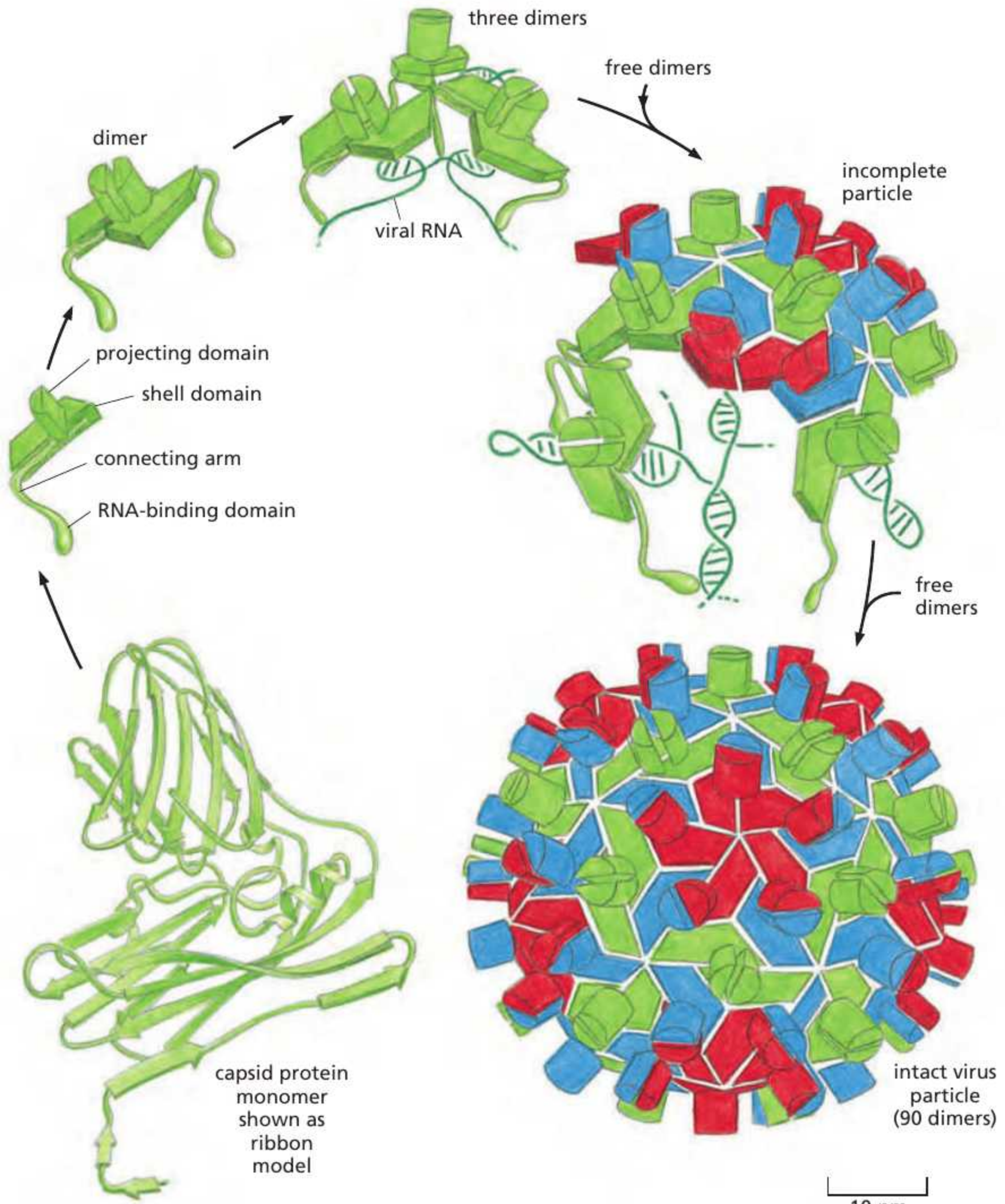


Figure 3–28 The structure of a spherical virus. In viruses, many copies of a single protein subunit often pack together to create a spherical shell (a capsid). This capsid encloses the viral genome, composed of either RNA or DNA (see also Figure 3–27). For geometric reasons, no more than 60 identical subunits can pack together in a precisely symmetric way. If slight irregularities are allowed, however, more subunits can be used to produce a larger capsid that retains icosahedral symmetry. The tomato bushy stunt virus (TBSV) shown here, for example, is a spherical virus about 33 nm in diameter formed from 180 identical copies of a 386-amino-acid capsid protein plus an RNA genome of 4500 nucleotides. To construct such a large capsid, the protein must be able to fit into three somewhat different environments. This requires three slightly different conformations, each of which is differently colored in the virus particle shown here. The postulated pathway of assembly is shown; the precise three-dimensional structure has been determined by x-ray diffraction. (Courtesy of Steve Harrison.)

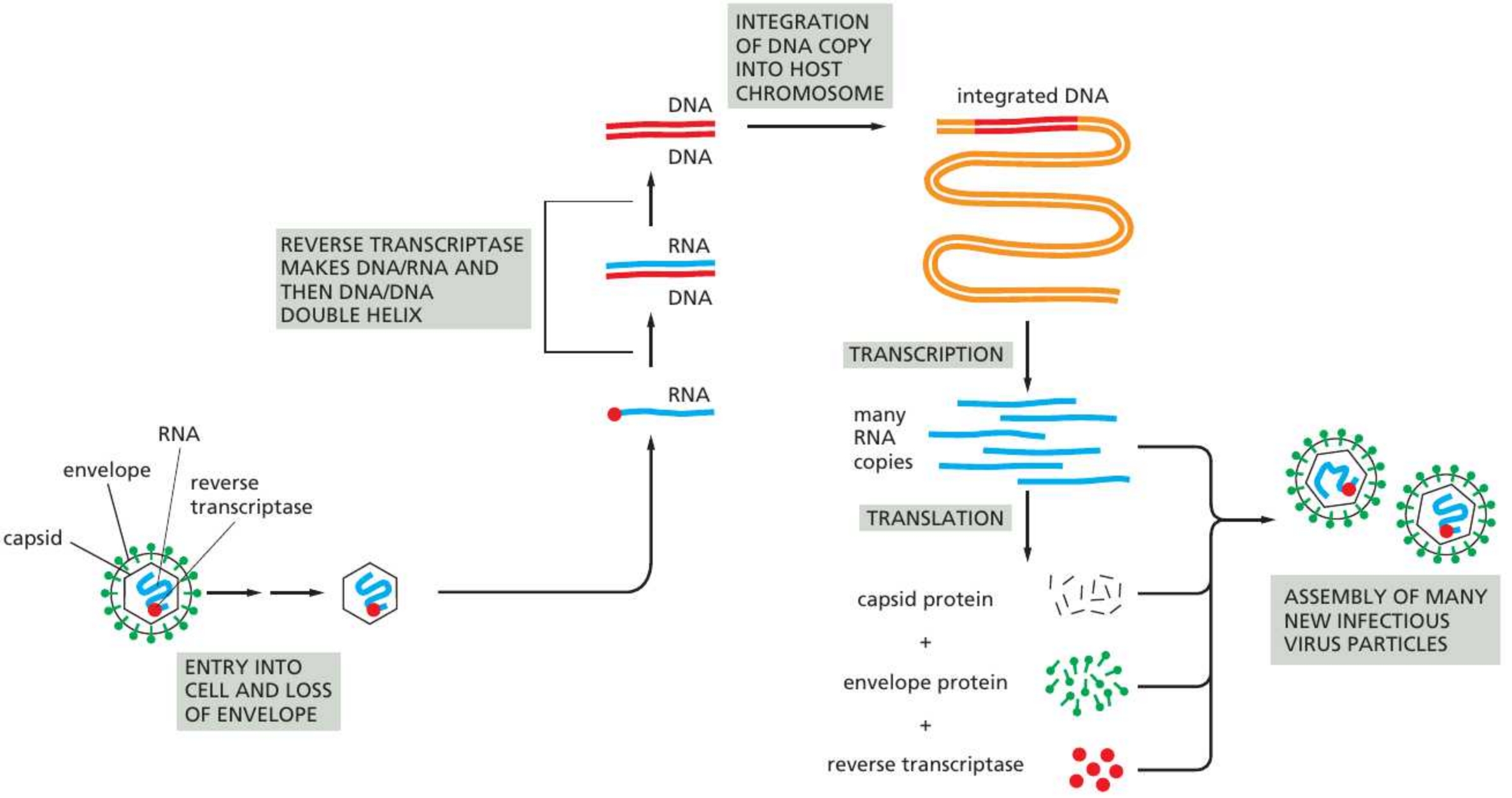


Figure 5–62 The life cycle of a retrovirus. The retrovirus genome consists of an RNA molecule *(blue)* that is typically between 7000 and 12,000 nucleotides in length. It is packaged inside a protein capsid, which is surrounded by a lipid-based envelope that contains virus-encoded envelope proteins *(green)*. Inside an infected cell, the enzyme reverse transcriptase *(red circle)* first makes a DNA copy of the viral RNA molecule and then a second DNA strand, generating a double-strand DNA copy of the RNA genome. The integration of this DNA double helix into the host chromosome is then catalyzed by a virus-encoded integrase enzyme. This integration is required for the synthesis of new viral RNA molecules by the host-cell RNA polymerase, the enzyme that transcribes DNA into RNA (discussed in Chapter 6).

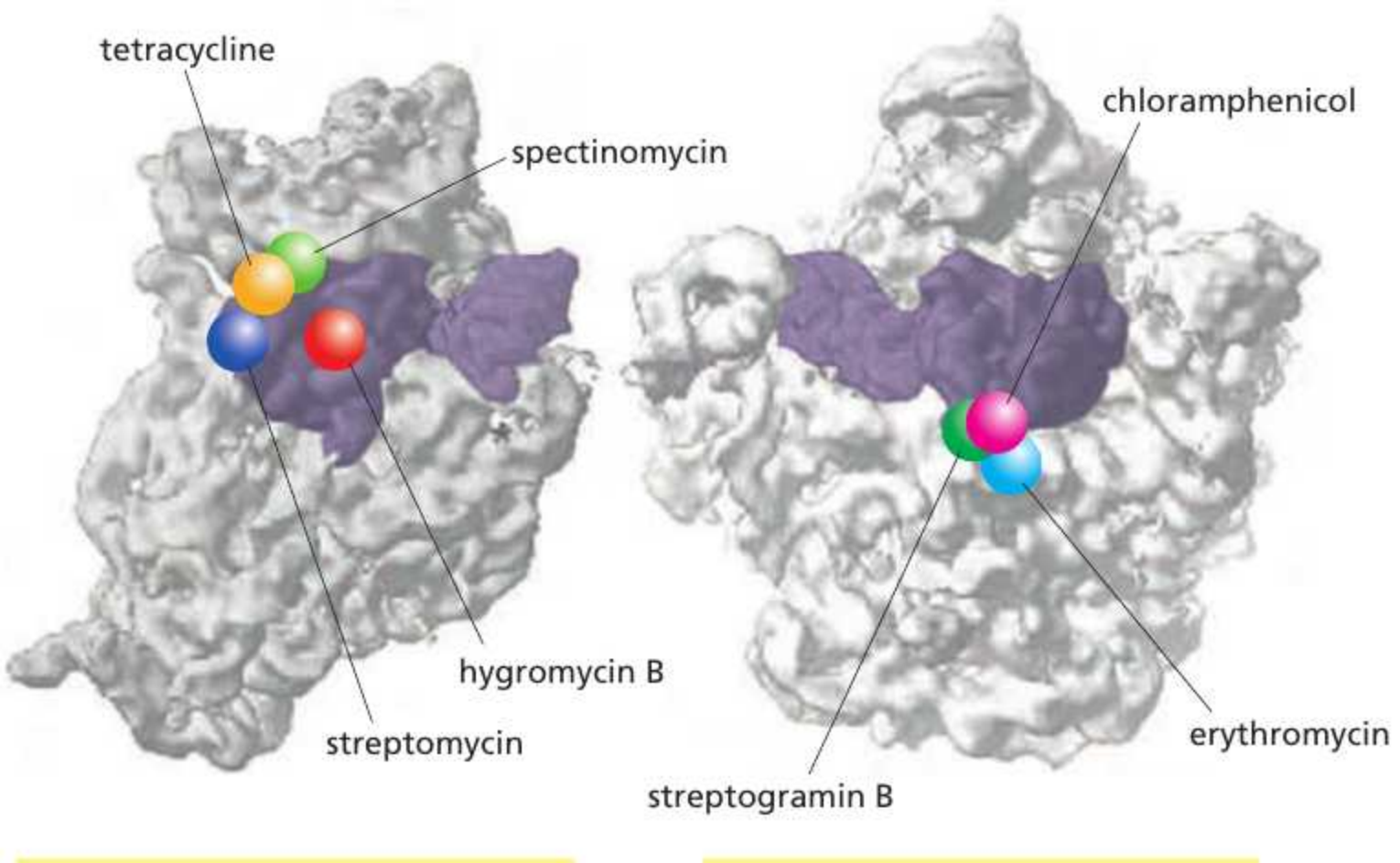


Figure 6–75 Binding sites for antibiotics on the bacterial ribosome. The small *(left)* and large *(right)* subunits of the ribosome are arranged as though the ribosome has been opened like a book. Antibiotic binding sites are marked with colored spheres, and the bound tRNA molecules are shown in *purple* (see Figure 6–62). Most of the antibiotics shown bind directly to pockets formed by the ribosomal RNA molecules. Hygromycin B induces errors in translation, spectinomycin blocks the translocation of the peptidyl-tRNA from the A site to the P site, and streptogramin B prevents elongation of nascent peptides. Table 6–4 lists the inhibitory mechanisms of the other antibiotics shown in the figure. (Adapted from J. Poehlsgaard and S. Douthwaite, *Nat. Rev. Microbiol.* 3:870–881, 2005. With permission from Macmillan Publishers Ltd.)

TABLE 6–4 Inhibitors of Protein or RNA Synthesis	
Inhibitor	Specific effect
Acting only on bacteria	
Tetracycline	Blocks binding of aminoacyl-tRNA to the A site of ribosome
Streptomycin	Prevents the transition from translation initiation to chain elongation and also causes miscoding
Chloramphenicol	Blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–64)
Erythromycin	Binds in the exit channel of the ribosome and thereby inhibits elongation of the peptide chain
Rifamycin	Blocks initiation of RNA chains by binding to RNA polymerase (prevents RNA synthesis)
Acting on bacteria and eukaryotes	
Puromycin	Causes the premature release of nascent polypeptide chains by its addition to the growing chain end
Actinomycin D	Binds to DNA and blocks the movement of RNA polymerase (prevents RNA synthesis)
Acting on eukaryotes but not bacteria	
Cycloheximide	Blocks the translocation reaction on ribosomes (step 3 in Figure 6–64)
Anisomycin	Blocks the peptidyl transferase reaction on ribosomes (step 2 in Figure 6–64)
α-Amanitin	Blocks mRNA synthesis by binding preferentially to RNA polymerase II

The ribosomes of eukaryotic mitochondria (and chloroplasts) often resemble those of bacteria in their sensitivity to inhibitors. Therefore, some of these antibiotics can have a deleterious effect on human mitochondria.

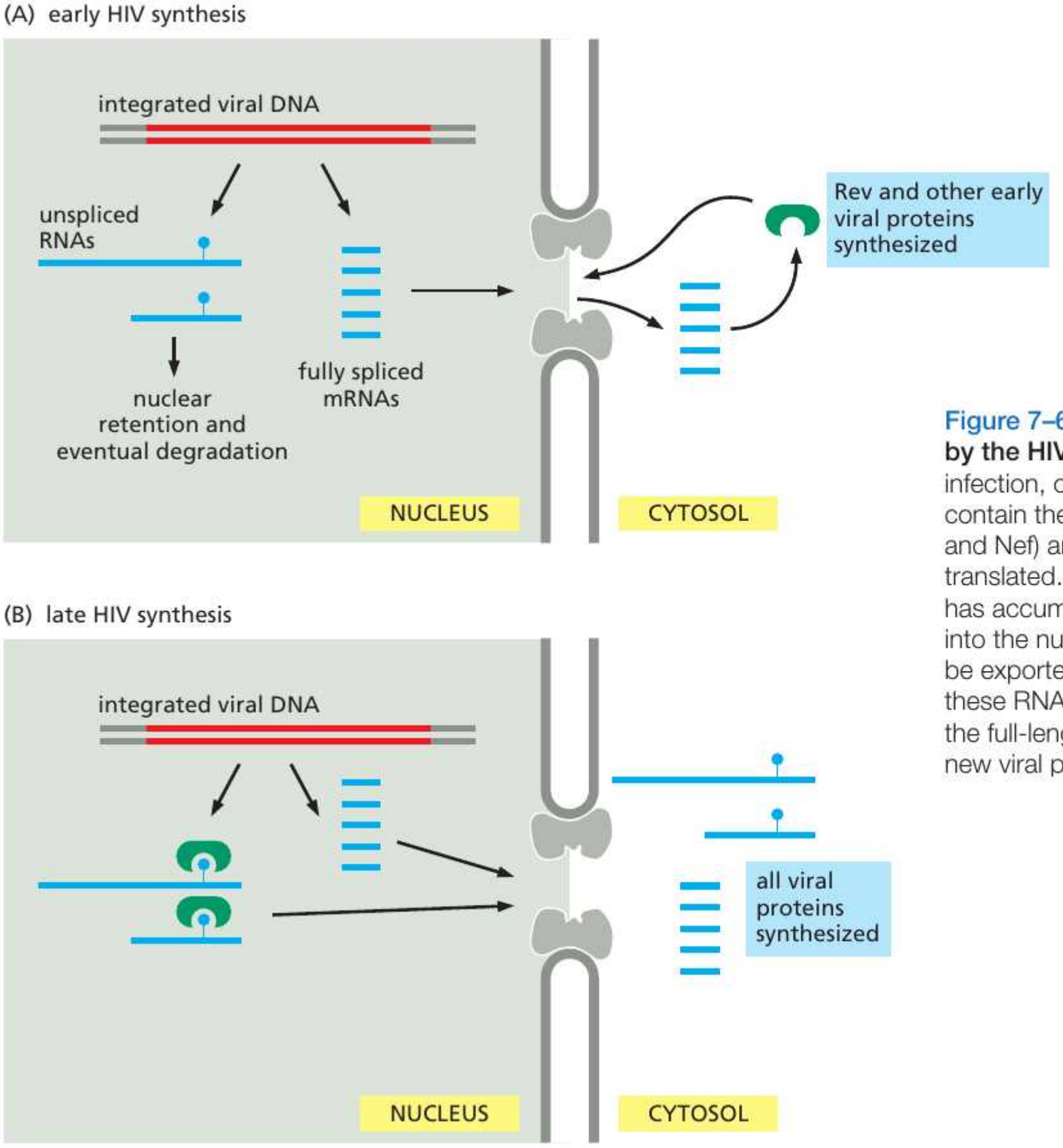
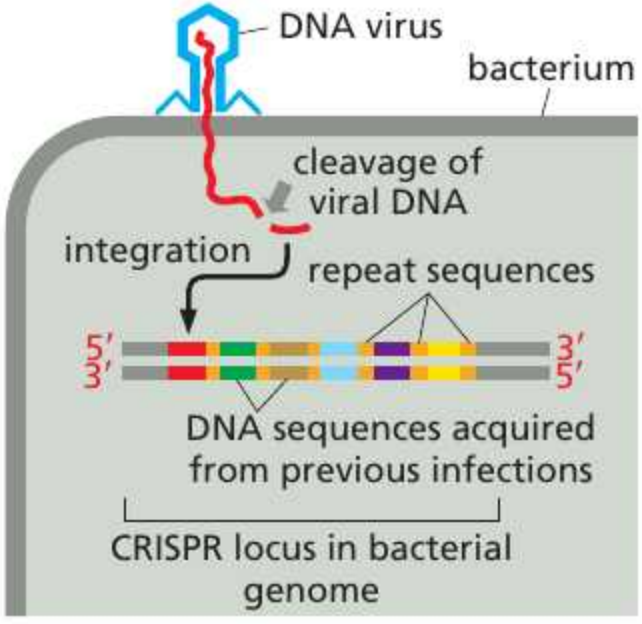
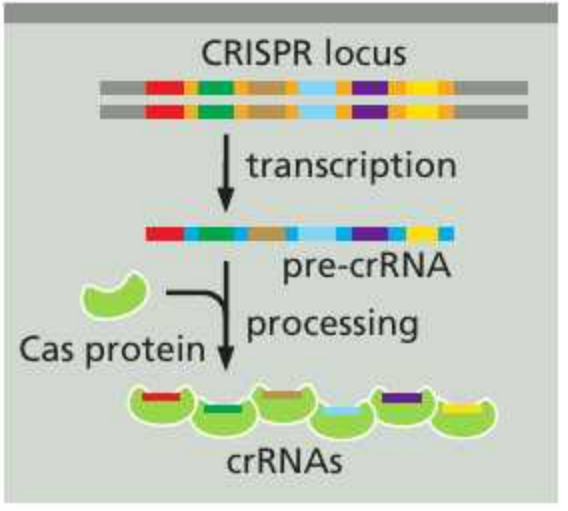


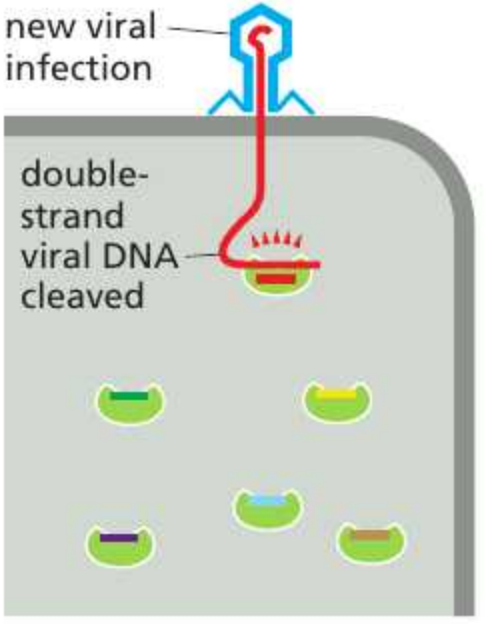
Figure 7–63 Regulation of nuclear export by the HIV Rev protein. (A) Early in HIV infection, only the fully spliced RNAs (which contain the coding sequences for Rev, Tat, and Nef) are exported from the nucleus and translated. (B) Once sufficient Rev protein has accumulated and been transported into the nucleus, unspliced viral RNAs can be exported from the nucleus. Many of these RNAs are translated into protein, and the full-length transcripts are packaged into new viral particles.



STEP 1: short viral DNA sequence is integrated into CRISPR locus



STEP 2: RNA is transcribed from CRISPR locus, processed, and bound to Cas protein



STEP 3: small crRNA in complex with Cas seeks out and destroys viral sequences

The **Ca^{2+} pump**, or **Ca^{2+} ATPase**, in the *sarcoplasmic reticulum* (SR) membrane of skeletal muscle cells is a well-understood P-type transport ATPase. The SR is a specialized type of endoplasmic reticulum that forms a network of tubular sacs in the muscle cell cytoplasm, and it serves as an intracellular store of Ca^{2+}. When an action potential depolarizes the muscle cell plasma membrane, Ca^{2+} is released into the cytosol from the SR through *Ca^{2+}-release channels*, stimulating the muscle to contract (discussed in Chapters 15 and 16). The Ca^{2+} pump, which accounts for about 90% of the membrane protein of the SR, moves Ca^{2+} from the cytosol back into the SR. The endoplasmic reticulum of nonmuscle cells contains a similar Ca^{2+} pump, but in smaller quantities.

Enzymatic studies and analyses of the three-dimensional structures of transport intermediates of the SR Ca^{2+} pump and related pumps have revealed the molecular mechanism of P-type transport ATPases in great detail. They all have similar structures, containing 10 transmembrane α helices connected to three cytosolic domains (**Figure 11–13**). In the Ca^{2+} pump, amino acid side chains protruding from the transmembrane helices form two centrally positioned binding sites for Ca^{2+}. As shown in **Figure 11–14**, in the pump's ATP-bound nonphosphorylated state, these binding sites are accessible only from the cytosolic side of the SR membrane. Ca^{2+} binding triggers a series of conformational changes that close the passageway to the cytosol and activate a phosphotransfer reaction in which the terminal phosphate of the ATP is transferred to an aspartate that is highly conserved among all P-type ATPases. The ADP then dissociates and is replaced with a fresh ATP, causing another conformational change that opens a passageway to the SR lumen through which the two Ca^{2+} ions exit. They are replaced by two H^+ ions and a water molecule that stabilize the empty Ca^{2+}-binding sites and close the passageway to the SR lumen. Hydrolysis of the labile phosphoryl-aspartate bond returns the pump to the initial conformation, and the cycle starts again. The transient self-phosphorylation of the pump during its cycle is an essential characteristic of all P-type pumps.

The Plasma Membrane Na^+-K^+ Pump Establishes Na^+ and K^+ Gradients Across the Plasma Membrane

The concentration of K^+ is typically 10–30 times higher inside cells than outside, whereas the reverse is true of Na^+ (see Table 11–1, p. 598). A **Na^+-K^+ pump**, or **Na^+-K^+ ATPase**, found in the plasma membrane of virtually all animal cells maintains

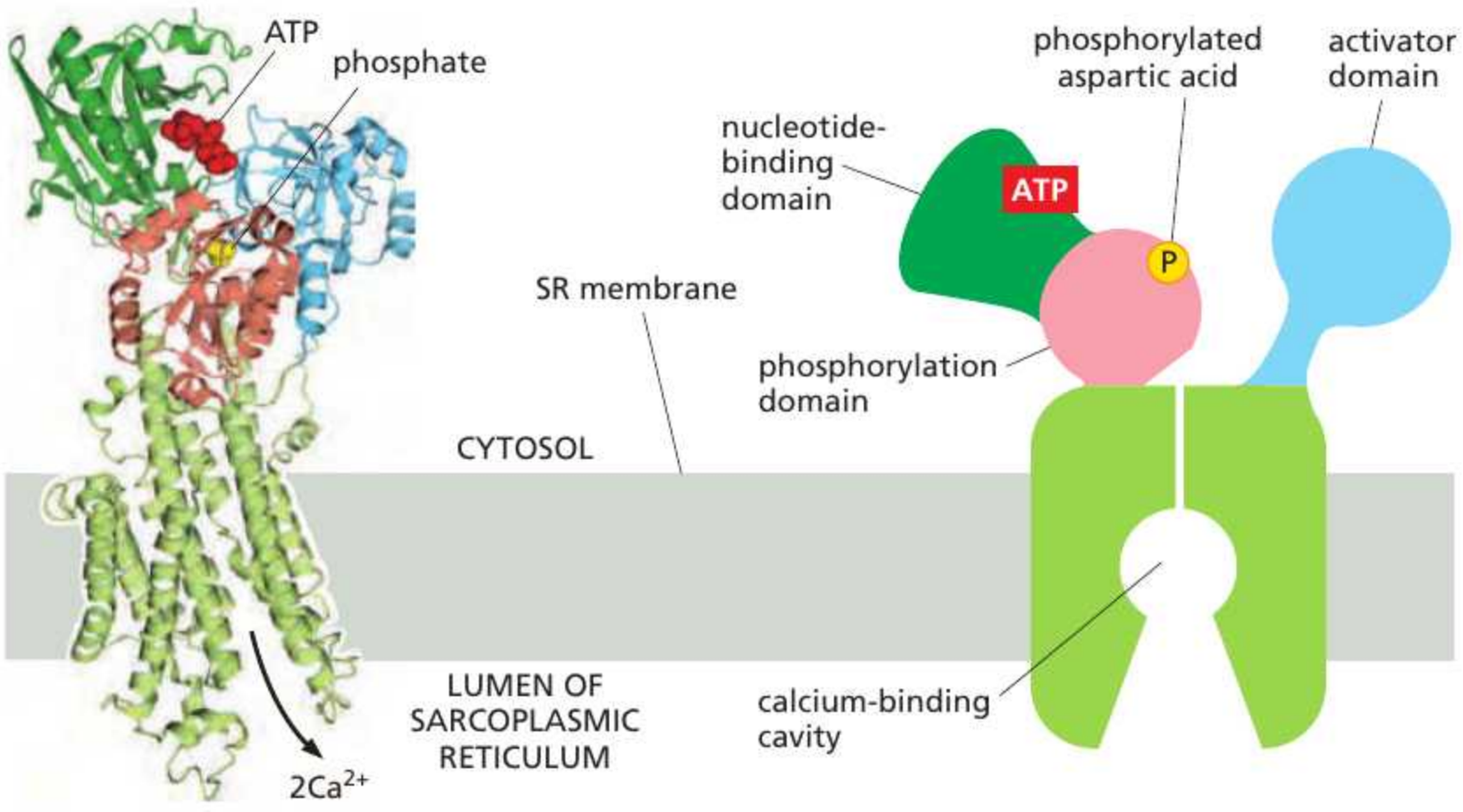


Figure 11–13 The structure of the sarcoplasmic reticulum Ca^{2+} pump. The ribbon model (*left*), derived from x-ray crystallographic analyses, shows the pump in its phosphorylated, ATP-bound state. The three globular cytosolic domains of the pump—the nucleotide-binding domain (*dark green*), the activator domain (*blue*), and the phosphorylation domain (*red*), also shown schematically on the *right*—change conformation dramatically during the pumping cycle. These changes in turn alter the arrangement of the transmembrane helices, which allows the Ca^{2+} to be released from its binding cavity into the SR lumen (**Movie 11.3**). (PDB code: 3B9B.)

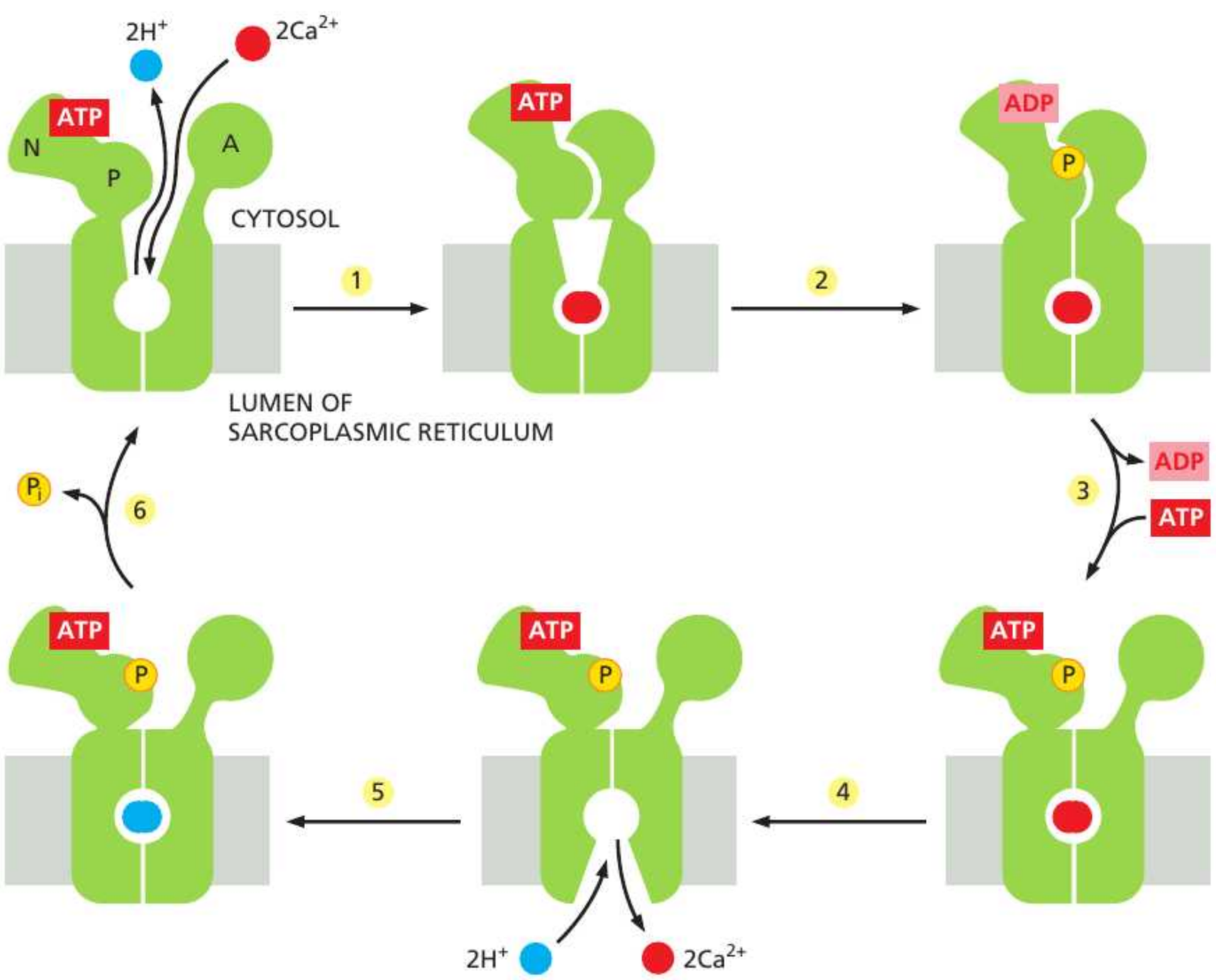


Figure 11–14 The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump. Ion pumping proceeds by a series of stepwise conformational changes in which movements of the pump's three cytosolic domains [the nucleotide-binding domain (N), the phosphorylation domain (P), and the activator domain (A)] are mechanically coupled to movements of the transmembrane α helices. Helix movement opens and closes passageways through which Ca^{2+} enters from the cytosol and binds to the two centrally located Ca^{2+} binding sites. The two Ca^{2+} then exit into the SR lumen and are replaced by two H^+, which are transported in the opposite direction. The Ca^{2+}-dependent phosphorylation and H^+-dependent dephosphorylation of aspartic acid are universally conserved steps in the reaction cycle of all P-type pumps: they cause the conformational transitions to occur in an orderly manner, enabling the proteins to do useful work. (Adapted from C. Toyoshima et al., *Nature* 432:361–368, 2004 and J.V. Møller et al., *Q. Rev. Biophys.* 43:501–566, 2010.)

these concentration differences. Like the Ca^{2+} pump, the Na^+-K^+ pump belongs to the family of P-type ATPases and operates as an ATP-driven antiporter, actively pumping Na^+ out of the cell against its steep electrochemical gradient and pumping K^+ in (**Figure 11–15**).

We mentioned earlier that the Na^+ gradient produced by the Na^+-K^+ pump drives the transport of most nutrients into animal cells and also has a crucial role in regulating cytosolic pH. A typical animal cell devotes almost one-third of its energy to fueling this pump, and the pump consumes even more energy in nerve cells and in cells that are dedicated to transport processes, such as those forming kidney tubules.

Since the Na^+-K^+ pump drives three positively charged ions out of the cell for every two it pumps in, it is *electrogenic*: it drives a net electric current across the membrane, tending to create an electrical potential, with the cell's inside being negative relative to the outside. This electrogenic effect of the pump, however, seldom directly contributes more than 10% to the membrane potential. The remaining 90%, as we discuss later, depends only indirectly on the Na^+-K^+ pump.

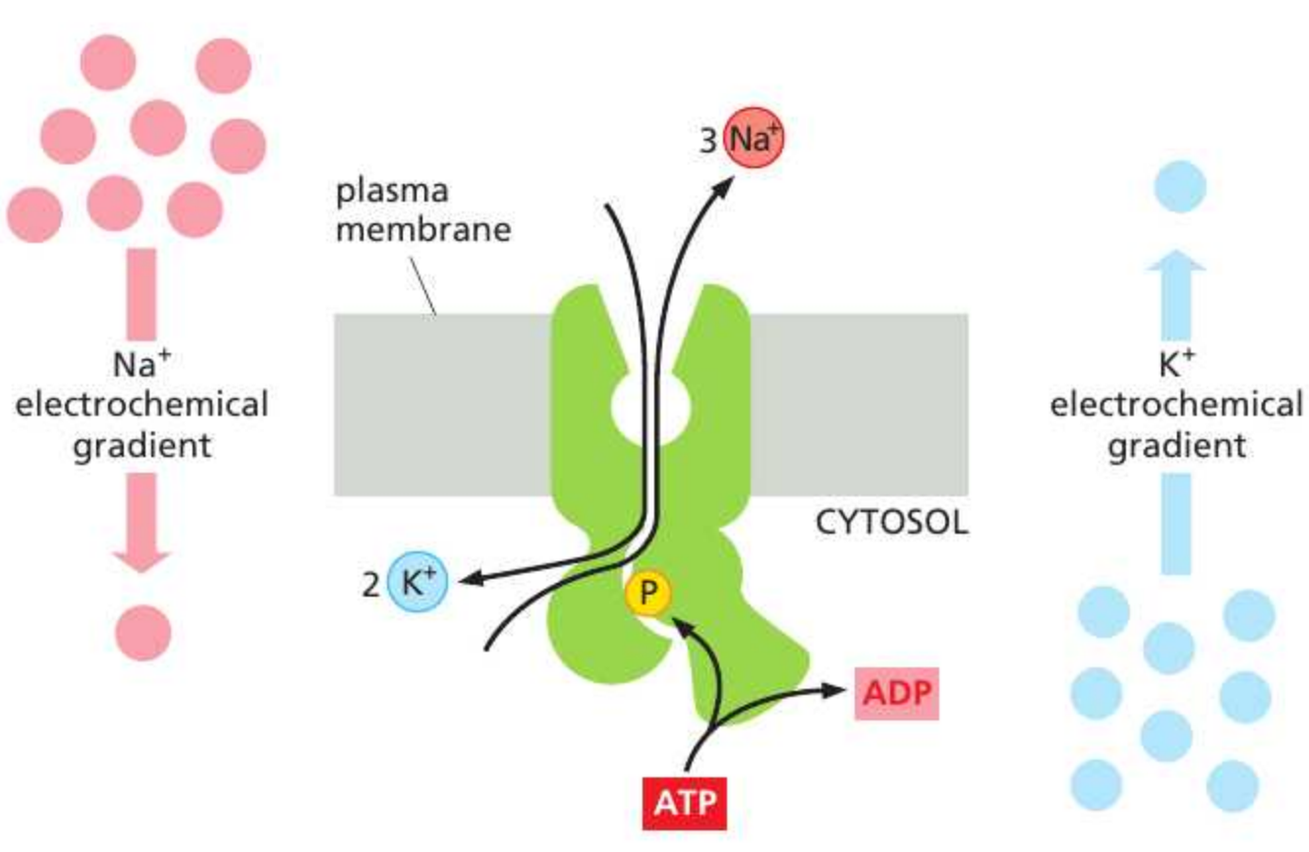


Figure 11–15 The function of the Na^+-K^+ pump. This P-type ATPase actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients. It is structurally closely related to the Ca^{2+} ATPase but differs in its selectivity for ions: for every molecule of ATP hydrolyzed by the pump, three Na^+ are pumped out and two K^+ are pumped in. As in the Ca^{2+} pump, an aspartate is phosphorylated and dephosphorylated during the pumping cycle (**Movie 11.4**).

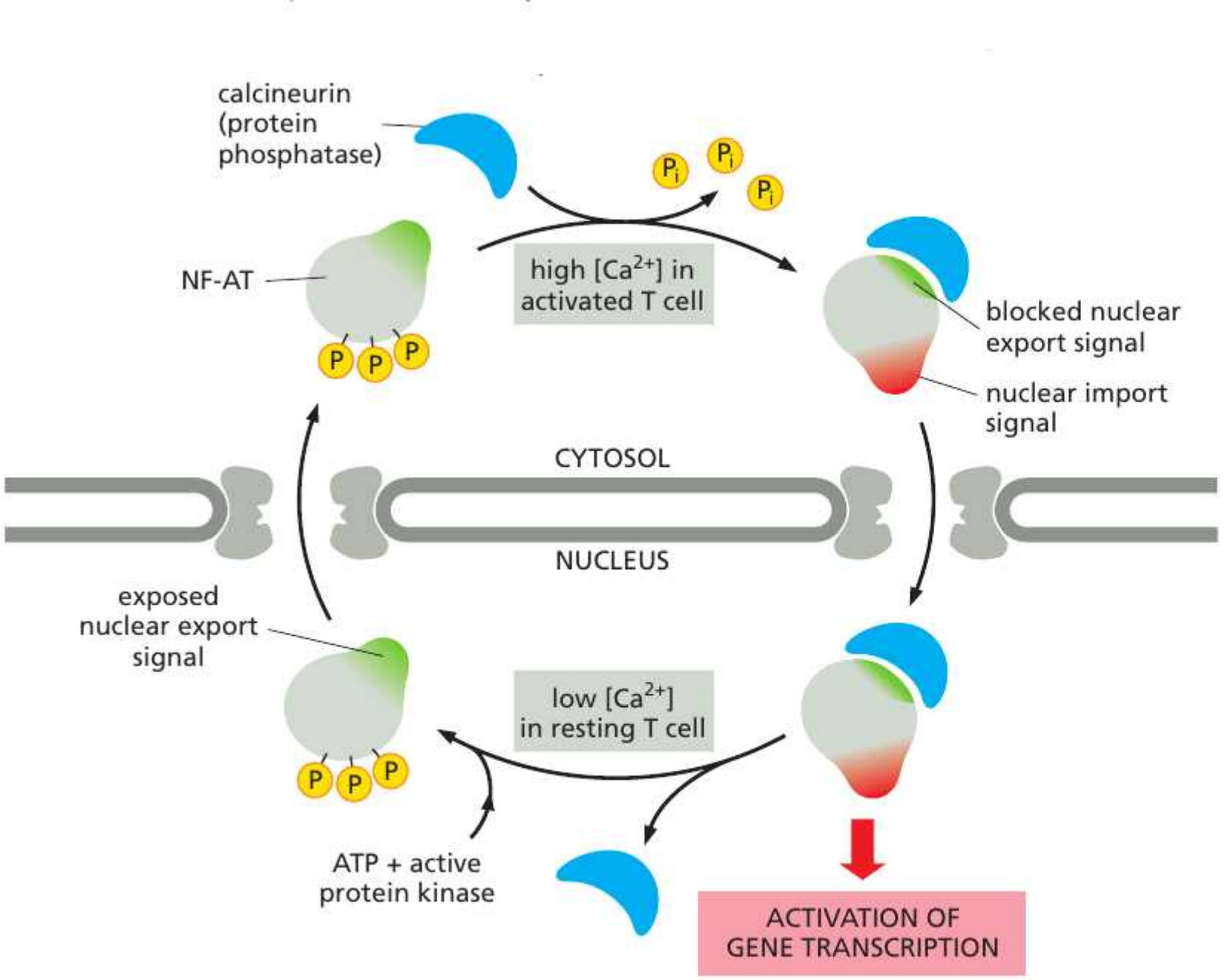


Figure 12–15 The control of nuclear import during T cell activation. The nuclear factor of activated T cells (NF-AT) is a transcription regulatory protein that, in the resting T cell, is found in the cytosol in a phosphorylated state. When T cells are activated by foreign antigen (discussed in Chapter 24), the intracellular Ca^{2+} concentration increases. In high Ca^{2+}, the protein phosphatase calcineurin binds to NF-AT and dephosphorylates it. The dephosphorylation exposes nuclear import signals and blocks a nuclear export signal. The complex of NF-AT and calcineurin is therefore imported into the nucleus, where NF-AT activates the transcription of numerous genes required for T cell activation.

The response shuts off when Ca^{2+} levels decrease, releasing NF-AT from calcineurin. Rephosphorylation of NF-AT inactivates the nuclear import signals and re-exposes the nuclear export signal, causing NF-AT to relocate to the cytosol. Some of the most potent immunosuppressive drugs, including cyclosporin A and FK506, inhibit the ability of calcineurin to dephosphorylate NF-AT and thereby block the nuclear accumulation of NF-AT and T cell activation (Movie 12.1).

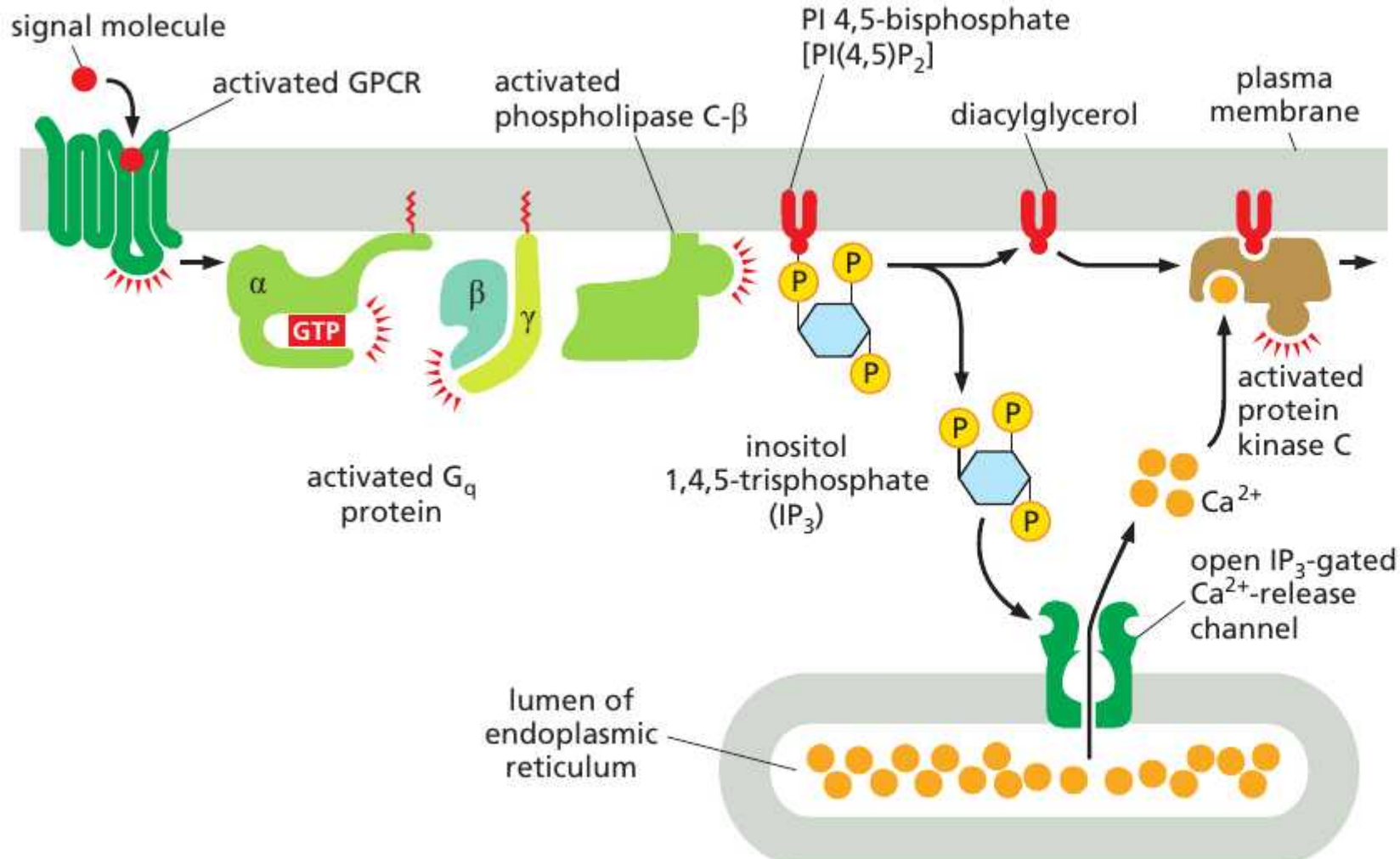
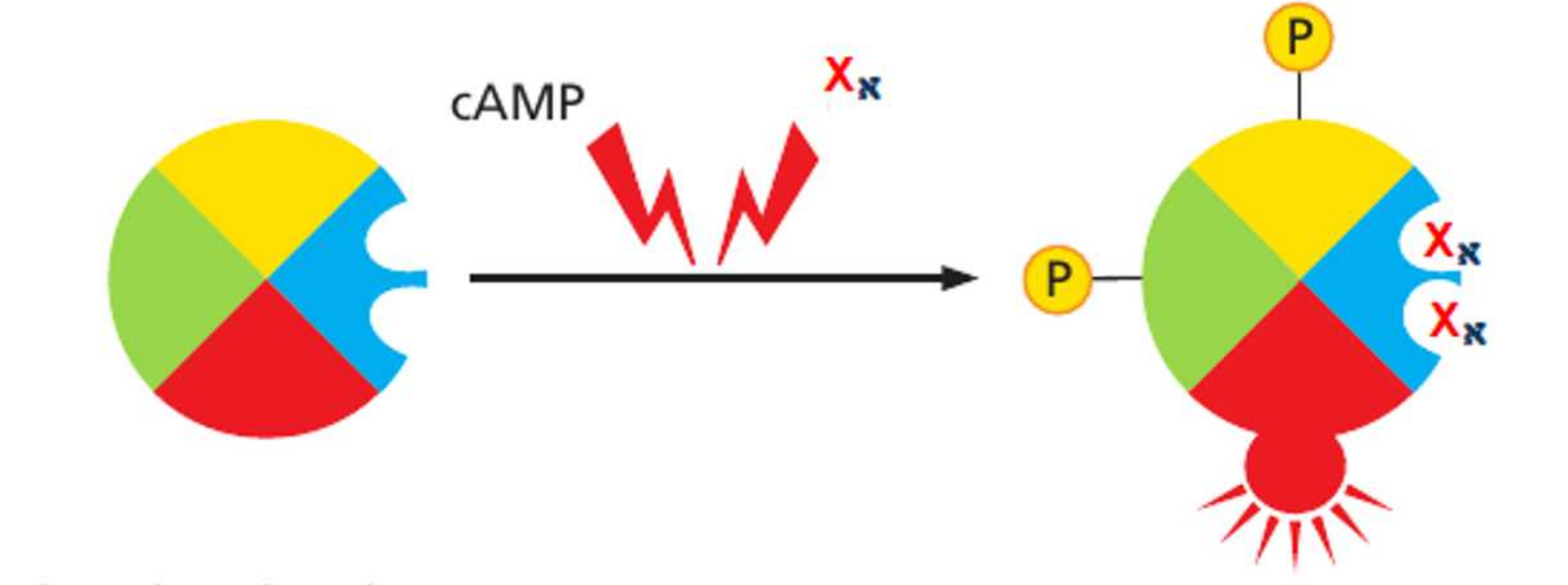


Figure 15–29 How GPCRs increase cytosolic Ca^{2+} and activate protein kinase C. The activated GPCR stimulates the plasma-membrane-bound phospholipase C-β (PLCβ) via a G protein called G_q. The α subunit and βγ complex of G_q are both involved in this activation. Two second messengers are produced when $PI(4,5)P_2$ is hydrolyzed by activated PLCβ. Inositol 1,4,5-trisphosphate (IP_3) diffuses through the cytosol and releases Ca^{2+} from the ER by binding to and opening IP_3-gated Ca^{2+}-release channels (IP_3 receptors) in the ER membrane. The large electrochemical gradient for Ca^{2+} across this membrane causes Ca^{2+} to escape into the cytosol when the release channels are opened. Diacylglycerol remains in the plasma membrane and, together with phosphatidylserine (not shown) and Ca^{2+}, helps to activate protein kinase C (PKC), which is recruited from the cytosol to the cytosolic face of the plasma membrane. Of the 10 or more distinct isoforms of PKC in humans, at least 4 are activated by diacylglycerol (Movie 15.3).

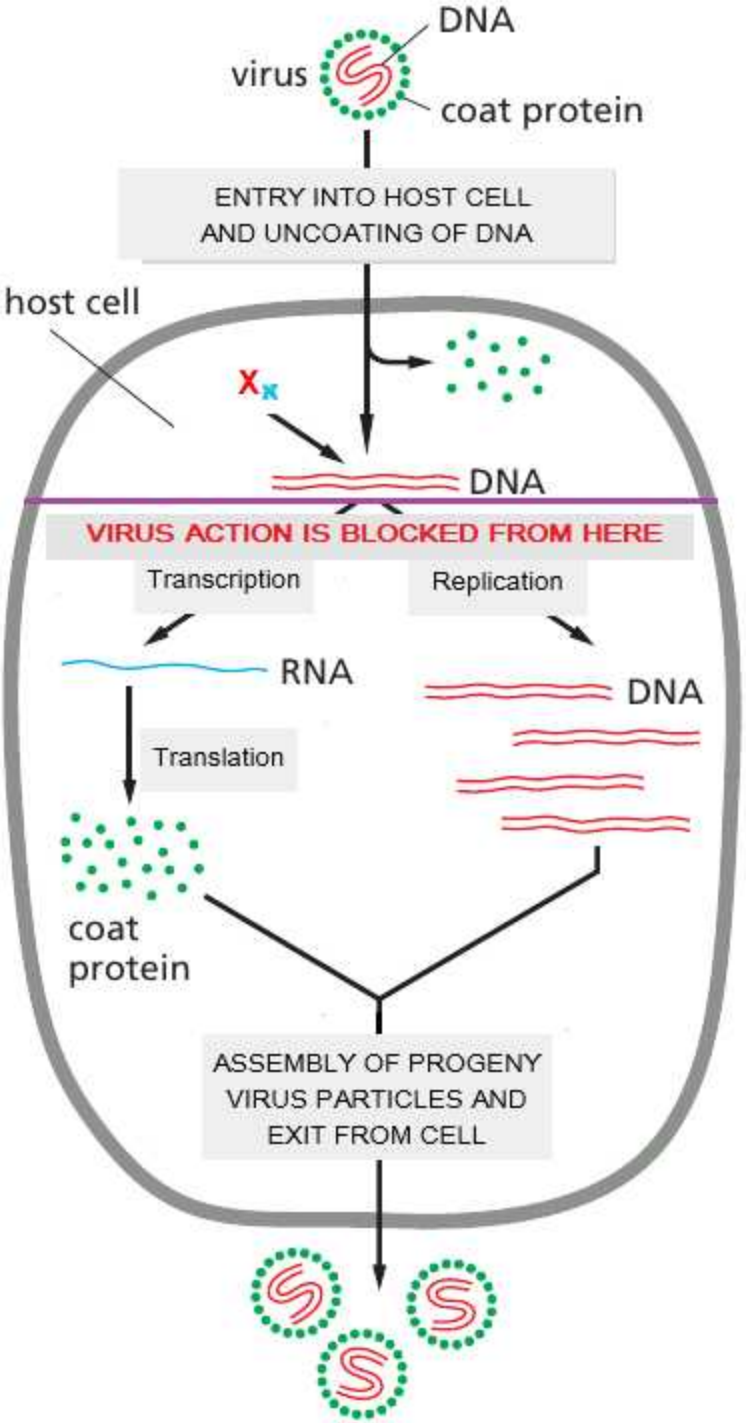


phosphorylase kinase
inactive

phosphorylase kinase
active

TABLE 23–1 Viruses That Cause Human Disease

Virus	Genome type	Disease
Herpes simplex virus 1	Double-stranded DNA	Recurrent cold sores
Epstein–Barr virus (EBV)	Double-stranded DNA	Infectious mononucleosis
Varicella-zoster virus	Double-stranded DNA	Chickenpox and shingles
Smallpox virus (Variola)	Double-stranded DNA	Smallpox
Human papillomavirus	Double-stranded DNA	Warts, cancer
Adenovirus	Double-stranded DNA	Respiratory disease
Hepatitis-B virus	Part single-, part double-stranded DNA	Hepatitis B
Human immunodeficiency virus (HIV-1)	Single-stranded RNA [+] strand	Acquired immune deficiency syndrome (AIDS)
Poliovirus	Single-stranded RNA [+] strand	Poliomyelitis
Rhinovirus	Single-stranded RNA [+] strand	Common cold
Hepatitis-A virus	Single-stranded RNA [+] strand	Hepatitis A
Hepatitis-C virus	Single-stranded RNA [+] strand	Hepatitis C
Yellow fever virus	Single-stranded RNA [+] strand	Yellow fever
Coronavirus	Single-stranded RNA [+] strand	Common cold, respiratory disease
Rabies virus	Single-stranded RNA [–] strand	Rabies
Mumps virus	Single-stranded RNA [–] strand	Mumps
Measles virus	Single-stranded RNA [–] strand	Measles
Influenza virus type A	Single-stranded RNA [–] strand	Respiratory disease (flu)


DNA
virus
coat protein
ENTRY INTO HOST CELL
AND UNCOATING OF DNA
host cell
X
DNA
VIRUS ACTION IS BLOCKED FROM HERE
Transcription
Replication
RNA
DNA
Translation
coat
protein
ASSEMBLY OF PROGENY
VIRUS PARTICLES AND
EXIT FROM CELL

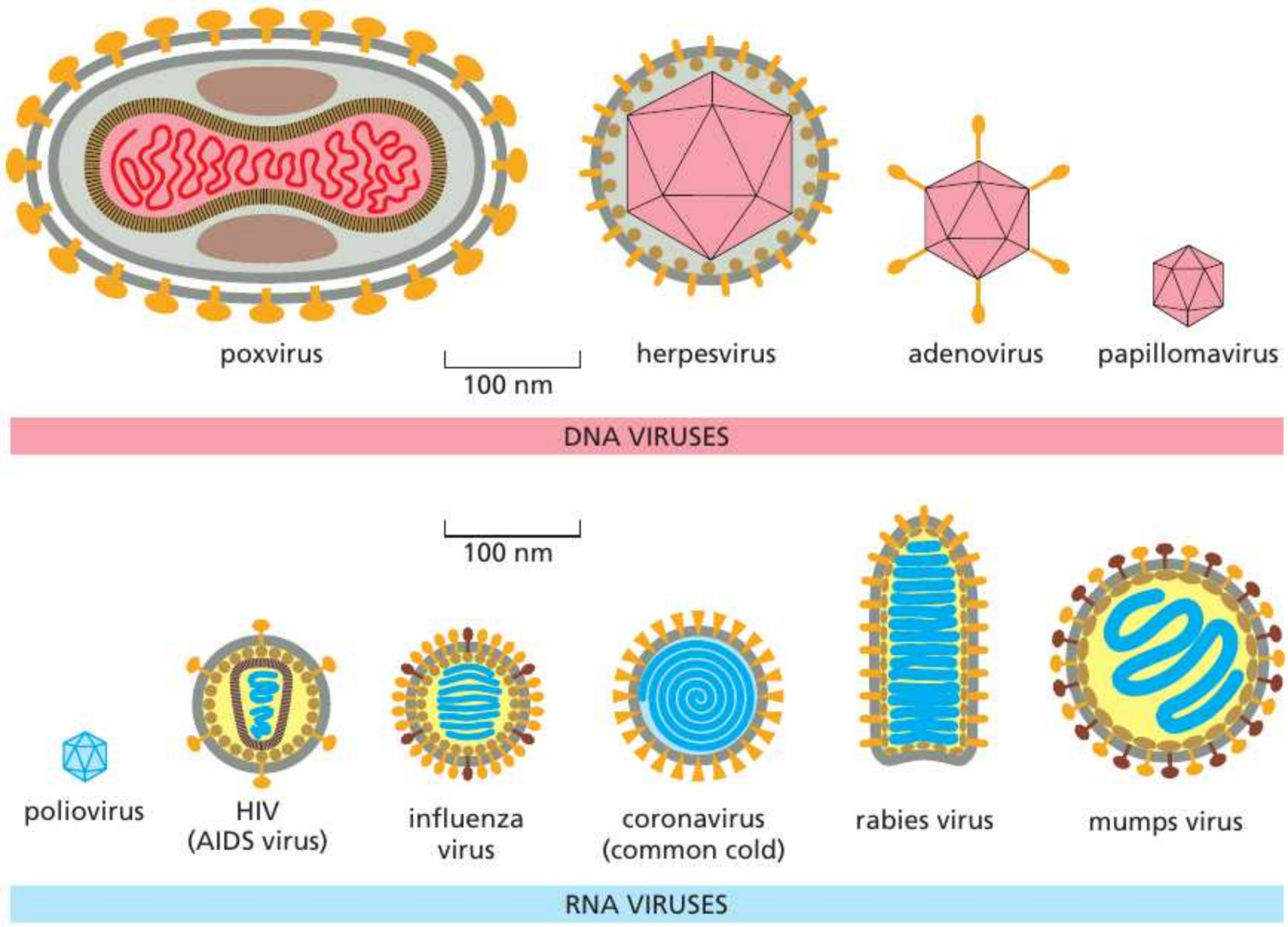
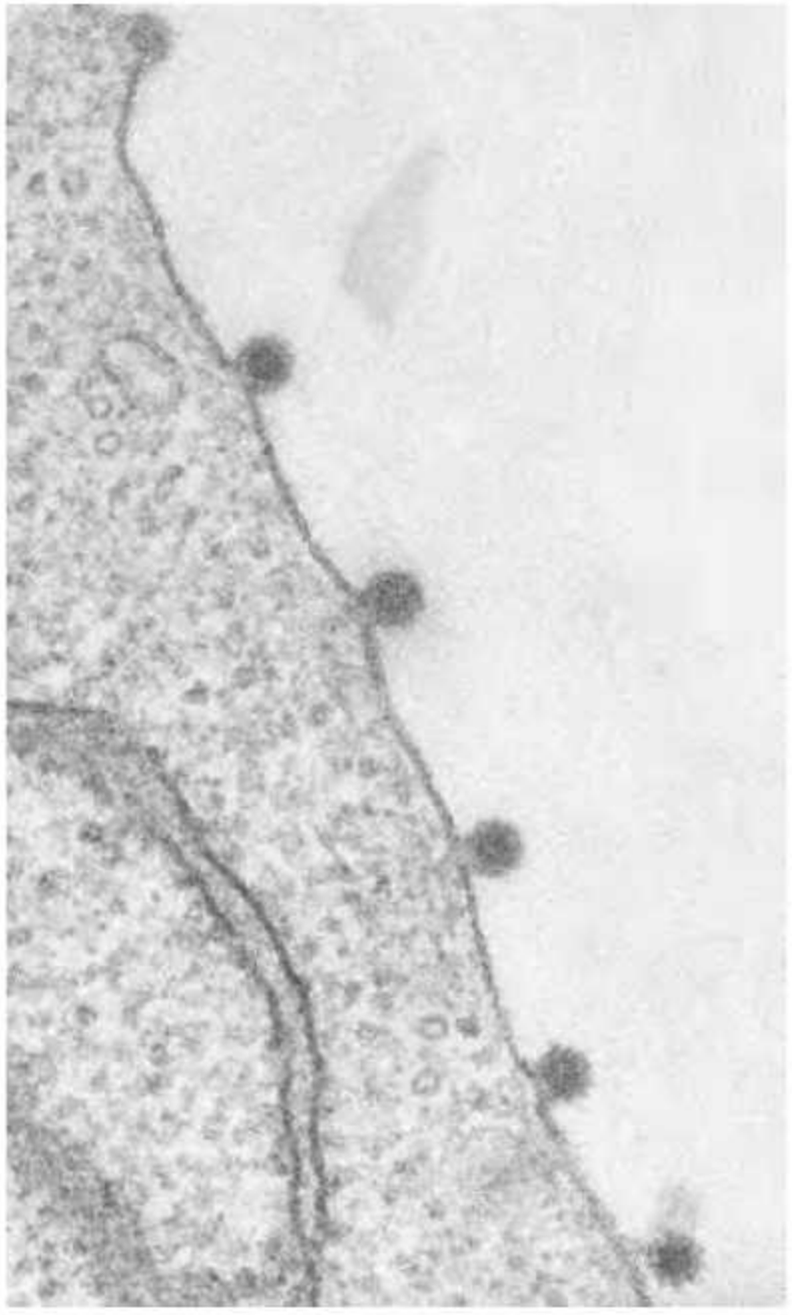


Figure 23–11 Examples of viral morphology. As shown, both DNA and RNA viruses vary greatly in both size and shape.



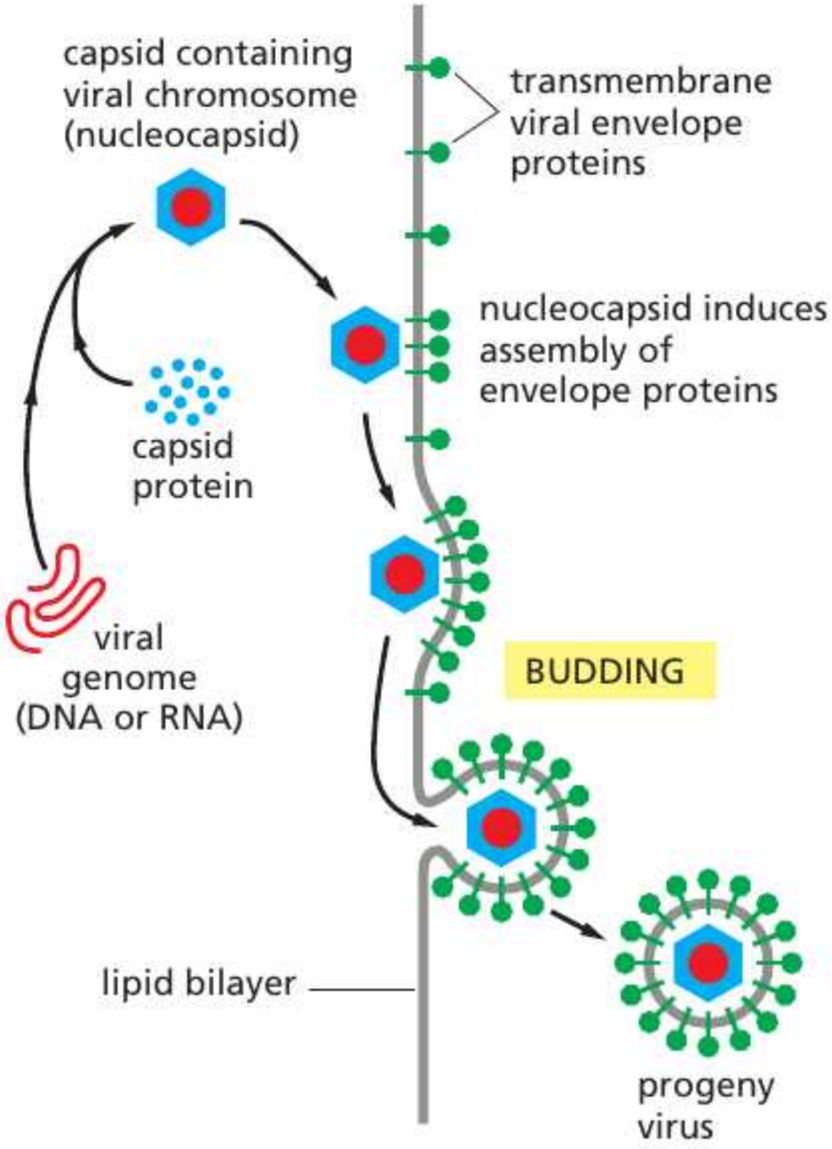


Figure 23–12 Acquisition of a viral envelope. (A) Electron micrograph of an animal cell from which six copies of an enveloped virus (*Semliki forest virus*) are budding. (B) Schematic drawing of the envelope assembly and budding processes. The lipid bilayer that surrounds the viral capsid is derived directly from the plasma membrane of the host cell. In contrast, the proteins in this lipid bilayer (shown in *green*) are encoded by the viral genome. (A, courtesy of M. Olsen and G. Griffith.)

Viruses Bind to Virus Receptors at the Host Cell Surface

The first step for any intracellular pathogen is to bind to the surface of the host target cell. Viruses accomplish this by the binding of viral surface proteins to **virus receptors** displayed on the host cell. The first virus receptor identified was an *E. coli* surface protein that is recognized by the bacteriophage lambda; the protein normally functions to transport the sugar maltose from outside the bacterium to the inside where it is used as an energy source. Receptors need not be proteins, however: an envelope protein of herpes simplex virus, for example, binds to heparan sulfate proteoglycans (discussed in Chapter 19) on the surface of certain vertebrate host cells, and simian virus 40 (SV40) binds to a glycolipid. The specificity of virus-receptor interactions often serves as a barrier preventing the spread of a virus from one species to another. Acquiring the ability to bind to a new receptor often requires multiple changes in a virus, but it can be crucial in allowing the cross-species transmission that can result in new disease outbreaks.

Viruses that infect animal cells generally exploit cell-surface receptor molecules that are either ubiquitous (such as the sialic-acid-containing oligosaccharides used by the influenza virus) or found uniquely on those cell types in which the virus replicates (such as the neuron-specific proteins used by rabies virus). Although a virus usually uses a single type of host-cell receptor, some viruses use more than one type. An important example is HIV-1, which requires two types of receptors to enter a host cell. Its primary receptor is CD4, a cell-surface protein on helper T cells and macrophages that is involved in immune recognition (discussed in Chapter 24). It also requires a co-receptor, which is either CCR5 (a receptor for β-chemokines) or CXCR4 (a receptor for α-chemokines), depending on the particular variant of the virus; macrophages are susceptible only to HIV variants that use CCR5 for entry, whereas helper T cells are most efficiently infected by variants that use CXCR4 (**Figure 23–17**). The viruses that are found within the first few months after HIV infection almost invariably use CCR5, which explains why individuals that carry a defective *CCR5* gene are less susceptible to HIV infection. In the later stages of infection, viruses often either switch to use CXCR4 or adapt to use both co-receptors through the accumulation of mutations; in this way, the virus can change the cell types it infects as the disease progresses. It may seem paradoxical that viruses would infect immune cells, as we might expect that virus binding would trigger an immune response; but invasion of an immune cell can be a useful way for a virus to weaken the immune response and travel around the body to infect other immune cells.

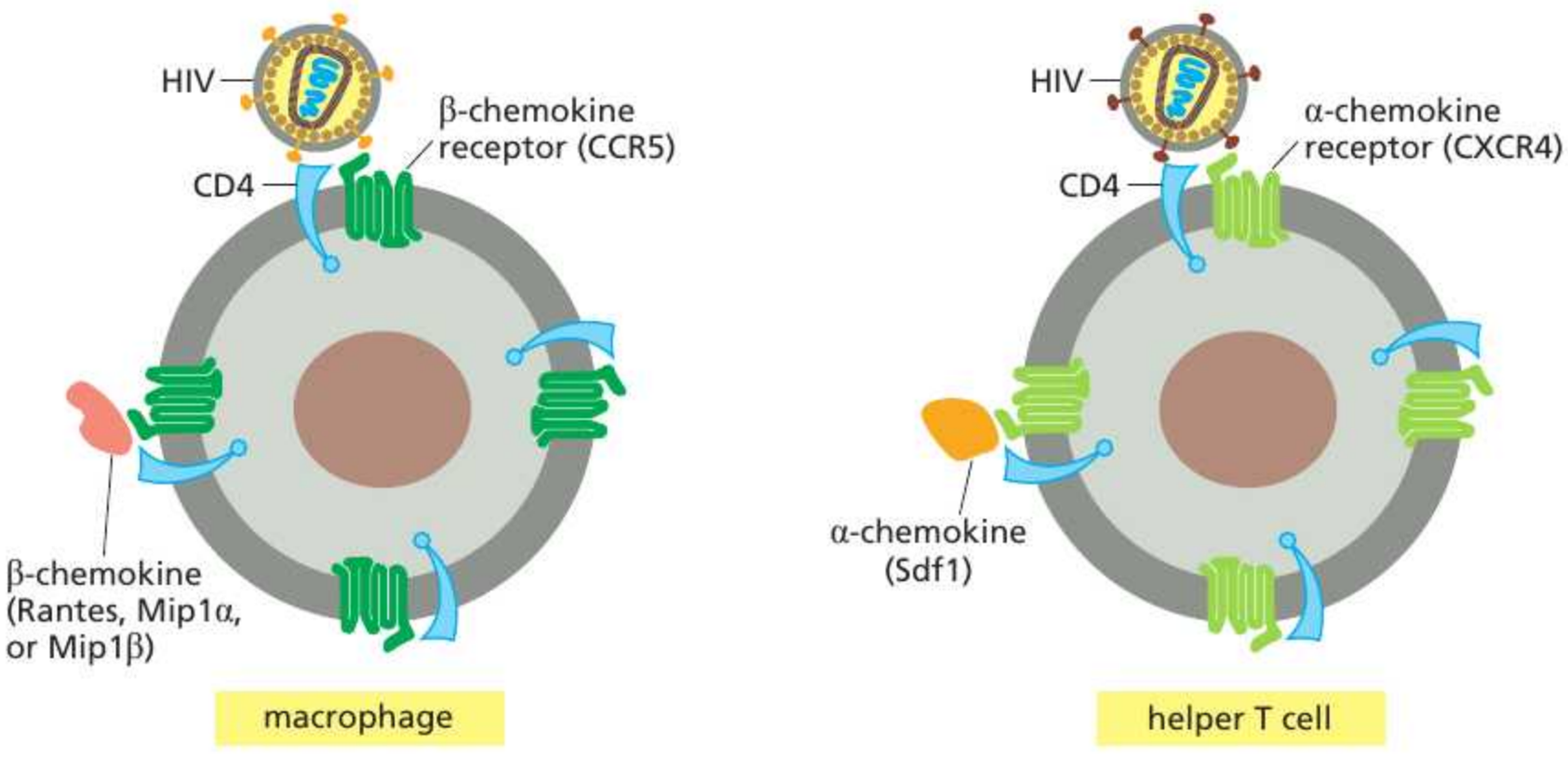


Figure 23–17 Receptor and co-receptors for HIV. All strains of HIV require the CD4 protein as a primary receptor. Early in an infection, most of the viruses use CCR5 as a co-receptor, allowing them to infect macrophages and their precursors, monocytes. As the infection progresses, mutant variants arise that now use CXCR4 as a co-receptor, enabling them to infect helper T cells efficiently. The natural ligand for the chemokine receptors (Sdf1 for CXCR4; Rantes, Mip1α, or Mip1β for CCR5) blocks co-receptor function and prevents viral invasion.

Viruses Enter Host Cells by Membrane Fusion, Pore Formation, or Membrane Disruption

After recognition and attachment to the host cell surface, the virus must enter the cell to replicate. Some **enveloped viruses** enter the host cell by fusing their envelope membrane with the plasma membrane. Most viruses, whether enveloped or nonenveloped, activate signaling pathways in the cell that induce endocytosis, commonly via clathrin-coated pits (see Figure 13–7), leading to internalization into endosomes. Large viruses that do not fit into clathrin-coated vesicles, such as poxviruses, often enter cells by *macropinocytosis*, a process by which membrane ruffles fold over and entrap fluid into macropinosomes (see Figure 13–50). Once inside endosomes, fusion of the viral envelope occurs from the lumenal side of the endosome membrane. The mechanism of membrane fusion mediated by viral spike glycoproteins has similarities with SNARE-mediated membrane fusion during normal vesicular trafficking (discussed in Chapter 13).

Enveloped viruses regulate fusion both to ensure that they fuse only with the appropriate host cell membrane and to prevent fusion with one another. For viruses such as HIV-1 that fuse at neutral pH with the plasma membrane (**Figure 23–18**A), binding to receptors or co-receptors usually triggers a conformational change in a viral envelope protein that exposes a normally buried fusion peptide (see Figure 13–21). Other enveloped viruses, such as influenza A virus, only fuse with a host cell membrane after endocytosis (Figure 23–18B); in this case, it is frequently the acid environment in the late endosome that triggers the conformational change in a viral surface protein that exposes the fusion peptide. The H^+

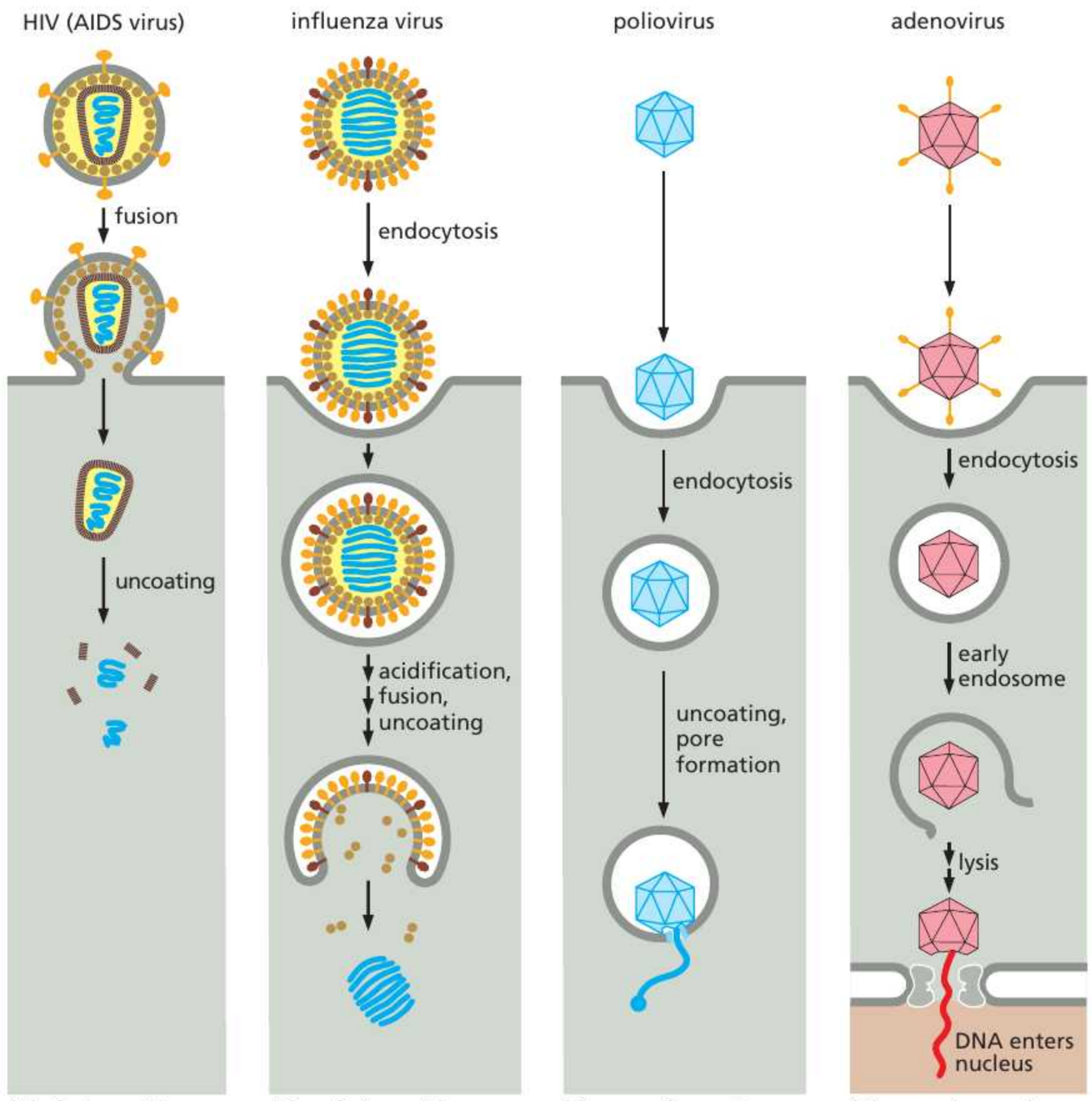
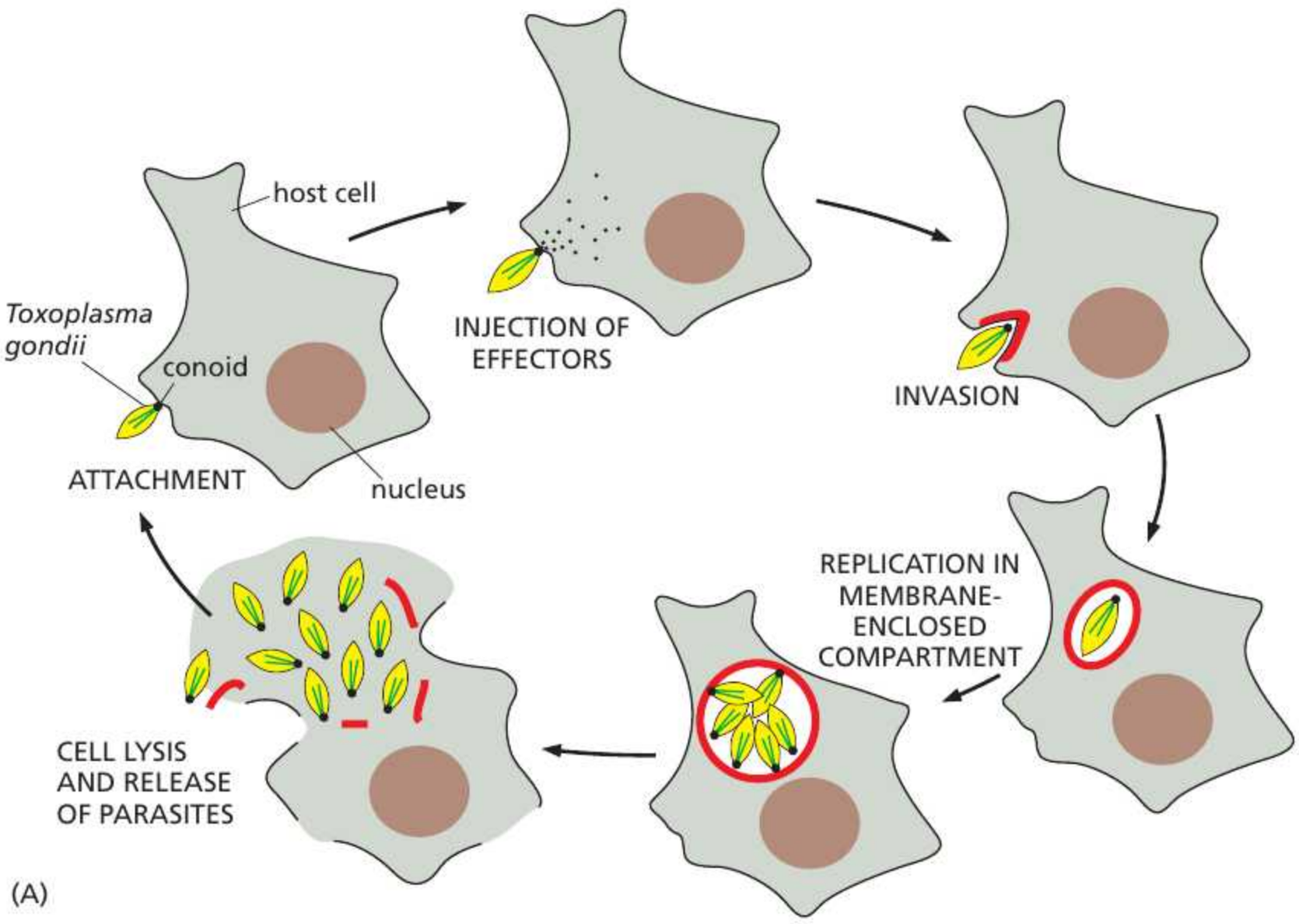


Figure 23–18 Four virus entry strategies. (A) Some enveloped viruses, such as HIV, fuse directly with the host-cell plasma membrane to release their RNA genome *(blue)* and capsid proteins *(brown)* into the cytosol. (B) Other enveloped viruses, such as influenza virus, first bind to cell-surface receptors, triggering receptor-mediated endocytosis; when the endosome acidifies, the virus envelope fuses with the endosomal membrane, releasing the viral RNA genome *(blue)* and capsid proteins *(brown)* into the cytosol. (C) Poliovirus, a nonenveloped virus, induces receptor-mediated endocytosis, and then forms a pore in the endosomal membrane to extrude its RNA genome *(blue)* into the cytosol. (D) Adenovirus, another nonenveloped virus, uses a more complicated strategy: it induces receptor-mediated endocytosis and then disrupts the endosomal membrane, releasing the capsid and its DNA genome into the cytosol; the trimmed-down virus eventually docks onto a nuclear pore and releases its DNA *(red)* directly into the nucleus (**Movie 23.5**).



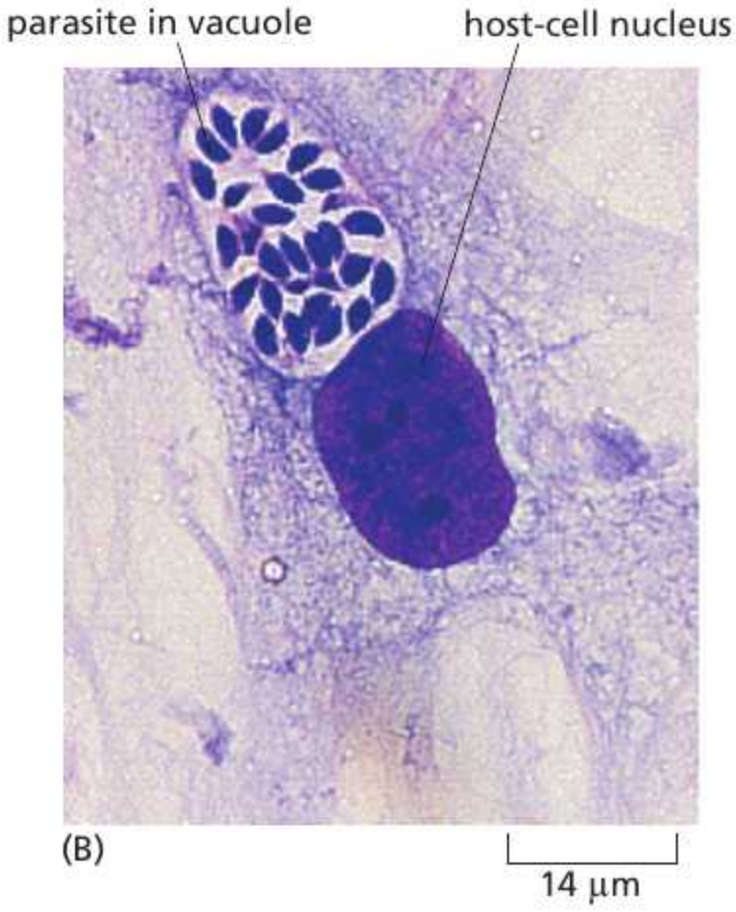


Figure 23–20 The life cycle of the intracellular parasite *Toxoplasma gondii*. (A) After attachment to a host cell, *T. gondii* uses its conoid to inject effector proteins that facilitate invasion. As the host cell's plasma membrane invaginates to surround the parasite, it somehow removes the normal host-cell membrane proteins, so that the compartment (shown in *red*) does not fuse with lysosomes. After several rounds of replication, the parasite causes the compartment to break down and the host cell to lyse, releasing the progeny parasites to infect other host cells (**Movie 23.6**). (B) Light micrograph of *T. gondii* replicating within a membrane-enclosed compartment (a vacuole) in a cultured cell. (B, courtesy of Manuel Camps and John Boothroyd.)

The protozoan *Trypanosoma cruzi,* which causes Chagas disease, in Mexico and Central and South America, uses two alternative invasion strategies. In a *lysosome-dependent pathway,* the parasite attaches to host cell-surface receptors, inducing a local increase in Ca^{2+} in the host cell's cytosol. The Ca^{2+} signal recruits lysosomes to the site of parasite attachment, and the lysosomes fuse with the host cell's plasma membrane, allowing the parasites rapid access to the lysosomal compartment (**Figure 23–21**). In a *lysosome-independent pathway,* the parasite penetrates the host-cell plasma membrane by inducing the membrane to invaginate, without the recruitment of lysosomes.

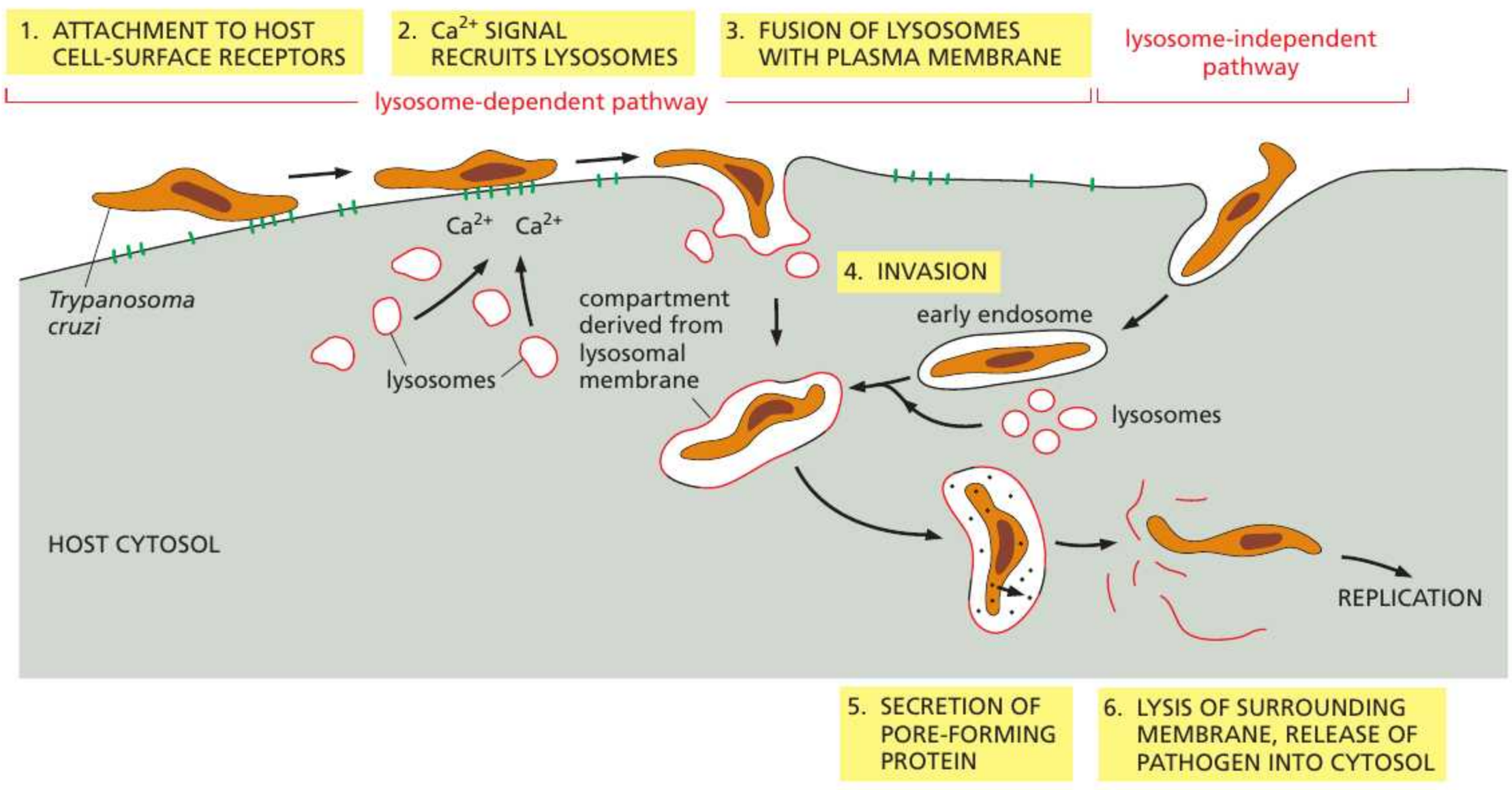
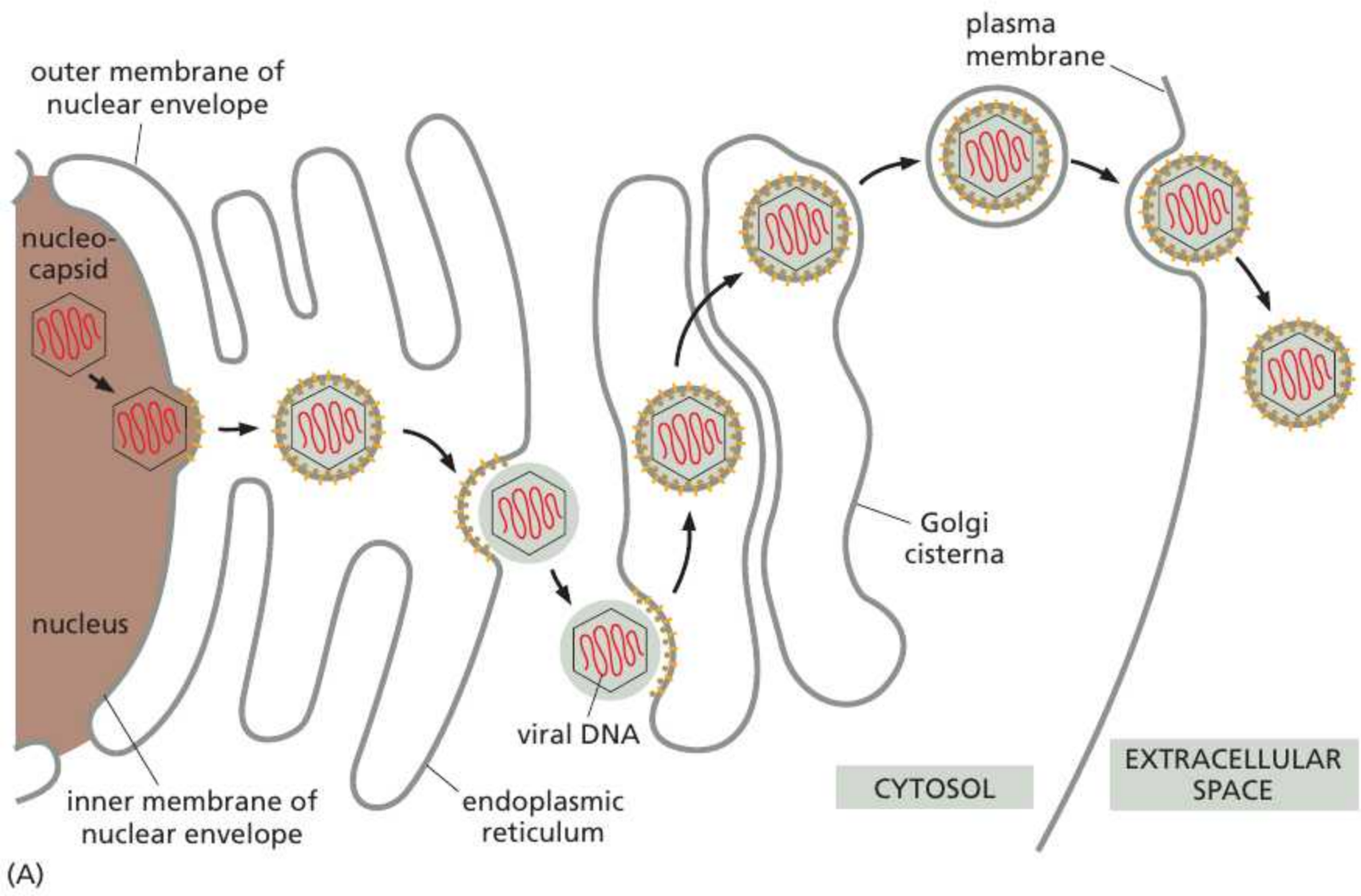


Figure 23–21 The two alternative strategies that *Trypanosoma cruzi* uses to invade host cells. In the lysosome-dependent pathway *(left),* *T. cruzi* recruits host-cell lysosomes to its site of attachment to the host cell. The lysosomes fuse with the invaginating plasma membrane to create an intracellular compartment constructed almost entirely of lysosomal membrane. After a brief stay in the compartment, the parasite secretes a pore-forming protein that disrupts the surrounding membrane, thereby allowing the parasite to escape into the host-cell cytosol and proliferate. In the lysosome-independent pathway *(right),* the parasite induces the host plasma membrane to invaginate and pinch off without recruiting lysosomes; then lysosomes fuse with the endosome prior to the parasite's escape into the cytosol.



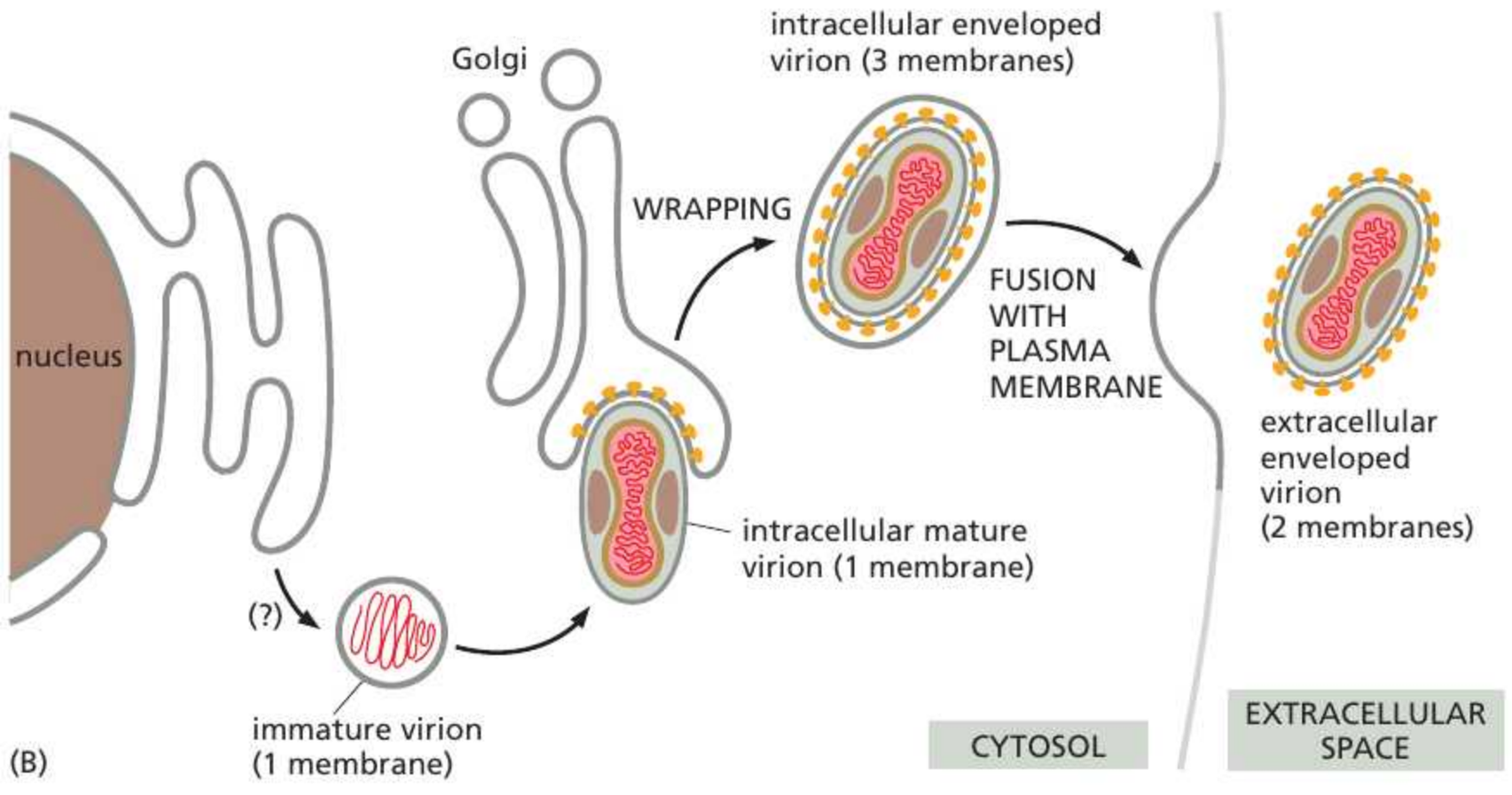


Figure 23–27 Complex strategies for viral envelope acquisition. (A) Herpesvirus nucleocapsids assemble in the nucleus and then bud through the inner nuclear membrane into the space between the inner and outer nuclear membranes, acquiring a lipid bilayer membrane coat. The virus particles then apparently lose this coat when they fuse with the endoplasmic reticulum membrane to escape into the cytosol. Subsequently, the nucleocapsids bud into the Golgi apparatus and bud out again on the other side, thereby acquiring two new membrane coats in the process. The virus then buds from the cell surface with a single membrane when its outer membrane fuses with the plasma membrane. (B) Vaccinia virus (which is closely related to the virus that causes smallpox and is used to vaccinate against smallpox) assembles in "replication factories" in the cytosol, far away from the plasma membrane. The immature virion, with one membrane, is then surrounded by two additional membranes, both acquired from the Golgi apparatus by a poorly understood wrapping mechanism, to form the intracellular enveloped virion. After fusion of the outermost membrane with the host-cell plasma membrane, the extracellular enveloped virion is released from the host cell.

Error-Prone Replication Dominates Viral Evolution

In contrast to the DNA rearrangements in bacteria and parasites, viruses rely on an error-prone replication mechanism for antigenic variation. Retroviral genomes, for example, acquire on average one point mutation every replication cycle, because the viral reverse transcriptase (see Figure 5–62) needed to produce DNA from the viral RNA genome lacks the proofreading activity of DNA polymerases. A typical, untreated HIV infection may eventually produce HIV genomes with every possible point mutation. By a process of mutation and selection within each host, most viruses change over time—from a form that is most efficient at infecting macrophages to one more efficient at infecting T cells, as described earlier (see Figure 23–17). Similarly, once a patient is treated with an antiviral drug, the viral genome can quickly mutate and be selected for its resistance to the drug. Remarkably, only about one-third of the nucleotide positions in the coding sequence of the viral genome are invariant, and nucleotide sequences in some parts of the genome, such as the *Env* gene (see Figure 7–62), can differ by as much as 30% from one HIV isolate to another. This extraordinary genomic plasticity greatly complicates attempts to develop vaccines against HIV. It has also led to the rapid emergence of new HIV strains. Nucleotide sequence comparisons between various strains of HIV and the very similar simian immunodeficiency virus (SIV) isolated from a variety of monkey species suggest that the most virulent type of HIV, HIV-1, may have jumped from primates to humans multiple independent times, starting as long ago as 1908 (**Figure 23–31**).

Influenza viruses are an important exception to the rule that error-prone replication dominates viral evolution. They are unusual in that their genome consists of several (usually eight) strands of RNA. When two strains of influenza infect the same host, the RNA strands of the two strains can reassort to form a new type of influenza virus. In normal years, influenza is a mild disease in healthy adults, although it can be life-threatening in the very young and very old. Different influenza strains infect fowl such as ducks and chickens, but only a subset of these strains can infect humans, and transmission from fowl to humans is rare. In 1918, however, a particularly virulent variant of avian influenza crossed the species barrier to infect humans, triggering the catastrophic pandemic of 1918 called the Spanish flu, which killed 20–50 million people worldwide. Subsequent influenza pandemics have been triggered by genome reassortment, in which a new RNA segment from an avian form of the virus replaced one or more of the viral RNA segments from the human form (**Figure 23–32**). In 2009, a new H1N1 swine virus emerged that derived genes from pig, avian, and human influenza viruses. Such recombination events allowed the new virus to replicate rapidly and spread through an immunologically naive human population. Generally, within two or three years, the human population develops immunity to a new recombinant strain of virus, and the infection rate drops to a steady-state level. Because the recombination events are unpredictable, it is not possible to know when the next influenza pandemic will occur or how severe it might be.

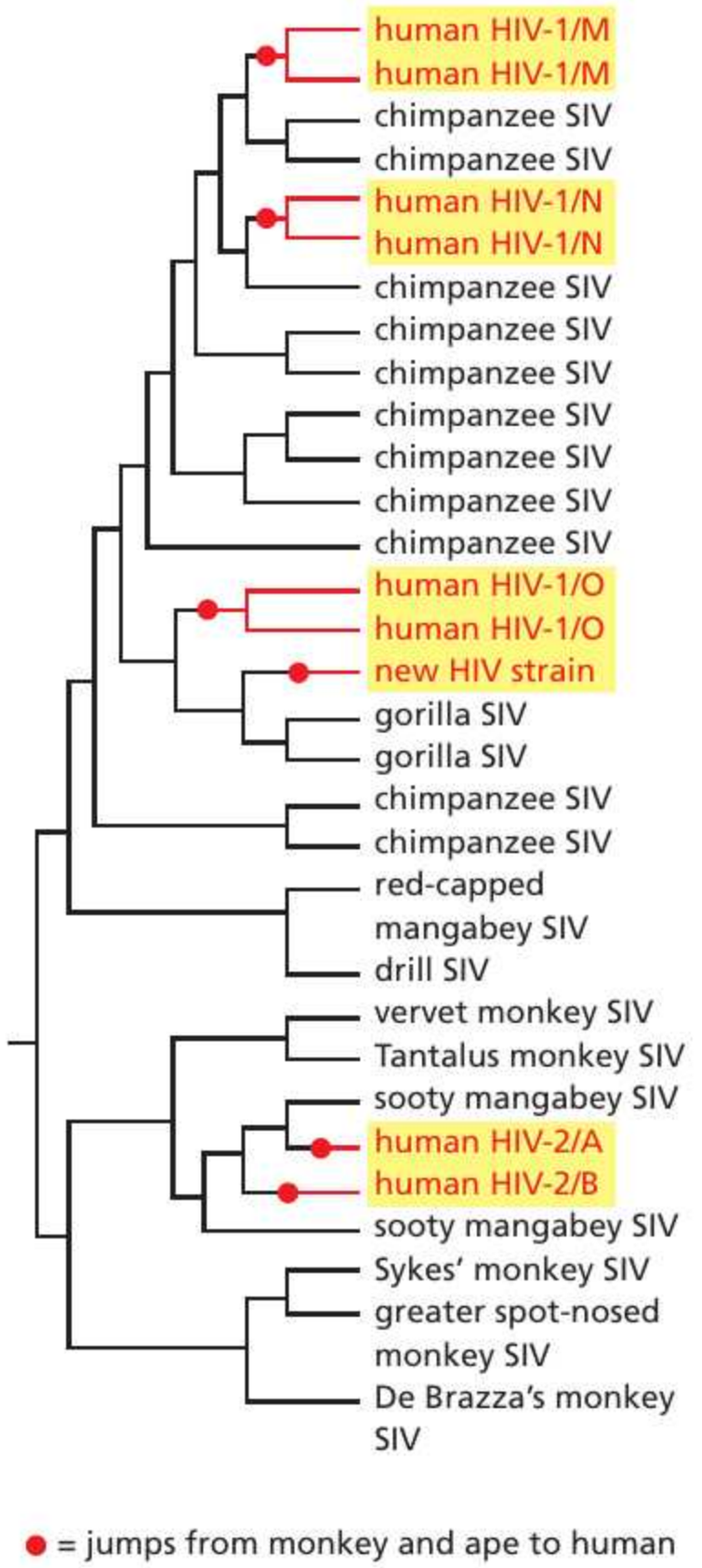


Figure 23–31 Diversification of HIV-1, HIV-2, and related strains of SIV. HIV comprises different viral families, all descended from SIV (simian immunodeficiency virus). On three separate occasions, SIV was passed from a chimpanzee to a human, resulting in three HIV-1 groups: major (M), outlier (O), and non-M non-O (N). The HIV-1 M group is the most common and is primarily responsible for the global AIDS epidemic. On two separate occasions, SIV was passed from a sooty mangabey monkey to a human, resulting in the two HIV-2 groups. In 2009, a new strain of HIV was discovered that appears to have resulted from SIV passage from a gorilla to a human.

Drug-Resistant Pathogens Are a Growing Problem

The development of drugs that cure rather than prevent infections has had a major impact on human health. **Antibiotics**, which are either bactericidal (they kill bacteria) or bacteriostatic (they inhibit bacterial growth without killing), are the most successful class of such drugs. Penicillin was one of the first antibiotics

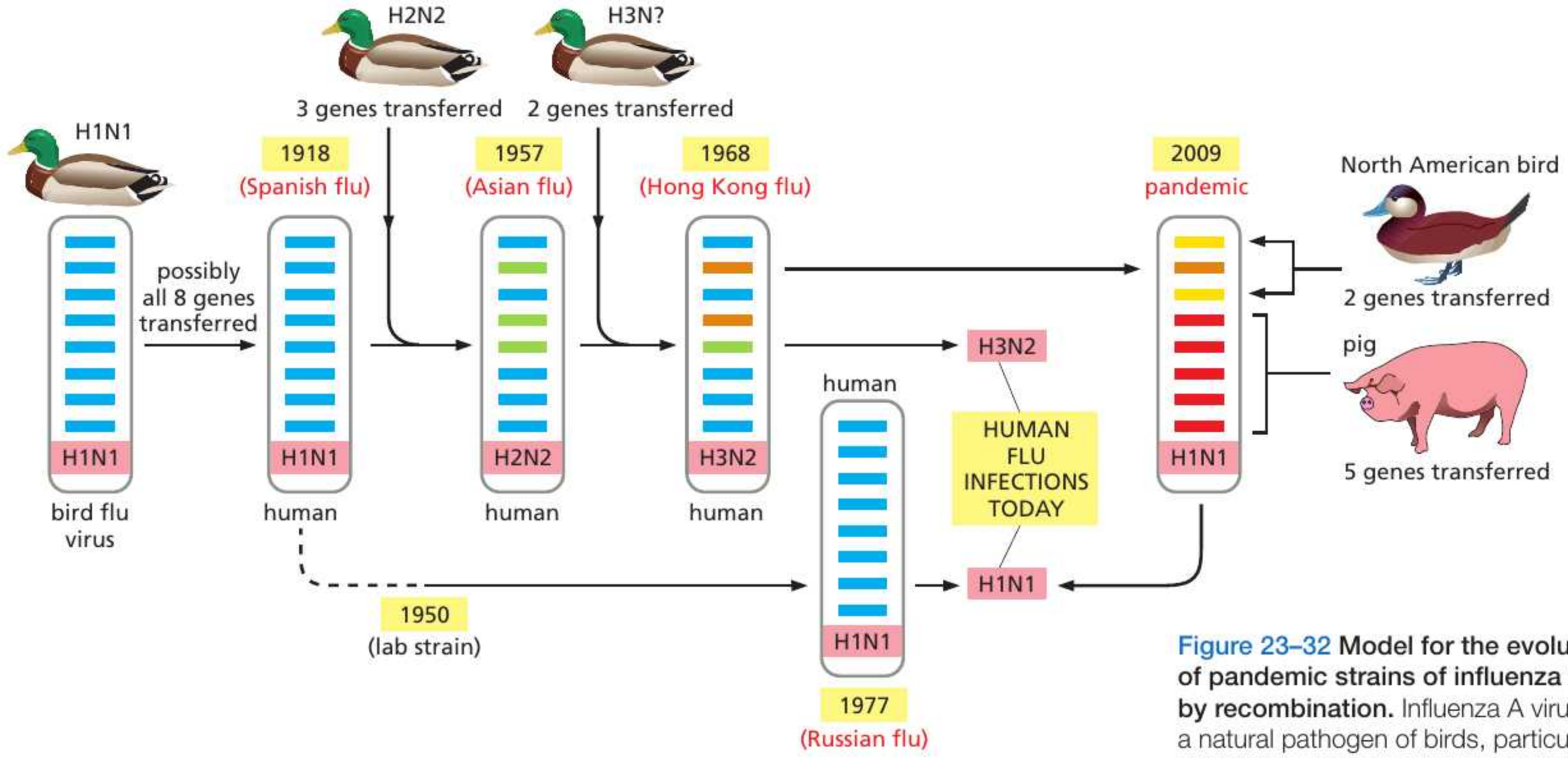


Figure 23–32 Model for the evolution of pandemic strains of influenza virus by recombination. Influenza A virus is a natural pathogen of birds, particularly waterfowl, and it is always present in wild bird populations. In 1918, a particularly virulent form of the virus crossed the species barrier from birds to humans and caused a devastating worldwide epidemic. This strain was designated H1N1, referring to the specific forms of its main antigens, hemagglutinin (H) and neuraminidase (N). Changes in the virus, rendering it less virulent, and the rise of adaptive immunity in the human population, prevented the pandemic from continuing in subsequent seasons, although H1N1 influenza strains continued to cause serious disease every year in very young and very old people. In 1957, a new pandemic arose when three genes were replaced by equivalent genes from an avian virus *(green bars)*; the new strain (designated H2N2) was not effectively cleared by antibodies in people who had previously contracted only H1N1 forms of influenza. In 1968, another pandemic was triggered when two genes were replaced from another avian virus; the new virus was designated H3N2. In 1977, there was a resurgence of H1N1 influenza, which had previously been almost completely replaced by the N2 strains. Molecular sequence information suggests that this minor pandemic may have been caused by an accidental release of an influenza strain that had been held in a laboratory since about 1950. In 2009, a new H1N1 swine virus emerged that had derived five genes from pig influenza viruses, two from avian influenza viruses, and one from a human influenza virus. As indicated, most human influenza today is caused by H1N1 and H3N2 strains.

used to treat infections in humans, just in time to prevent tens of thousands of deaths from infected battlefield wounds in World War II. Because bacteria (see Figure 1–17) are not closely related evolutionarily to the eukaryotes they infect, much of their basic machinery for DNA replication and transcription, RNA translation, and metabolism differs from that of their host. These differences enable us to develop antibacterial drugs that exhibit *selective toxicity*, in that they specifically inhibit these processes in bacteria without disrupting them in the host. Most of the antibiotics that we use to treat bacterial infections are small molecules that inhibit macromolecular synthesis in bacteria by targeting bacterial enzymes that either are distinct from their eukaryotic counterparts or are involved in pathways such as cell wall biosynthesis that are absent in animals (**Figure 23–33** and see Table 6–4).

However, bacteria continuously evolve and strains resistant to antibiotics rapidly develop, often within a few years of the introduction of a new drug. Similar drug resistance also arises rapidly when treating viral infections with antiviral drugs. The virus population in an HIV-infected person treated with the reverse transcriptase inhibitor AZT, for example, will acquire complete resistance to the drug within a few months. The current protocol for treatment of HIV infections involves the simultaneous use of three drugs, which helps to minimize the acquisition of resistance for any one of them.

There are three general strategies by which a pathogen can develop drug resistance: (1) it can alter the molecular target of the drug so that it is no longer sensitive to the drug; (2) it can produce an enzyme that modifies or destroys the drug; or (3) it can prevent the drug's access to the drug target by, for example, actively pumping the drug out of the pathogen (**Figure 23–34**).

Once a pathogen has chanced upon an effective drug-resistance strategy, the newly acquired or mutated genes that confer the resistance are frequently spread throughout the pathogen population by horizontal gene transfer. They may even spread between pathogens of different species. The highly effective but expensive antibiotic *vancomycin*, for example, is used as a treatment of last resort for many severe, hospital-acquired, Gram-positive bacterial infections that are resistant to most other known antibiotics. Vancomycin prevents one step in bacterial cell wall synthesis—the cross-linking of peptidoglycan chains in the bacterial cell wall (see Figure 23–3B). Resistance can arise if the bacterium synthesizes a cell wall using different subunits that do not bind vancomycin. The most effective form of vancomycin resistance depends on the acquisition of a transposon (see Figure 5–60) containing seven genes, the products of which work together to sense the

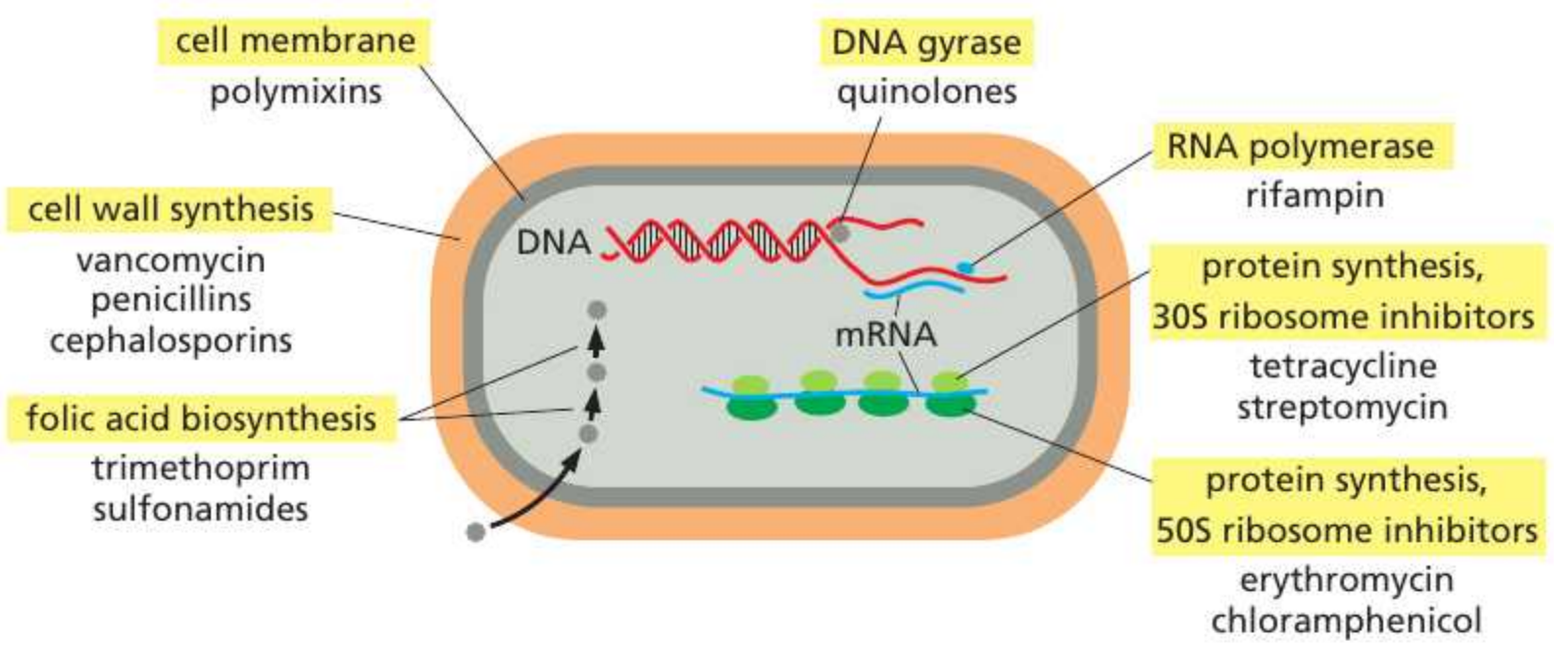


Figure 23–33 Antibiotic targets. Although there are many antibiotics in clinical use, they have a narrow range of targets, which are highlighted in *yellow*. A few representative antibiotics in each class are listed. Nearly all antibiotics used to treat human infections fall into one of these categories. The vast majority inhibit either bacterial protein synthesis or bacterial cell wall synthesis.

presence of vancomycin, shut down the normal pathway for bacterial cell wall synthesis, and produce a different type of cell wall.

Drug-resistance genes acquired by horizontal transfer frequently come from environmental microbial reservoirs. Nearly all antibiotics used to treat bacterial infections today are based on natural products produced by fungi or bacteria. Penicillin, for example, is made by the mold *Penicillium*, and more than 50% of the antibiotics currently used in the clinic are made by Gram-positive bacteria of the genus *Streptomyces*, which reside in the soil. It is believed that microorganisms produce antimicrobial compounds, many of which have probably existed on Earth for hundreds of millions of years, as weapons in their competition with other microorganisms in the environment. Surveys of bacteria taken from soil samples that have never been exposed to antibiotic drugs used in modern medicine reveal that the bacteria are typically already resistant to about seven or eight of the antibiotics widely used in clinical practice. When pathogenic microorganisms are faced with the selective pressure provided by antibiotic treatments, they can apparently draw upon this immense source of genetic material to acquire resistance.

Like most other aspects of infectious disease, human behavior has exacerbated the problem of drug resistance. Many patients take antibiotics for symptoms that are typically caused by viruses (flu-like illnesses, colds, sore throats, and earaches) and these drugs have no effects. Persistent and chronic misuse of antibiotics can eventually result in antibiotic-resistant normal flora, which can then transfer the resistance to pathogens. Antibiotics are also misused in agriculture, where they are commonly employed as food additives to promote the growth and health of farm animals. An antibiotic closely related to vancomycin was commonly added to cattle feed in Europe; the resulting resistance in the normal flora of these animals is widely believed to be one of the original sources for vancomycin-resistant bacteria that now threaten the lives of hospitalized patients.

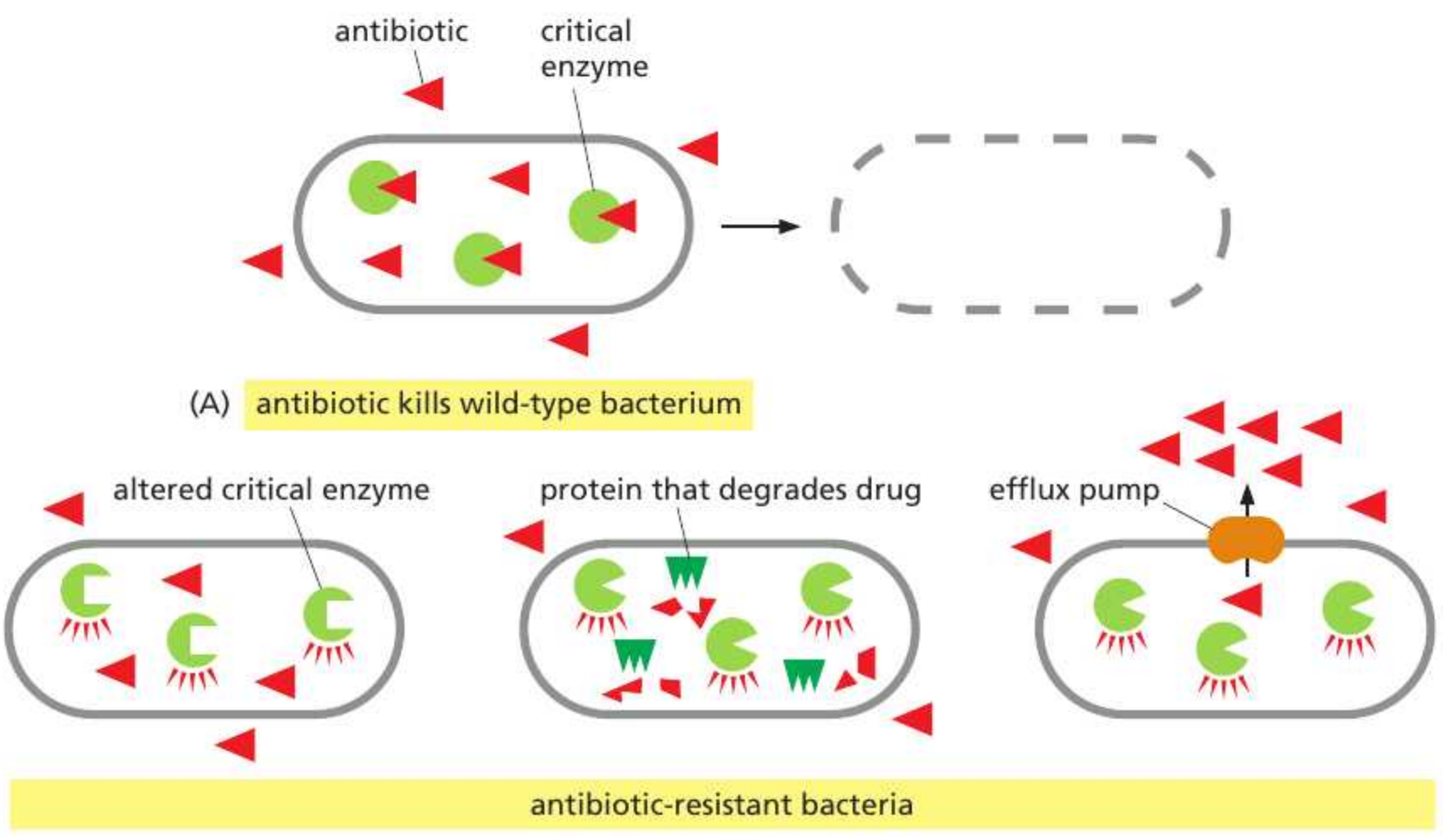


Figure 23–34 Three general mechanisms of antibiotic resistance. (A) A nonresistant wild-type bacterial cell bathed in a drug *(red triangles)* that binds to and inhibits an essential enzyme *(light green)* will be killed due to enzyme inhibition. (B) A bacterium that has altered the drug's target enzyme so that the drug no longer binds to the enzyme will survive and proliferate. In many cases, a single point mutation in the gene encoding the target protein can generate resistance. (C) A bacterium that expresses an enzyme *(dark green)* that either degrades or covalently modifies the drug will survive and proliferate. Some resistant bacteria, for example, make β-lactamase enzymes, which cleave penicillin and similar molecules. (D) A bacterium that expresses or up-regulates an efflux pump that ejects the drug from the bacterial cytoplasm (using energy derived from either ATP hydrolysis or the electrochemical gradient across the bacterial plasma membrane) will survive and proliferate. Some efflux pumps, such as the TetR efflux pump, are specific for a single drug (in this case, tetracycline), whereas others, called multidrug resistance (MDR) efflux pumps, are capable of exporting a wide variety of structurally dissimilar drugs. Upregulation of an MDR pump can render a bacterium resistant to a very large number of different antibiotics in a single step.

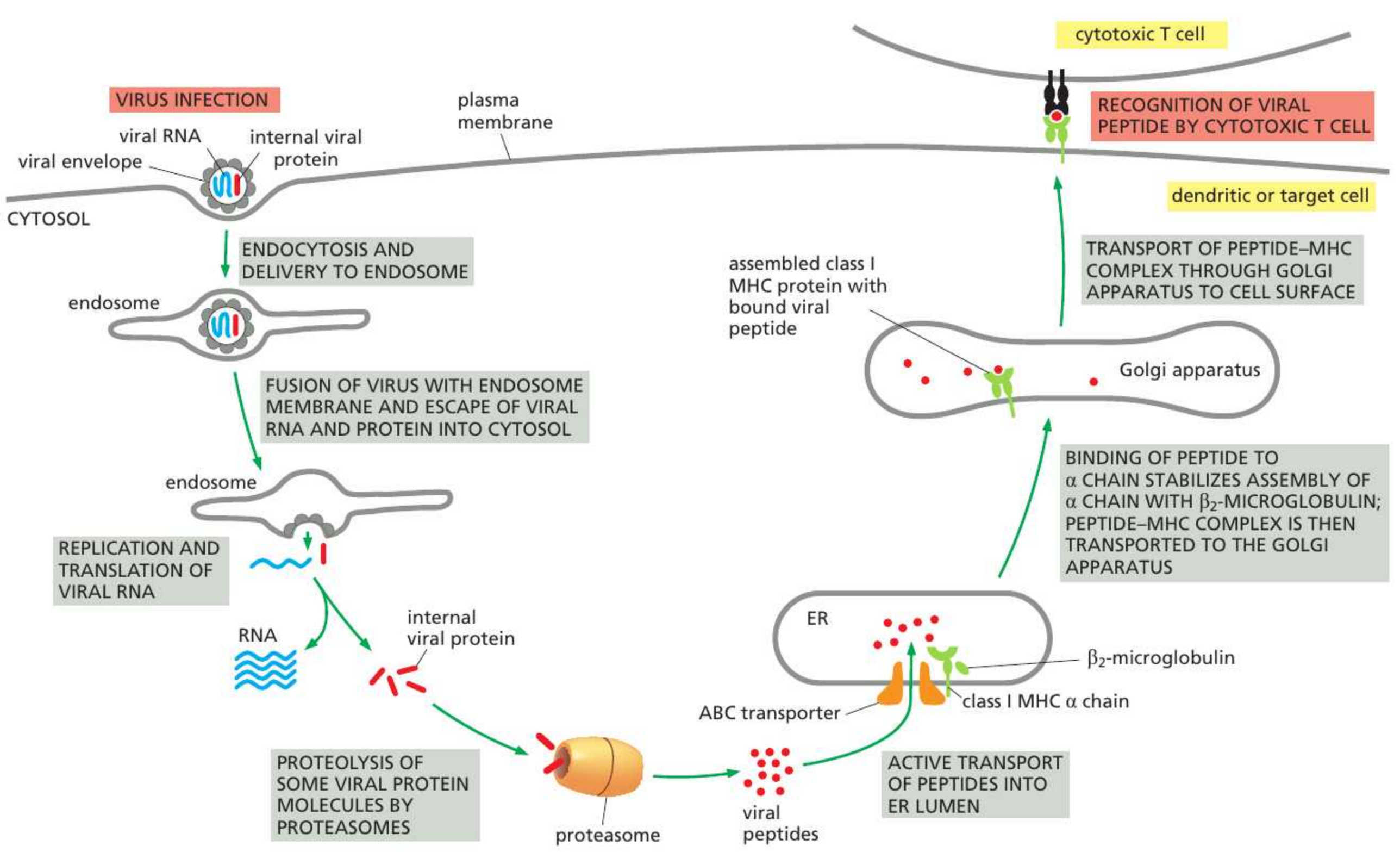
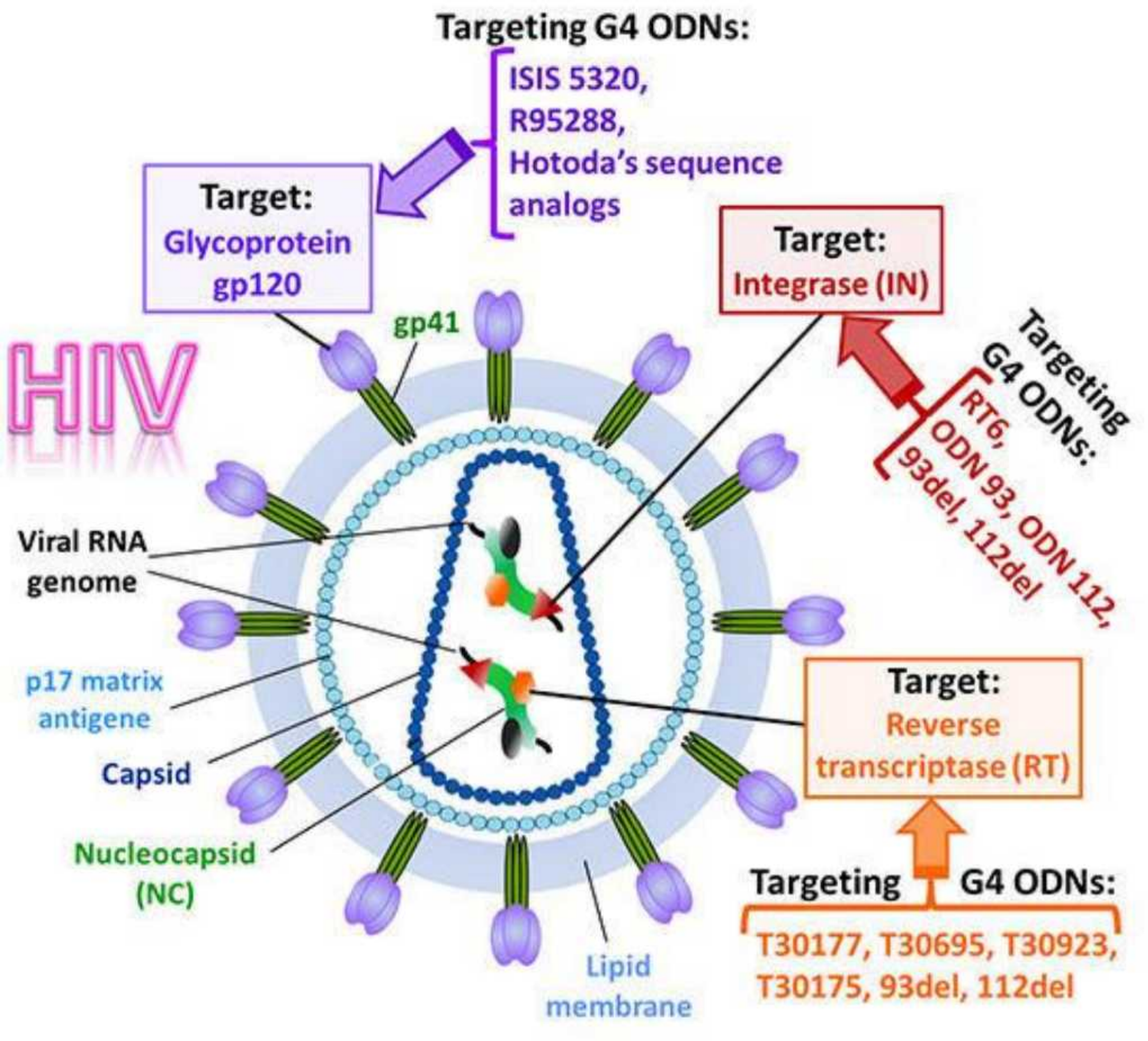


Figure 24–38 The processing of an extracellular foreign protein for presentation to cytotoxic T cells. An effector cytotoxic T cell kills a virus-infected cell when it recognizes fragments of an internal viral protein bound to class I MHC proteins on the surface of the infected cell. Not all viruses enter the cell in the way that this enveloped RNA virus does, but fragments of internal viral proteins always follow the pathway shown. Only a small proportion of the viral proteins synthesized in the cytosol are degraded and transported to the cell surface, but this is sufficient to attract an attack by a cytotoxic T cell. Several chaperone proteins in the ER lumen aid the folding and assembly of class I MHC proteins (not shown). The assembly of class I MHC proteins and their transport to the cell surface require the binding of either a self or foreign peptide (Movie 24.10).


Targeting G4 ODNs:
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R95288,
Hotoda's sequence
analogs
Target:
Glycoprotein
gp120
Target:
Integrase (IN)
Targeting
G4 ODNs:
RT6,
ODN 93, ODN 112,
93del, 112del
HIV
gp41
Viral RNA
genome
p17 matrix
antigene
Capsid
Nucleocapsid
(NC)
Target:
Reverse
transcriptase (RT)
Targeting
G4 ODNs:
T30177, T30695, T30923,
T30175, 93del, 112del
Lipid
membrane