6 The Beginning and End of the Virus Replication Cycle

CHAPTER

Outline of the virus replication cycle

All viruses share the same basic replication cycle, but the time involved depends on a number of factors, including the size and genetic complexity of the virus itself as well as the nature of the host cell. As outlined briefly in Part I, the basic replication process involves the following steps:

1 Virus recognition, attachment, and entry into the cell. Viruses must be able to utilize specific features of the host cell in which they will replicate to introduce their genome into that cell and ensure its being transported by cellular functions to where the virus replication cycle can continue. This requires either inducing the cell to engulf the whole virus particle in some specific way, or in the case of many bacterial viruses, injecting the viral genome into the host cell.

2 Viral gene expression and genome replication. Viral genes must be decoded from nucleic acid and translated into viral protein. This requires generation of mRNA. Different types of genomes obviously will require different mechanisms. One of the functions of viral gene expression is to allow the cell to carry out viral genome replication. It should be clear that the process for DNA viruses is different from that for RNA viruses.

3 Viral capsid formation and virion assembly. At the time that viral genomes are replicated, viral capsid proteins must be present to form virus structures. Often another stage of viral gene expression is required, and virion assembly may require scaffolding proteins (viral proteins that are needed to form the capsid structure, but are not part of the capsid structure). Following the formation of capsids, the virus must be released. Such release would involve an enveloped virus obtaining a membrane envelope.

Within this general pattern there is a wealth of variation and difference in detail. Consider virus

entry: While there is no known instance of a plant virus utilizing a specific cellular receptor for its entry, all animal and bacterial viruses do. The viral entry process for some bacterial viruses is an extremely complex one involving biochemical reactions between components of the virus capsid proteins to achieve injection of the viral genome.

There also is a lot of variation in the details of the virus release step. Here, most variations are seen among viruses being released from eukaryotic cells. In some infections, virus release results in cell death (a *cytocidal* infection). Such cell death might or might not involve cell lysis (**cytolysis**), depending on the virus. An infection leading to cell lysis is termed a cytolytic infection. Other changes to the cell (**cytopathology**) also occur. Cytopathic effects due to viral infection can be used to measure the biological activity of some viruses.

Despite this type of variability, the process of capsid maturation and assembly is generally determined by symmetry of the virus. Thus, icosahedral bacterial viruses mature following steps that are quite similar to those characterized for herpesviruses. Again, helical plant, animal, and bacterial viruses all assemble much in the same way.

Animal virus entry into cells – the role of the cellular receptor

Animal viruses must enter the cell in an appropriate manner through a complex plasma membrane composed of a lipid bilayer in which membrane-associated proteins "float" in the upper or lower surface (Fig. 6.1). Some integral membrane proteins form pores (*channels*) in the membrane for transport of ions and small molecules. Other proteins project from the cell's surface and are modified by the addition of sugar residues (glycosylation). Such **glycoproteins** serve many functions, including mediating immunity, cellular recognition, cell signaling, and cell adhesion.

Virus infection requires interaction between specific proteins on the surface of the virion and specific proteins on the cell's surface. Cell surface proteins are termed *receptors*. It should be kept in mind that the physiological functions of a cell surface protein utilized as a virus receptor really are for purposes other than viral attachment and entry; some identified viral receptors and their known functions are shown in Table 6.1. The term *receptor* is just a way of defining the protein by the effect that is being studied—in this case, entry of a virus into a cell.

The ability of a virus to replicate in specific cells (often termed the **host range** or tissue tropism) is largely determined by the type and distribution of receptors it utilizes. For example, certain surface proteins on $CD4^+$ T lymphocytes that are involved in the immune response are recognized by HIV to allow an infection of these lymphocytes. The virus has evolved to recognize the specific protein and "subvert" its function. Poliovirus utilizes an interaction with a major **intercellular adhesion molecule (ICAM)** in its infection. The slow progression of rabies virus up the neural net into the CNS is accomplished by its use of acetylcholine receptors as its vehicle of entry into neurons. These receptors are concentrated at the synapses between neurons, and thus, the virus can "jump" from neuron to neuron without causing destruction of the neuron. This pattern of progression minimizes tissue damage and inflammation resulting in virus "leakage" into the host's circulatory system with ensuing immune response. Finally, influenza virus and some other respiratory viruses use sialic acid residues to specifically target host cells. Sialic acid residues are enzymatically added as are ubiquitous modifications to the glycoproteins of secretory cells, especially of the nasopharynx and respiratory system.

Tissue tropism for a given virus also is determined in part by whether the receptor is available for recognition by the virus at a specific cell surface. For example, HIV interacts with two proteins found only on a limited subset of cells in the body—certain classes of T lymphocytes and certain neuronal cells. Poliovirus, on the other hand, infects only primates and can attach to and penetrate only specific cells of the small intestine's lining and motor neurons. The poliovirus receptor is not found on nonprimate cells; however, poliovirus-specific ICAMs are present on many primate cells,

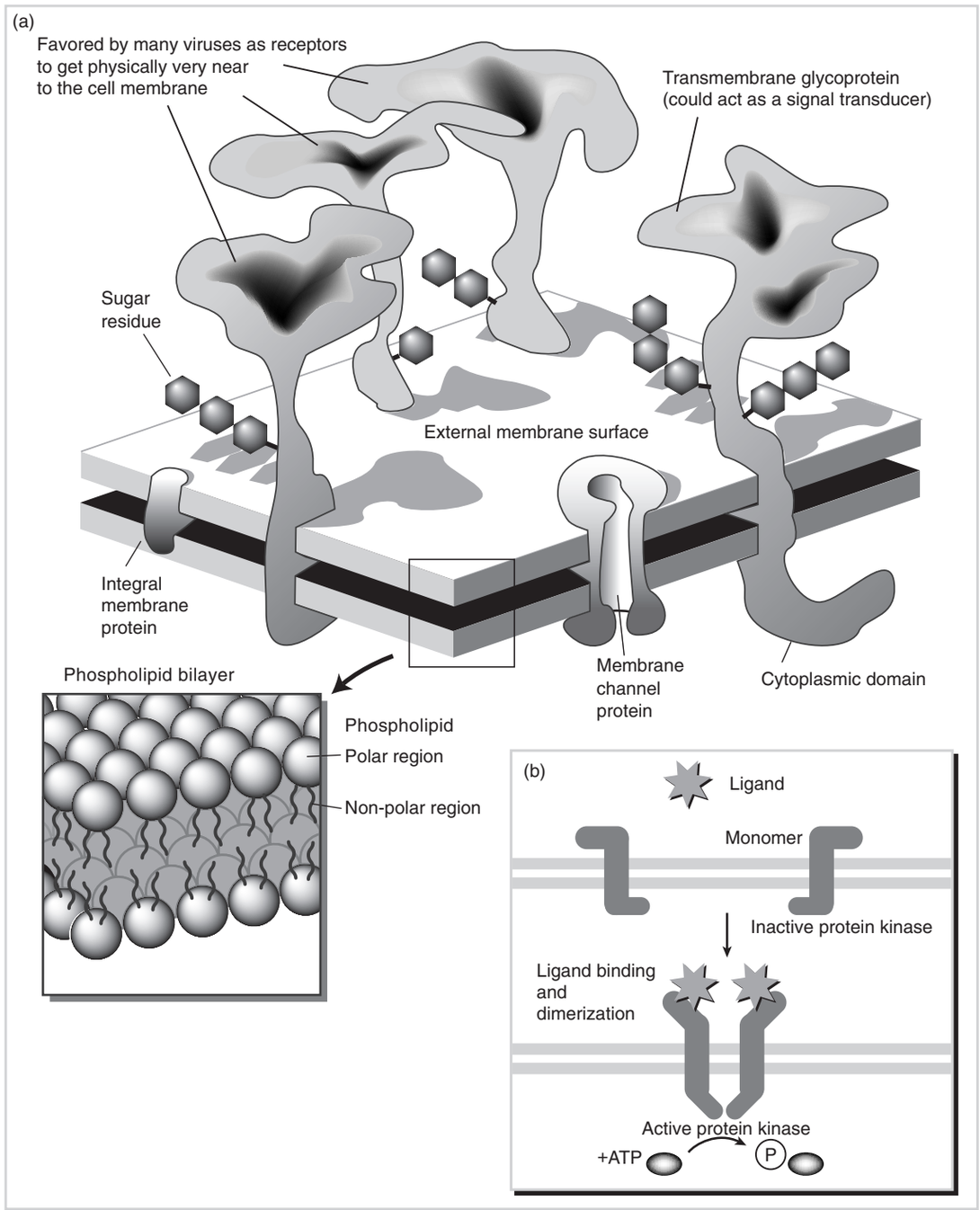

Fig. 6.1 (*a*) The surface of a "typical" animal cell. The lipid bilayer plasma membrane is penetrated by cell surface proteins of various functions. The proteins that extend from the surface (mainly glycoproteins) can be utilized by different viruses as "tether points" or "anchors" for bringing the virus close enough to the cell surface to initiate the entry process. This interaction between a cell surface protein serving as a virus receptor and the virus itself is highly specific between proteins. Integral membrane proteins, such as those mediating transport of small molecules and ions across the plasma membrane, tend not to project as far into the extracellular matrix and are not as commonly utilized by viruses as receptors. Some viral receptors are listed in Table 6.1. (*b*) The interaction between a cellular surface protein (receptor) and a ligand or co-receptor can lead to chemical and structural changes that transmit signals between the exterior and interior of the cell. This is the process of signal transduction. Here, for example, the binding of ligand with two monomeric receptor proteins leads to dimerization, which activates a protein kinase in the cytoplasm. This in turn leads to phosphorylation of a target protein, leading to further changes in the cell.

Table 6.1 Some cellular receptors for selected animal viruses.

Name	Cellular function	Virus receptor for
ICAM-1	Intracellular adhesion	Poliovirus
CD4	T-lymphocyte functional marker	HIV
MHC-I	Antigen presentation	Togavirus, SV40
MHC-II	Antigen presentation/stimulation of B-cell differentiation	Visnavirus (lentivirus)
Fibronectin	Integrin	Echovirus (picornavirus)
Cationic amino acid transporter	Amino acid transport	Murine leukemia virus (oncornavirus)
LDL receptor	Intracellular signaling receptor	Subgroup A avian leukosis virus (oncornavirus)
Acetylcholine receptor	Neuronal impulse transducer	Rabies virus
EGF	Growth factor	Vaccinia virus
CR2/CD21	Complement receptor	Epstein-Barr virus
HVEM	Tumor necrosis factor receptor family	Herpes simplex virus
Sialic acid	Ubiquitous component of extracellular glycosylated proteins	Influenza virus, reovirus, coronavirus

but are apparently "masked" by other membrane proteins on the surface of those cells refractory to infection.

There is another very important factor in entry-mediated tissue tropism in virus infections. Many viruses utilize other proteins on the surface of cells as *co-receptors* in addition to the major receptor. In the case of HIV, an important co-receptor is one of a group of surface proteins that are receptors for proteins known as *chemokines*, which normally function as receptors for chemical messengers passed between cells. There must be a molecular interaction between both the $CD4^+$ receptor and the co-receptor for efficient HIV infection. With HIV, the co-receptor also determines tissue tropism. In addition to CD4, macrophages express a protein, **CCR5**, which allows HIV variants that recognize this protein to show a marked tropism for these cells. Alternatively, T lymphocytes express CD4 and a second HIV co-receptor, **CXCR4**; some strains of HIV show a marked tropism for these cells.

Finally, peripheral mononucleocytes circulating in the blood express co-receptor proteins and can be infected by both HIV strains. Thus, a given virus may utilize a major receptor protein, but require the presence of one or several other proteins in addition. If a certain cell possesses the major receptor but not the co-receptor, infection cannot occur or occurs with impaired efficiency so that cell and tissue tropism are altered.

It is also important to understand that some viruses exhibit alternative methods of initiating infection in a cell neighboring the one initially infected via the receptor-mediated route. For example, infection of a cell may lead to membrane changes that allow fusion with a neighboring cell or cells. Then virus can pass freely into the cytoplasm of the uninfected cell without having to pass the plasma membrane; this is a well-established feature of infections with some strains of HSV that cause the formation of large groups of fused cells or **syncytia**. This and other aspects of virus-induced modifications to the infected cell are discussed in Chapter 10.

The contact between cells allowing virus spread need not be complete fusion. The close interaction between macrophages and other cells of the lymphatic system in induction of the immune response, which is described in Chapter 7, may facilitate the passage of viruses that were taken up but not destroyed by the macrophage. This is clearly an important feature in the pathogenesis of HIV.

The virus itself may possess a surface protein involved in recognition and receptor-mediated entry that is dispensable under certain conditions. An excellent example is the situation with HSV-1 mutants that lack glycoprotein C (gC) on their envelope. As described in somewhat more detail in Chapter 18, this glycoprotein interacts with heparan sulfate on the surface of the cell to allow it to come into close proximity of the ultimate receptor.

Mutant viruses lacking gC demonstrate significant alterations in the details of their infection and pathogenesis in laboratory animals, but they replicate with excellent efficiency in many cultured cells in the laboratory. Here the culturing and frequent passage of the cells lead to alterations in the cell surface so that gC-negative viruses can "find" their receptors with little difficulty.

Viruses may inefficiently utilize other proteins on the surface to infect cells that do not bear the efficient receptor protein. Provided conditions are optimized, these proteins can substitute for the efficient receptor. This substitution is one reason why some viruses can be induced to infect certain cells in culture even though they do not possess the ideal receptor. An example is the ability of SV40 virus to infect inefficiently certain murine and hamster cells in culture.

Such infections can be observed with ease in the laboratory, and there is good suggestive evidence that such atypical infections can occur with some frequency under natural conditions. The emergence of new infectious viruses in the environment is often associated with the appearance of a virus infecting a host previously unaffected by it. The emergence of novel infectious viruses is discussed in Chapter 22.

Some such occurrences can be inferred to result from an "inappropriate" infection followed by the novel virus adapting to utilize a previously unrecognized receptor. A rare "inappropriate" infection of an animal virus into a human with subsequent changes in the genetic properties of the virus was suggested to explain the relatively sudden appearance of HIV in the human community. Another example of such an occurrence may explain the sudden appearance of the novel chicken influenza virus that has apparently adapted to human transmission in Hong Kong in 1997.

Mechanisms of entry of nonenveloped viruses

Nonenveloped virus particles must be incorporated into the cell via a process called **translocation** across the lipid bilayer. This process is one in which the capsid or a cell-modified capsid physically crosses the cell plasma membrane. It involves receptor-mediated **endocytosis** and is illustrated for poliovirus in Fig. 6.2. The acidic environment of the endocytotic vesicle causes specific changes to the poliovirus capsid so that the internal genome (positive-sense RNA) is released into the cytoplasm where it can be translated and begin gene expression.

A nuclear replicating nonenveloped virus, such as SV40 (a papovavirus), begins entry in a similar fashion, but the interaction between viral capsid proteins and the vesicle, along with other **intracellular trafficking proteins**, allows the modified virion to be transported to the nuclear membrane. Once there, the viral genome is released and viral DNA interacts with cellular transcription factors to begin gene expression. Because specific genetic alterations (**mutations**) in the SV40 capsid protein will interfere with this transport, it is known that the process is controlled by the virus.

Entry of enveloped viruses

Enveloped viruses interact with cell receptors via the action of membrane-associated viral glycoproteins that project beyond the viral envelope. The viral glycoproteins are glycosylated with

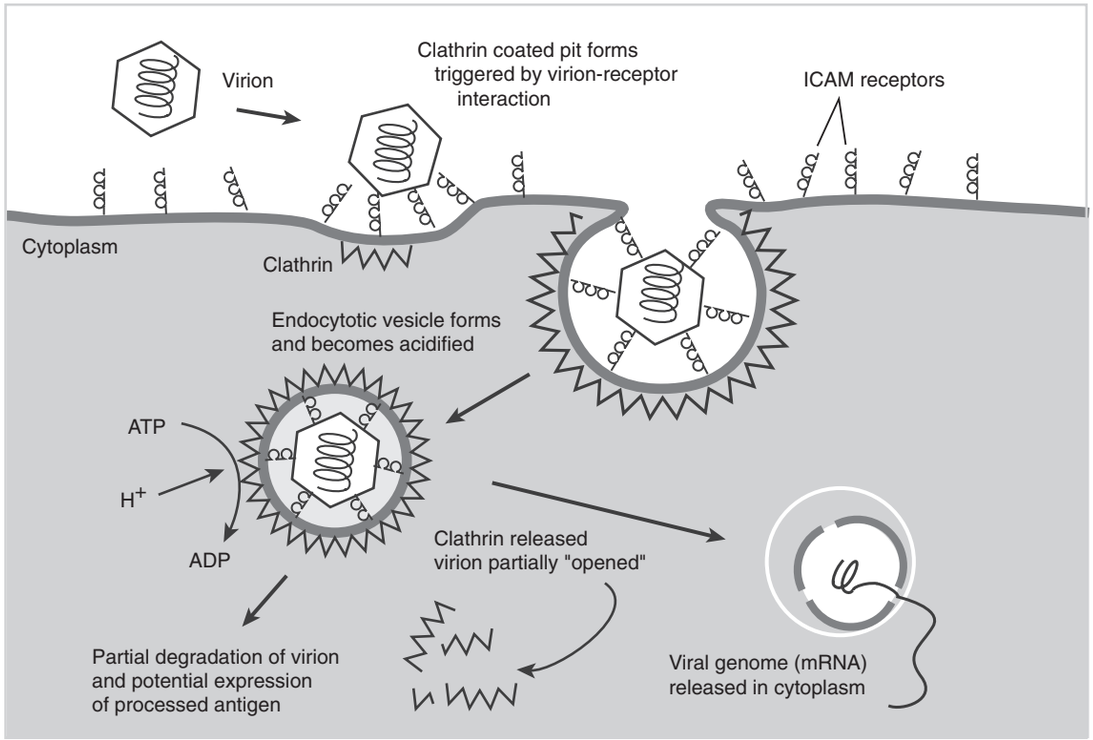

Fig. 6.2 Schematic of receptor-mediated endocytosis utilized by poliovirus for entry into the host cell. The endocytotic vesicle forms as a consequence of close association between the poliovirus-receptor complex and the plasma membrane.

sugars in the cell Golgi apparatus during viral maturation. The process is similar to that carried out by the cell on its own glycoproteins.

Virus entry can involve **fusion** of the viral membrane at the cell's surface, or it can involve receptor-mediated endocytosis. These two processes are shown in schematic form in Fig. 6.3a. As with nonenveloped viruses, the acidic pH of the endocytotic vesicle can lead to modifications of the viral envelope so that fusion between it and the vesicle's membrane can occur. Fusion of the pseudorabies virus (a close relative of HSV) with the plasma membrane of the cultured cells being infected is shown in the electron micrographs of Fig. 6.3b.

The fusion interaction between the viral and plasma or vesicular membrane can be a simple one between one viral glycoprotein and one cellular receptor, or it can be a complex cascade of linked protein interactions. For example, with a herpesvirus such as HSV, five or six viral glycoproteins first bring the virus near the cell, and then allow entry, which requires interaction with a specific cellular surface receptor. The first interaction appears to be an association between viral glycoproteins and sulfated sugar molecules (polyglycans) like heparan sulfate, which is found attached to many surface proteins of the cell. Only then can the virion be brought close enough to the plasma membrane to allow interaction with the actual receptor.

Entry of plant virus into cells

A plant cell's special architecture, namely the presence of a rigid and fairly thick cell wall, presents a unique challenge for virus entry. Initial entry into the plant cell must take advantage of some break in integrity of the cell wall. Apparently, when the virus enters such a break and becomes situated in

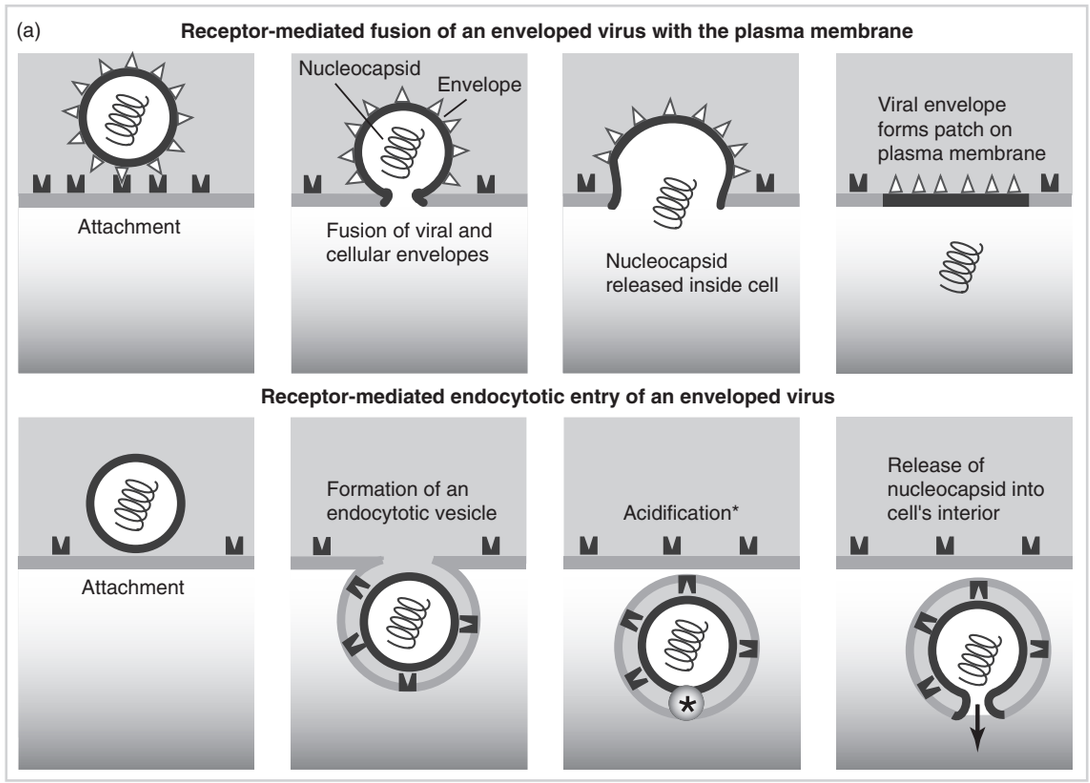

Fig. 6.3 *a.* The two basic modes of entry of an enveloped animal virus into the host cell. Membrane-associated viral glycoproteins either can interact with cellular receptors to initiate a fusion between the viral membrane and the cell plasma membrane, or can induce endocytosis. The fate of the input virus membrane differs in the two processes. *b.* The fusion of pseudorabies virus with the plasma membrane of an infected cultured cell is shown in this series of electron micrographs (the bars represent 150 nm). Although each electron micrograph represents a single event "frozen in time," a logical progression from the initial association between viral envelope glycoproteins and the cellular receptor on the plasma membrane through the fusion event is shown. The final micrograph contains colloidal gold particles bound to antibodies against the viral envelope glycoproteins (dense dots). With them, the envelope can be seen clearly to remain at the surface of the infected cell. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow, H., Weiland, F., Jöns, A., Klupp, B., Karger, A., and Mettenleiter, T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. *Journal of Virology* 1997;71:2072–82.)

close proximity to the plant cell's plasma membrane, it can enter the cell without interaction with specific receptors.

Breaks or lesions in the plant cell's wall are most often produced by organisms that feed on the plant or by mechanical means. Above ground, invertebrates, such as aphids, leafhoppers, white flies, and thrips, are known vectors for a number of plant viruses. Nematodes, which feed on the root system of the plant, are another source of viral infection. In some cases, the virus is transferred from the invertebrate to the plant without growing in the vector. This is the case for *Geminivirus* transmission by white flies. Alternatively, viruses may replicate in both their invertebrate and plant hosts. This is seen with tomato spotted wilt virus (a plant bunyavirus) and its thrip vector. In all cases the viruses gain entry to cell cytoplasm after the insect has begun to feed on plant tissue.

Mechanical damage to the plant's cell wall also can be a means of entry for plant viruses. This approach is used most often in experimental settings when the leaf surface is scratched or abraded prior to inoculation with a virus suspension. This also may happen in nature as a result of agricultural applications, such as harvesting. Brome mosaic virus, transmitted by beetles, can also gain entry into the plant during cutting operations.

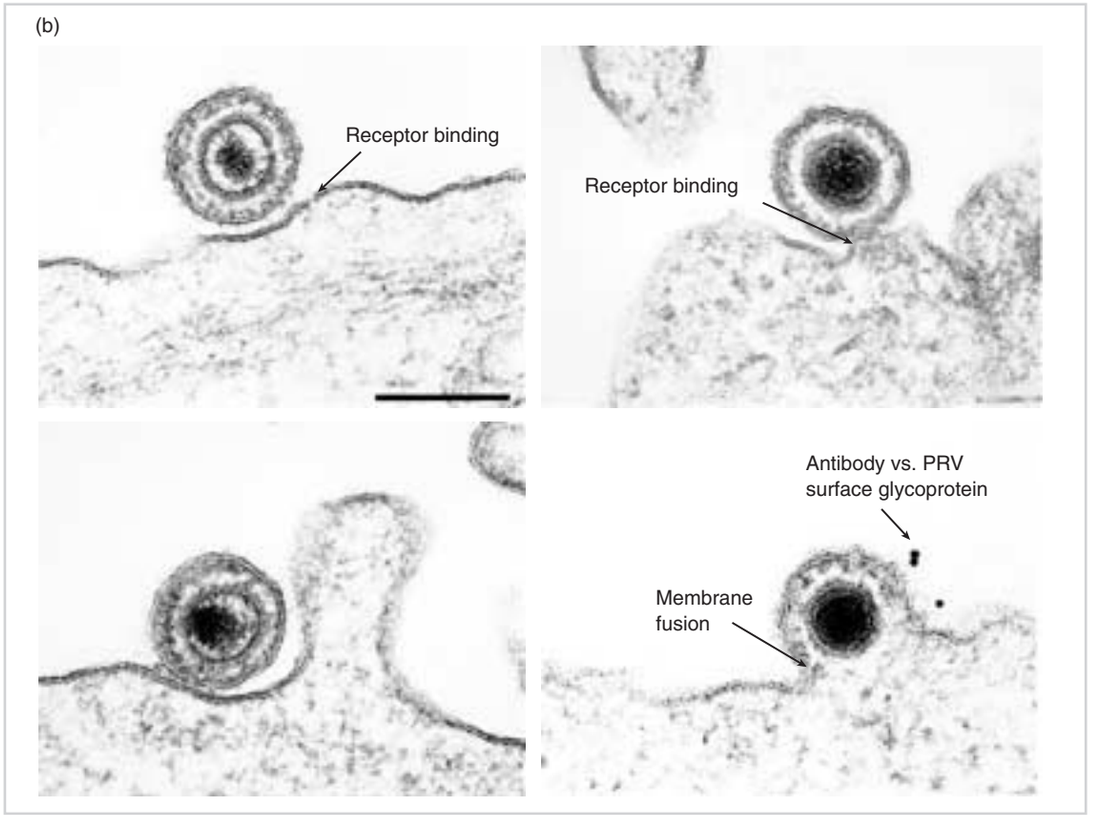

Fig. 6.3 *Continued*

Once inside the plant cell cytoplasm, viruses are uncoated and gene expression begins following patterns similar to those described for animal viruses. Passage of progeny virus from the initial site of infection to new host cells takes place through cell-to-cell connections called **plasmodesmata** and through the plant's circulatory system, the phloem. For this reason, most plant virus infections end up as systemic infections of the whole organism; thus, a single lesion and virus entry can result in virus lesions appearing throughout the plant.

The injection of bacteriophage DNA into *Escherichia coli*

Bacteriophages must interact with a receptor on the bacterial cell surface to successfully initiate replication. The outer surface of a prokaryotic cell presents a set of features to the external environment that includes structural materials (glycoproteins and lipopolysaccharides), transport machinery (amino acid or sugar transport complexes), and cell-to-cell interaction apparatus—the F or **sex pilus**. Sex pili are used by the bacteria in conjugation and exchange of genetic material with other bacteria of the opposite "sex." Attachment of the phage to host cells may employ any one of these structures, depending on the particular virus. Some features utilized by bacteriophages replicating in *Escherichia coli* are shown in Table 6.2.

In some cases, attachment of phage to the host cell involves a physical rearrangement of the virus particle. For example, attachment of bacteriophage T4 to the surface of susceptible *E. coli* cells occurs in two steps, which are shown in Fig. 6.4. First, there is a relatively weak interaction between the tips of the phage tail fibers and lipopolysaccharide residues on the surface of the cell's outer membrane. This triggers a second, stronger, and irreversible interaction. In this, tail pins on the

Table 6.2 Some *E. coli* bacteriophage receptors.

Virus	Structure	Normal function
T2	OmpF	Porin protein
	Lipopolysaccharide	Outer membrane structure
T4	OmpC	Porin protein
	Lipopolysaccharide	Outer membrane structure
T6	Tsx	Nucleoside transport protein
T1 and T5	TonA	Ferrichrome transport
λ	LamB	Maltose transport protein
MS2	F pilus	Conjugation

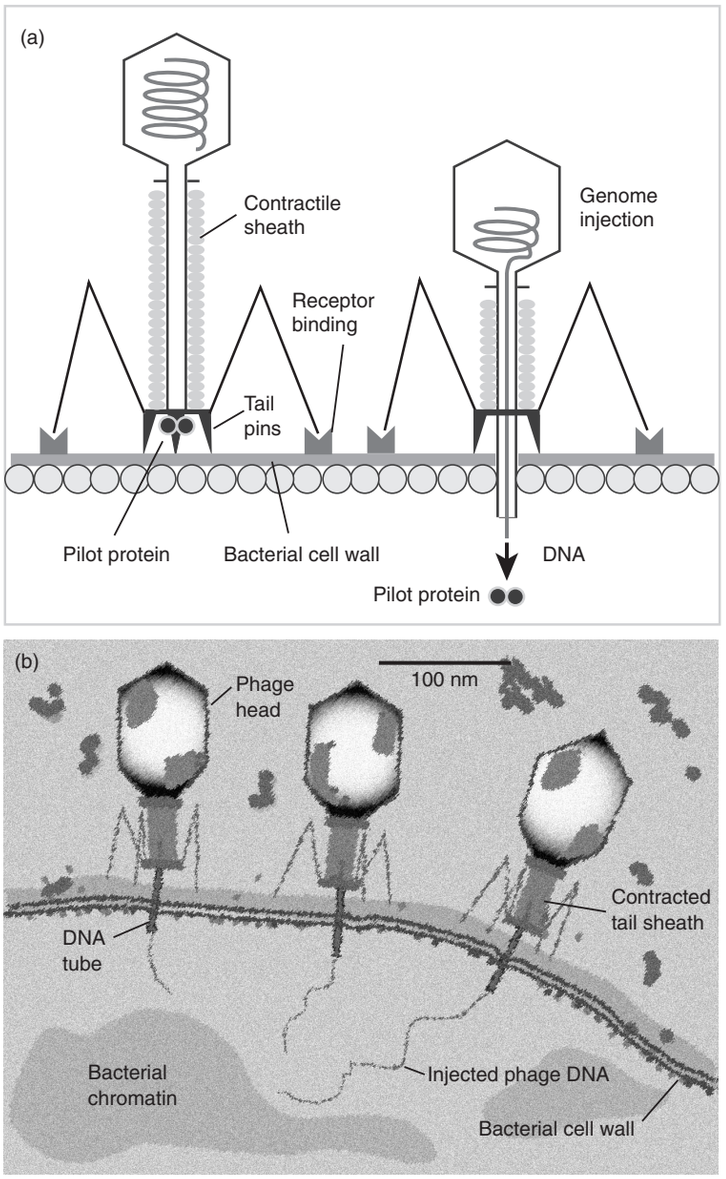

Fig. 6.4 Entry of T4 bacteriophage DNA into an *E. coli* cell. Initial attachment is between the fibers to the ompC lipopolysaccharide receptor on the bacterial cell wall (*a*). The binding of protein pins on the base plate to the cell wall leads to contraction of the tail fibers and sheath proteins, leading to insertion of the tail tube through the cell wall. As shown in the electron micrograph (*b*), phage pilot protein (arrow) allows the highly charged viral DNA genome to penetrate the bacterial plasma membrane and enter the cell. Phage DNA can be seen as shadowy lines emanating from the tail tube. (From Dimmock, N.J., and Primrose, S.B. *Introduction to Modern Virology*, 4th edn. Boston: Blackwell Science, 1994.)

base plate of the virion interact with structures in the outer membrane itself, requiring a change in conformation of the tail fibers. This ultimately results in compression of the phage tail's contractile sheath and injection of phage DNA into the host cell. In this process, the phage tail tube penetrates the cell wall, but phage DNA must still cross the inner cell membrane. This last step is carried out with the help of a viral gene product called a **pilot protein**.

With some other phages, the interaction between virion and cell results in no immediate alterations to the phage structure, for instance, in attachment of bacteriophage λ to LamB. Again, the attachment of MS2 bacteriophage to the F pilus does not result in changes to the virus structure. Since cells with a pilus structure (the product of an F-plasmid) are called *male*, MS2 and similar phages are sometimes termed *male specific*.

The actual amount of bacteriophage that enters the host cell is quite variable. In the case of tailed phage, only phage DNA and certain accessory proteins enter the host cell. For a nontailed phage such as MS2, however, the entire phage particle enters the cell and is uncoated in the cytoplasm.

Nonspecific methods of introducing viral genomes into cells

Clearly, the process of infection of a cell by a virus essentially involves the efficient insertion of the viral genome into an appropriate location within the cell so that viral genes can be expressed. The fact that viruses can be internalized into plant cells without the benefit of receptors suggests that other methods for the introduction of viral genomes can take place, if only rarely. In the laboratory for example, cells can be made permeable by chemical or physical methods so that they can take up quite large particles. Appropriate treatment of cells and addition of high concentrations of virus particles can lead to virus uptake. The process will be inefficient, and most virus particles may be destroyed. Despite this, it is often possible to initiate productive infection in a few cells if enough virus particles are taken up so that an intact viral genome or two can get to the appropriate portion of the cell to initiate infection.

A similarly inefficient and nonspecific process called **transfection** is often used to introduce viral genomes (especially DNA genomes) into cells. Isolated genomes can be aggregated into the proper-sized particles by precipitation into aggregates using calcium phosphate ($Ca_3(PO_4)_2$), and cells can be treated to readily incorporate the aggregates. Alternatively, viral genomes can be concentrated inside lipid vesicles called *liposomes* in solution and these can be readily assimilated by cells that have been specifically treated with mild detergents so that their plasma membrane can fuse with the liposome. Again, although the process is inefficient, if a few viral genomes are presented to the proper intercellular location, productive infection can be initiated.

An example of the use of transfection to examine the properties of a viral protein is illustrated in Fig. 6.5. Here, cells were transfected with a fragment of DNA containing the gene for the varicella-zoster virus (herpes zoster virus) glycoprotein, gL. This gene is controlled by a promoter that can be expressed by transcriptional machinery of the uninfected cell (see Chapter 13). The three micrographs shown in Fig. 6.5b were taken just after, 12 hours after, and 24 hours after transfection. Cells were incubated with a fluorescent antibody against gL (see Chapter 12). The expression of this protein in the cytoplasm is quite evident at the later times.

LATE EVENTS IN VIRAL INFECTION: CAPSID ASSEMBLY AND VIRION RELEASE

Assembly of helical capsids

The capsids of helical viruses must assemble around the genome. This process is relatively well studied in tobacco mosaic virus (TMV) of plants. As noted previously, the basic process appears

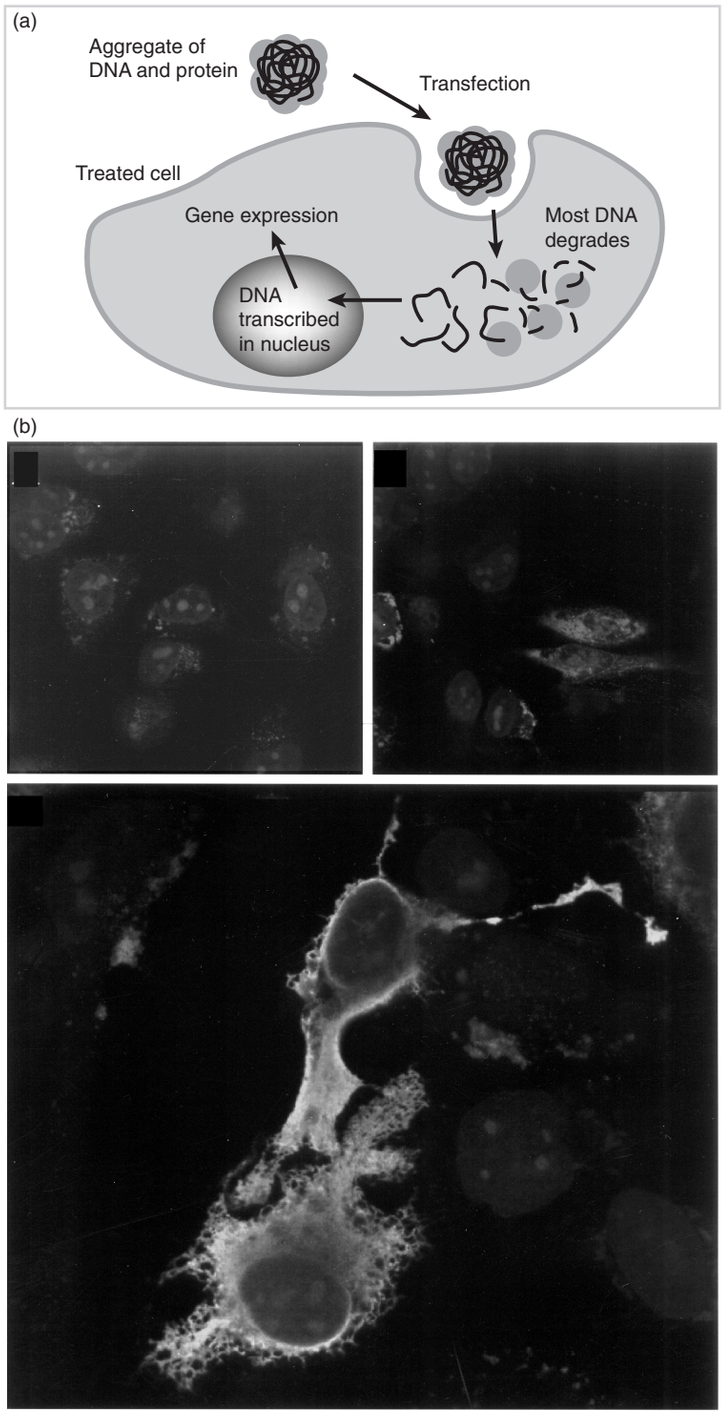

Fig. 6.5 Expression of a varicella-zoster virus protein following transfection of a cell with the viral gene under the control of a promoter that is active in the uninfected cell. *a.* The basic process. The cell membrane is treated with agents that allow it to readily take up large aggregates of protein and nucleic acids by phagocytosis. The transfecting DNA is caused to form aggregates with the use of calcium phosphate ($Ca_3(PO_4)_2$), and then mixed with cells that have been appropriately treated. While most of the DNA taken up by the cell is degraded, some gets to the nucleus by nonspecific cellular transport of macromolecules, and this DNA can be transcribed and any genes within it expressed as proteins. *b.* An actual experiment. Cells were made permeable and then transfected with DNA containing the varicella-zoster virus glycoprotein L gene. The protein encoded in this gene was expressed following its transcription into mRNA (see Chapter 13). Cells were treated with fluorescent antibody reactive with the glycoprotein at (clockwise from the top left) 0, 12, and 24 hours after infection. The expression of the glycoprotein in the cytoplasm is clearly evident from the green fluorescence. (See Chapter 12 for a description of the method.) See Plate 3 for color image. (Photographs courtesy of C. Grose.)

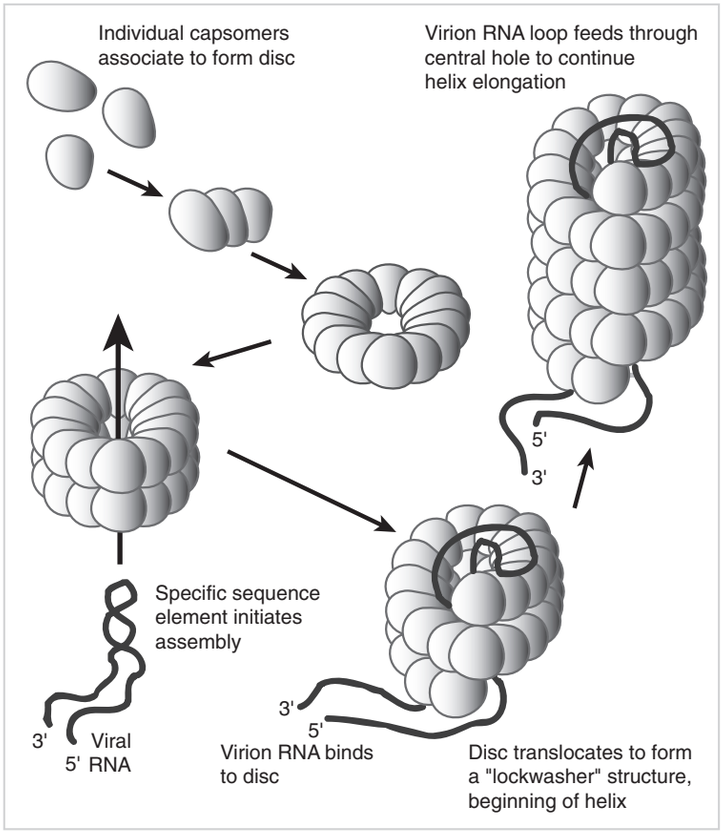

Fig. 6.6 Assembly of the helical tobacco mosaic virus. Steps in the preassembly of the capsomer disk, insertion of viral RNA, and the translational "screwlike" helix assembly process with sequential addition of more capsomers are shown. (Adapted from Dimmock, N.J., and Primrose S.B. *Introduction to Modern Virology*, 4th edn. Boston: Blackwell Science, 1994.)

to be similar for all helical viruses. This similarity depends on the fact that single- or double-stranded RNA (or DNA, for that matter) can readily form a helical structure when associated with the proper type of protein.

The assembly of the helical capsid and RNA genome of TMV is shown in Fig. 6.6. Capsomers self-assemble to form disks, and the disks formed by the capsomers initially interact with a specific sequence in the genome called *pac* (for **packaging signal**). Interaction with the RNA itself converts the disk into a "lockwasher" conformation, and subsequent capsomer assemblies then thread onto the growing helical array to form the complete capsid. Note that, for TMV, the RNA forms the equivalent of a "screw," which penetrates the disk assembly of capsomers. This penetration allows translocation to a helical arrangement that grows by continued association with the genomic RNA.

Assembly of icosahedral capsids

In the majority of cases studied in detail, maturation of the icosahedral capsid from an immature *procapsid* to final state involves specific proteolytic cleavage of one or several capsid proteins that were assembled into the immature virus particle. This cleavage results in subtle changes in structure or increased capsid stability, and often accompanies inclusion of the viral genome. These cleavage steps are quite limited. Only one or a few discrete peptide bonds are hydrolyzed, and the process is accomplished by virus-encoded proteins called *maturational* proteases. Thus, a fairly good general rule has the assembly of icosahedral capsids involving both preassembly of procapsids and specific covalent modifications of the virion proteins by proteolytic processing. The high specificity of maturational proteases and the fact that they are encoded by the viral genome make them attractive tar-

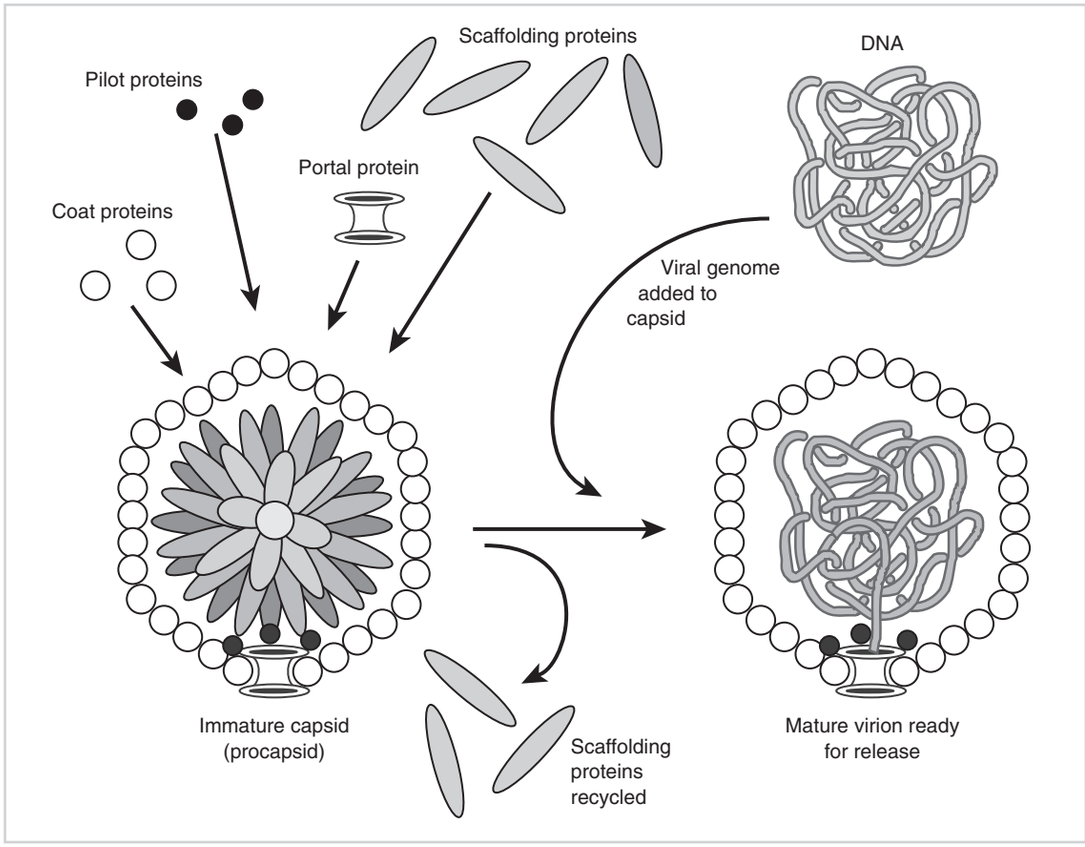

Fig. 6.7 Assembly of the phage P22 capsid and maturation by insertion of viral genomic DNA. Individual capsomer subunits preassemble into a procapsid around scaffolding protein. This latter protein is recycled with phage P22 but can be proteolytically removed with a maturational protease with other icosahedral viruses. The empty head then associates with viral genomes. Genome insertion requires both energy and a conformational change in the procapsid.

gets for antiviral therapy; protease inhibitors of HIV have been found to have great therapeutic value (see Chapter 8).

Some of the general models for assembly of an icosahedral capsid were based on early studies on poliovirus, a small RNA-containing virus. One characteristic of poliovirus infection in the laboratory is the formation of empty capsids. Thus, it is clear that the viral capsomers can self-assemble. This observation was interpreted as indicating that empty capsids assemble *before* the genome enters the virion. Ironically, some recent studies on the assembly of poliovirus and related viruses suggested that the procapsid assembles directly around the viral RNA, and empty capsids are a nonfunctional by-product of the assembly process. Despite this, empty capsids can form a stable structure spontaneously.

With larger icosahedral viruses, the process of capsid assembly is complex, with scaffolding proteins forming a "mold" or pattern for the final capsid. In either case, capsid assembly occurs *before* entry of the viral genome into the capsid, and one of the hallmarks of icosahedral virus maturation is the generation of empty capsids.

Assembly of the head of bacteriophage P22 is shown in Fig. 6.7 as an illustration of this process. The process is quite similar to the assembly of herpesvirus capsids. Note, the pilot proteins, which

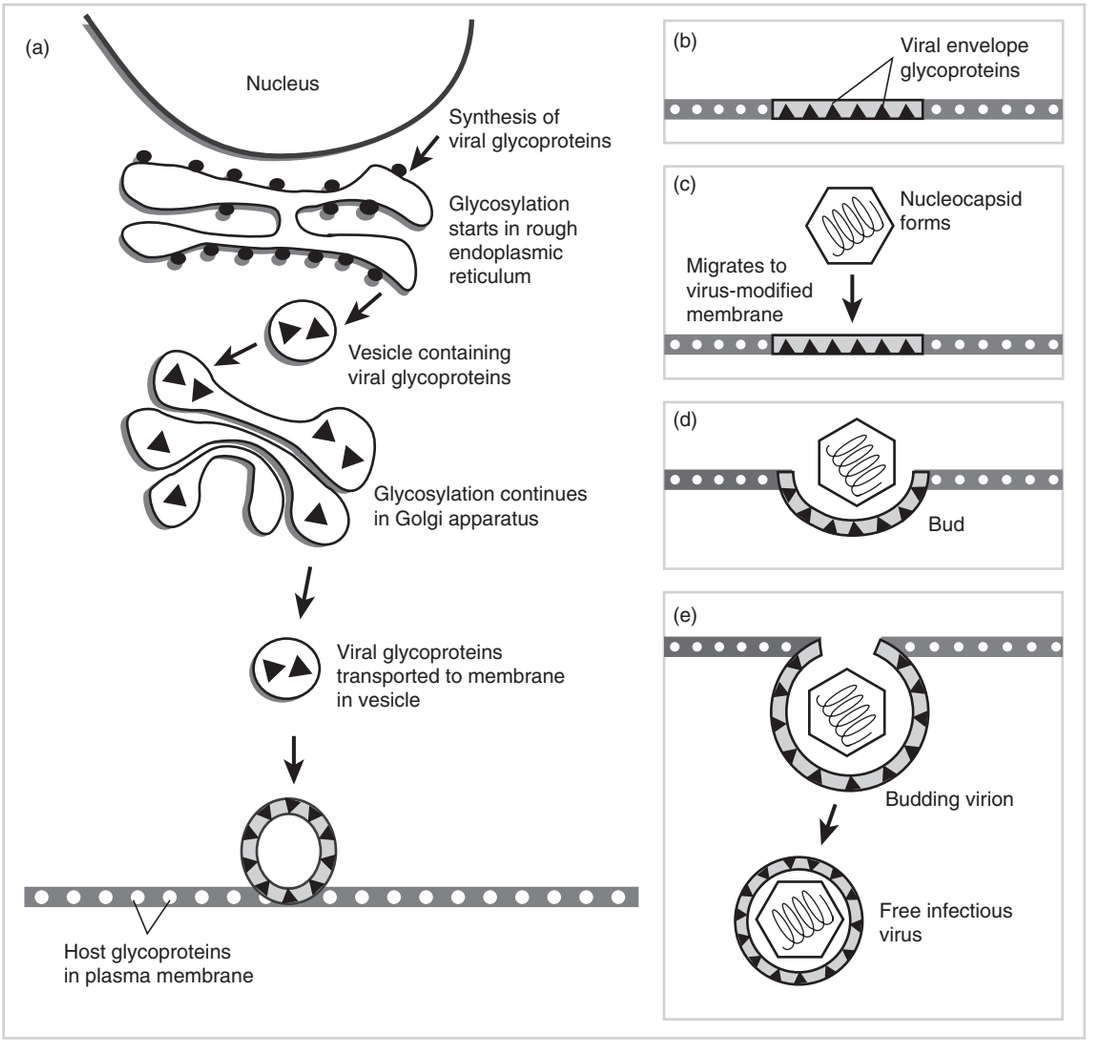

Fig. 6.8 Insertion of glycoproteins into the cell's membrane structures and formation of the viral envelope. The formation of viral glycoproteins on the rough endoplasmic reticulum parallels that of cellular glycoproteins except that viral mRNA is translated. (*a*). Full glycosylation takes place in the Golgi bodies, and viral glycoproteins are incorporated into transport vesicles for movement to the cell membrane where they are inserted (*b*). At the same time (*c*), viral capsids assemble and then associate with virus-modified membranes. This can involve the interaction with virus-encoded matrix proteins that serve as "adapters." Budding takes place (*d*, *e*) as a function of the interaction between viral capsid and matrix proteins and the modified cellular envelope containing viral glycoproteins.

are important for injection of the genome (see Fig. 6.4), may also help the capsid proteins assemble. The scaffolding proteins can recycle and function in the assembly of more than one capsid. Also note that the term *pilot protein* here has a completely different meaning than when used in the T-even bacteriophage infection discussed previously.

Retrovirus proteases "activate" virion-associated enzymes during the final stages of virion maturation following release from the infected cell. These retrovirus proteases form part of the virion's structural protein. Antiviral drugs targeting the HIV protease have shown significant therapeutic benefit, and other viral proteases are targets for drug development because they are specific to the virus encoding them. This is discussed in more detail in Chapter 20.

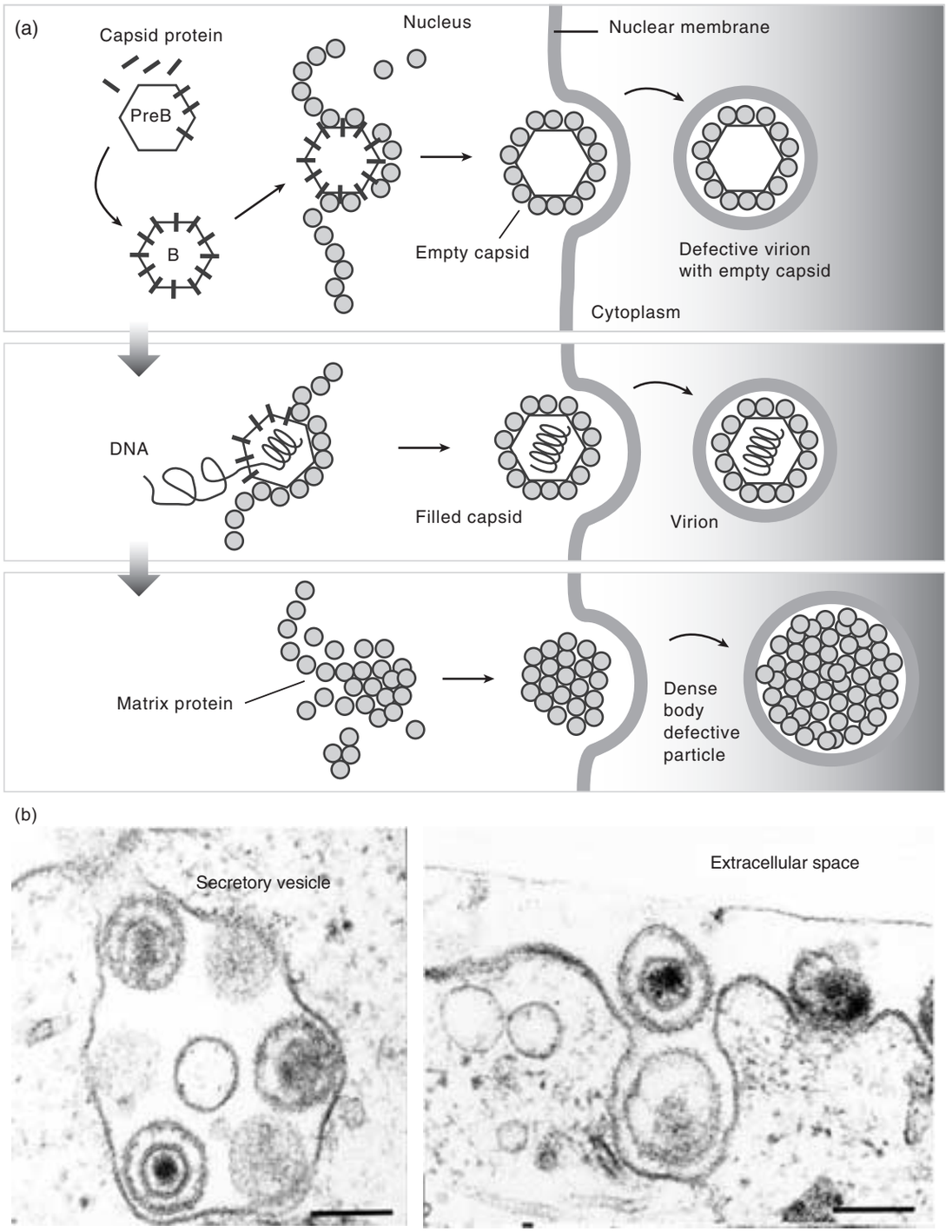

Fig. 6.9 The envelopment and egress of herpesvirus. *a.* A schematic representation of the nuclear envelopment step for human cytomegalovirus (HCMV). Viral capsids form in a manner similar to that shown in Fig. 6.6, and virus-modified nuclear membranes are generated as described in Fig. 6.7. Matrix (tegument) protein serves as the adapter or "glue" to allow the association between capsid and virus-modified envelope, and mature virions bud through the nuclear membrane into the infected cell's cytoplasm. The process is inefficient and empty capsids can be enveloped. Further, matrix protein can aggregate and itself become enveloped, forming "dense bodies." (Based on a drawing by D.W. Gibson.) *b.* Electron micrographs of exocytosis of pseudorabies virus in the cytoplasm of the infected cell; release of enveloped virions is clearly shown. The bars represent 150 nm. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow, H., Weiland, F., Jöns, A., Klupp, B., Karger, A., and Mettenleiter, T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. *Journal of Virology* 1997;71:2072–82.)

Generation of the virion envelope and egress of the enveloped virion

The lipid bilayer of the membrane envelope of the viruses that bear them is derived from the infected cell. Few (if any) viral genes directed toward lipid biosynthesis or membrane assembly are yet identified. While the lipid bilayer is entirely cellular, the envelope is made virus specific by the insertion of one or several virus-encoded membrane proteins that are synthesized during the replication cycle.

Some of the patterns of envelopment at the plasma membrane for viruses that assemble in the cytoplasm are shown in Fig. 6.8. Viral glycoproteins, originally synthesized at the rough **endoplasmic reticulum** and then processed through the **Golgi apparatus**, arrive at the site of budding with their carboxy termini in the cytoplasm and their amino termini on the outside of the cell.

For viruses budding at other subcellular locations (such as the bunyaviruses, which bud into the Golgi itself; or herpesviruses, which bud from the nuclear membrane and then into **exocytotic vesicles**), a similar arrangement occurs. In each case, the viral glycoproteins contain trafficking signals that direct the protein to its destination, using host cell machinery for this purpose. The plasma membrane of many cells in organized tissue is asymmetrical, and some viruses have evolved to utilize this asymmetry. Thus, certain viruses (e.g., influenza viruses) bud from the **apical surface** of such cells while others (e.g., vesicular stomatitis virus) bud from the **basolateral surface**. Using elegant recombinant DNA techniques to produce hybrid versions of the relevant proteins, the trafficking signals in these cases were shown to reside in the amino terminal portion of the viral glycoprotein.

Specific details of envelope formation and virion release are complex for nuclear replicating enveloped viruses exemplified by the herpesviruses. As outlined earlier, capsid formation takes place in the nucleus and full capsids presumably associate with **tegument** (matrix) proteins near the nuclear membrane that has become modified by inclusion of viral glycoproteins glycosylated in the cellular Golgi apparatus. The envelopment of HCMV is outlined in Fig. 6.9a. Empty capsids also can be enveloped, and some enveloped particles contain no capsids at all, just matrix proteins—these are often termed *dense bodies*. Since neither of these **defective virus particles** contains a genome, neither can initiate a normal infection. A fuller discussion of defective virus particles is presented in Chapter 14.

Two models have been proposed for what happens to HSV following its envelopment at the nuclear membrane. Either the virion retains its nuclear membrane upon egress, or this nuclear membrane is lost by fusion with the endoplasmic reticulum prior to exocytosis. This latter model posits that the virus reacquires an envelope from the trans-Golgi network on the plasma membrane. While experimental evidence has been variously interpreted to support either one or the other model, recently Mettenleiter and colleagues have provided persuasive evidence that the second model; i.e., double envelopment, is the correct one. Viral capsids bud into the lumen between the inner and outer nuclear membranes, and then enter the cytoplasm through fusion with the outer nuclear membrane. Subsequently, the capsids acquire their mature envelope by budding into exocytotic vesicles, and enveloped virus is transported to the cell surface for release. The process is very elegantly shown in the electron micrographs of the exocytosis of pseudorabies virus included in Fig. 6.9b. This process will be described in a bit more detail in Chapter 19, where herpesvirus replication is detailed.

QUESTIONS FOR CHAPTER 6

1 Briefly describe the two modes that enveloped viruses use for entry into their host cells.

2 How do nonenveloped viruses enter their host cells? Describe in detail one example.

3 How do plant viruses enter their host cells? What feature of the plant cell's architecture dictates these modes of entry?

4 Describe how the T-even bacteriophage attaches and enters the host cells. Which part of the virus particle enters the cell?

5 Simple virus capsids are found in two types of structural arrangements: helical and icosahedral. What are the key features in the assembly of these two kinds of particles?

6 How do enveloped viruses acquire their membranes during their maturation in animal cells?