Attachment and entry of viruses into cells

Overview Of Virus Replication

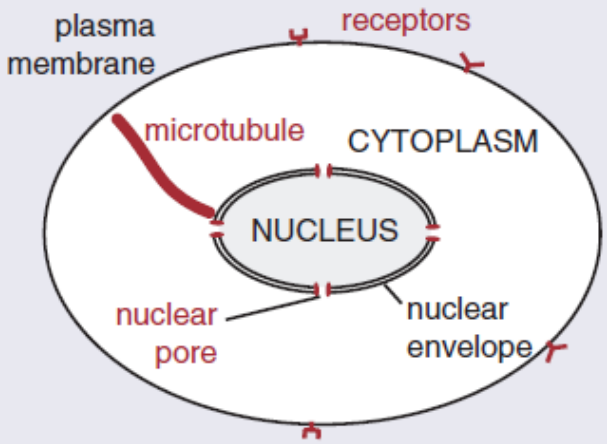
1. Attachment
2. Entry
3. Transcription
4. Translation
5. Genome replication
6. Assembly
7. Exit

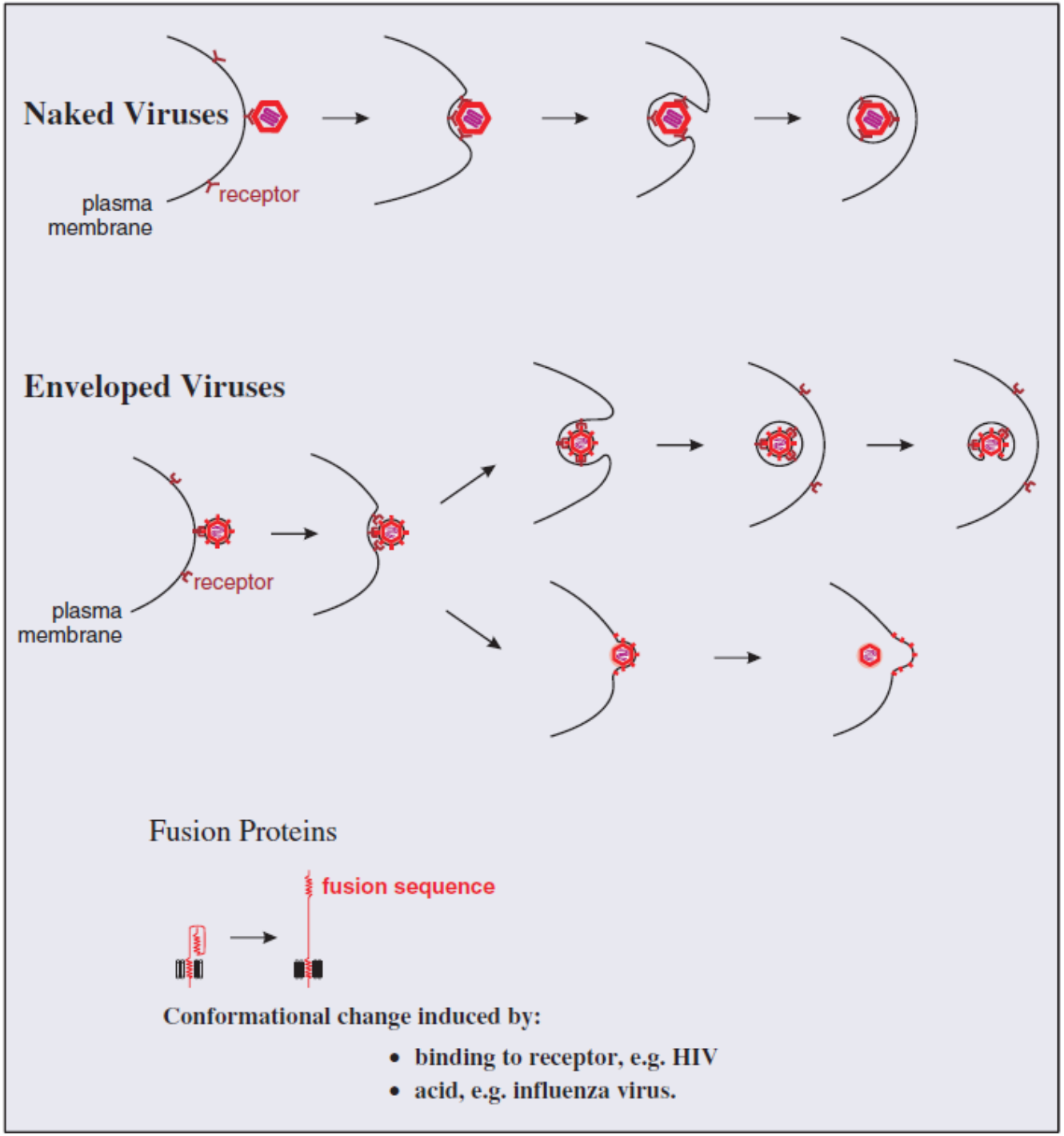
Animal Viruses

All animal viruses must cross the **plasma membrane**.

Some are transported in the cytoplasm via **microtubules**.

Some must cross the **nuclear envelope**, usually via a **nuclear pore**.




Naked Viruses
plasma membrane
receptor
Enveloped Viruses
plasma membrane
receptor
Fusion Proteins
fusion sequence
Conformational change induced by:
• binding to receptor, e.g. HIV
• acid, e.g. influenza virus.

The aim of a virus is to replicate itself, and in order to achieve this aim it needs to enter a host cell, make copies of itself and get the new copies out of the cell. In general the process of virus replication can be broken down into seven steps:

1. Attachment of a virion to a cell

2. Entry into the cell

3. Transcription of virus genes into messenger RNA molecules (mRNAs)

4. Translation of virus mRNAs into virus proteins

5. Genome replication

6. Assembly of proteins and genomes into virions.

7. Exit of the virions from the cell.

The first letter from each step gives the acronym AETTGAE, which may provide a memory aid.

The first step, attachment of a virion to a cell, applies to viruses that infect animal and bacterial hosts. Before these viruses can cross the bounding membrane or wall of the host cell they must first bind to specific molecules on the cell surface. Most plant viruses, on the other hand, are delivered directly into a cell by a vector

Cell receptors and co-receptors

A virion attaches via one or more of its surface proteins to specific molecules on the surface of a host cell. These cellular molecules are known as receptors and the recognition of a receptor by a virion is highly specific, like a key fitting in its lock. It has been found that some viruses need to bind to a second type of cell surface molecule (a co-receptor) in order to infect a cell.

Receptors and co-receptors are cell surface molecules, usually glycoproteins, with a wide range of functions that include

- **acting as receptors for chemokines and growth factors;**
- **mediating cell-to-cell contact and adhesion.**

Virus attachment sites

Each virion has multiple sites that can bind to receptors, and each site is made up of regions of one or more protein molecules.

The virus attachment sites of naked viruses are on the capsid surface, sometimes within depressions (e.g. poliovirus) and sometimes on ridges (e.g. foot and mouth disease virus).

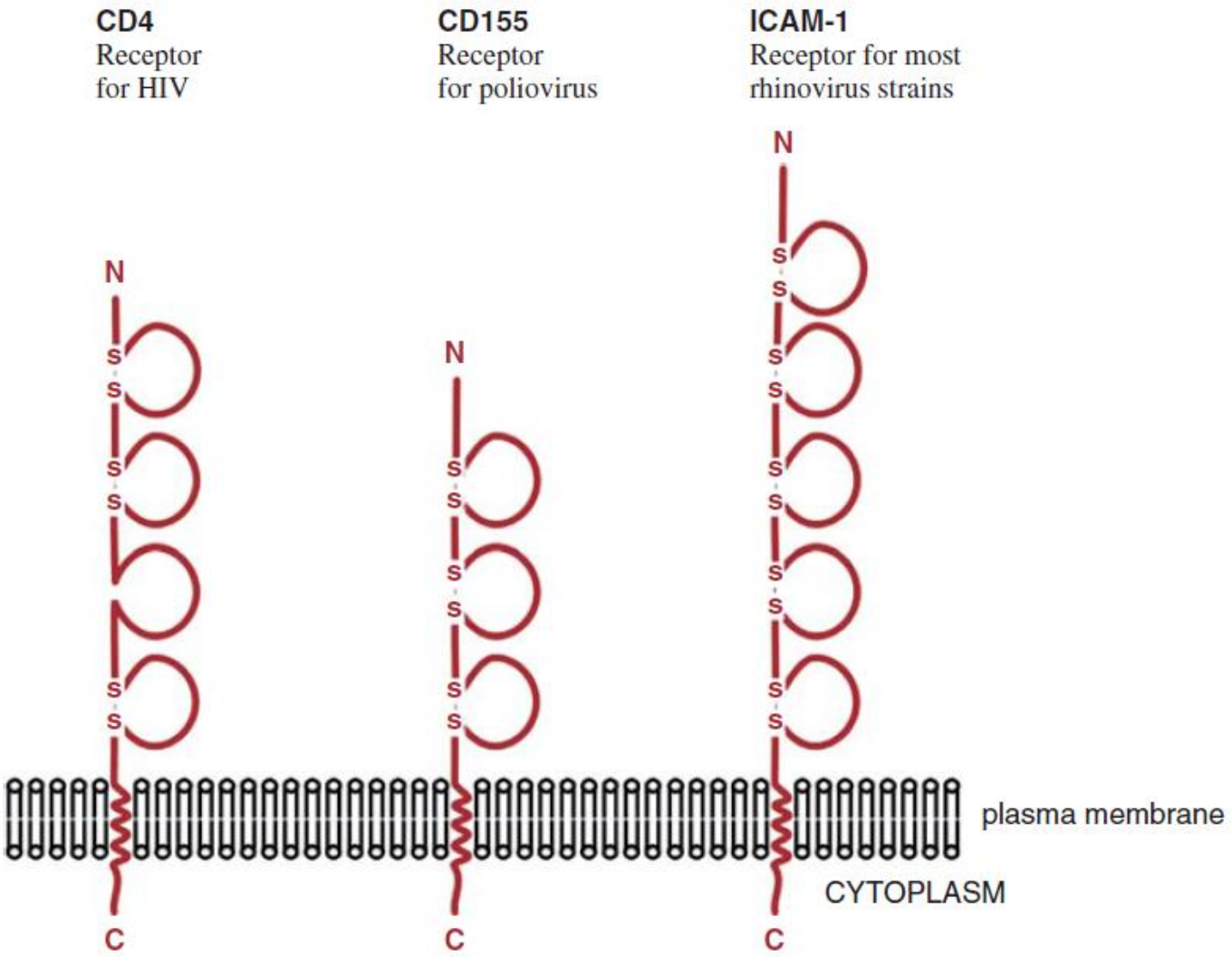


Figure 5.1 *Cell receptors with immunoglobulin-like domains.* Each loop indicates an immunoglobulin-like domain. Most of the domains are stabilized by one or two disulphide (–S–S–) bonds. In each receptor the virus binding site is located in the outermost domain.

Table 5.1 Examples of cell receptors, virus proteins involved in attachment, and (for enveloped viruses) fusion proteins

Virus	Cell receptor	Virus protein(s) involved in	
		attachment to receptor	fusion
Naked viruses			
Approx. 90% of human rhinoviruses	Intercellular adhesion molecule-1 (ICAM-1)	VP1 + VP3	
Approx. 10% of human rhinoviruses	Low-density lipoprotein receptors	VP1	
Poliovirus	CD155	VP1	
Enveloped viruses			
Murine leukaemia viruses	Mouse cationic amino acid transporter	SU (surface glycoprotein)	TM (transmembrane glycoprotein)
HIV-1	CD4	gp120	gp41
Influenza viruses A & B	Sialic-acid-containing glycoproteins	Haemagglutinin	Haemagglutinin
Measles virus	Signalling lymphocyte activation molecule (CD150)	Haemagglutinin	Fusion

The virus attachment sites of some naked viruses are on specialized structures, such as the fibers and knobs of adenoviruses and the spikes of rotaviruses, while the virus attachment sites of enveloped viruses are on the surface glycoproteins.

Some virion surface proteins that bear the virus attachment sites are able to bind strongly to red blood cells of various species and cause them to clump, a phenomenon known as haemagglutination. The proteins responsible for haemagglutination are called haemagglutinins. Examples of viruses that can haemagglutinate are influenza viruses and measles virus.

Attachment of virions to receptors

The forces that bind a virus attachment site to a receptor include hydrogen bonds, ionic attractions and van der Waals forces. Sugar moieties, when present on the receptor and/or on the virus attachment site, are commonly involved in the forces that bind the two.

No covalent bonds are formed between virions and receptors. Initially, a virion is weakly bound to a cell at only one or a few receptors. At this stage the attachment is reversible and the virion may detach, but if it remains attached there are opportunities for more virus attachment sites to bind to more receptors, and if sufficient bind then the attachment to the cell becomes irreversible.

Entry of animal viruses into cells

After binding to receptors animal viruses must cross the plasma membrane to gain entry to the host cell. They may do this either at the cell surface or they may cross the membrane of an endosome, which is a vesicle formed by part of the plasma membrane pinching off into the cytoplasm. This process (endocytosis) is used by cells for a variety of functions, including nutrient uptake and defense against pathogens.

An endosome may fuse with other vesicles such as lysosomes, which have a pH of 4.8–5.0, thus lowering the pH within the vesicle. The pH may be further lowered by a process that pumps hydrogen ions across the membrane. This acidification of the environment of the virion is important for those enveloped viruses that need to carry out acid-triggered fusion of the envelope with the vesicle membrane.

Entry of naked viruses

It is possible that some naked viruses deliver their genomes into their host cells through a pore formed in the plasma membrane, but for most naked viruses irreversible attachment of the virion to the cell surface leads to endocytosis.

The plasma membrane 'flows' around the virion, more receptors bind, and eventually the virion is completely enclosed in membrane, which pinches off as an endosome (figure 5.2).

Entry of enveloped viruses

Reversible attachment of an enveloped virion may lead to irreversible attachment, as for naked viruses. There are then two processes whereby infection of the cell may occur: either fusion of the virion envelope with the plasma membrane, or endocytosis followed by fusion of the virion envelope with the endosome membrane (Figure 5.3).

Both processes involve the fusion of the virion envelope with a cell membrane, either the plasma membrane or a vesicle membrane. Lipid bilayers do not fuse spontaneously and each enveloped virus has a specialized glycoprotein responsible for membrane fusion (Figure 5.4).

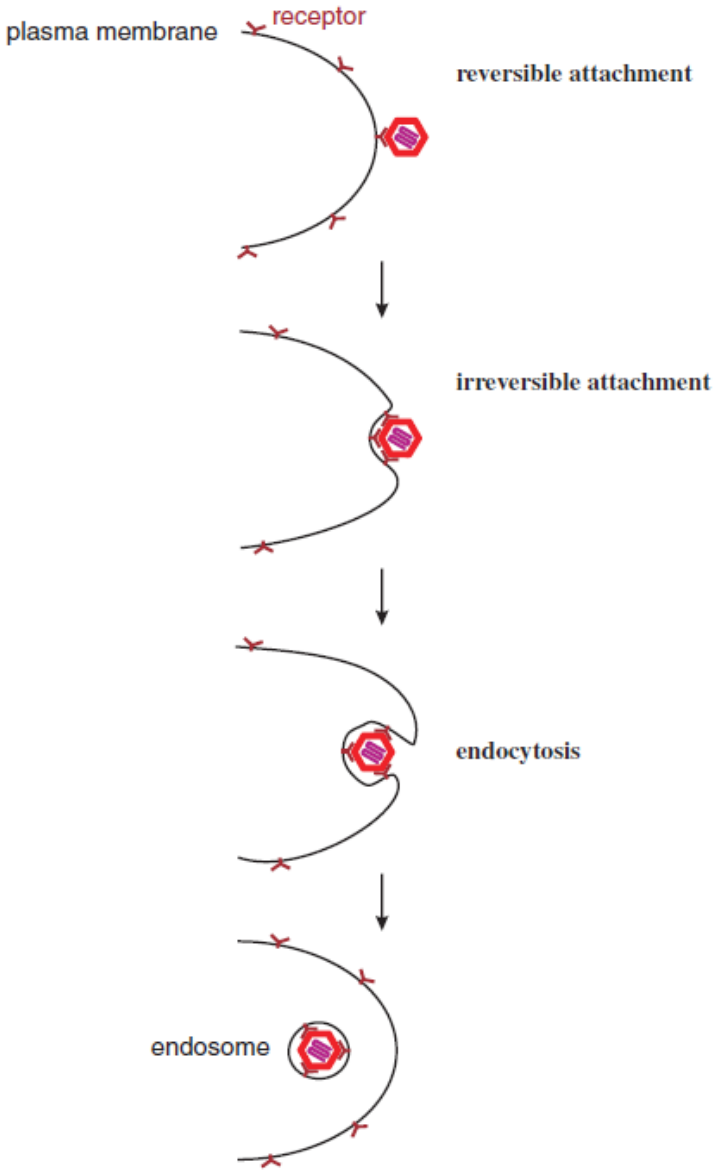


Figure 5.2 *Attachment and entry of a naked virion.* As more receptors bind to the virion its attachment becomes irreversible. The virion is taken into an endosome, the membrane of which is formed by pinching off from the plasma membrane.

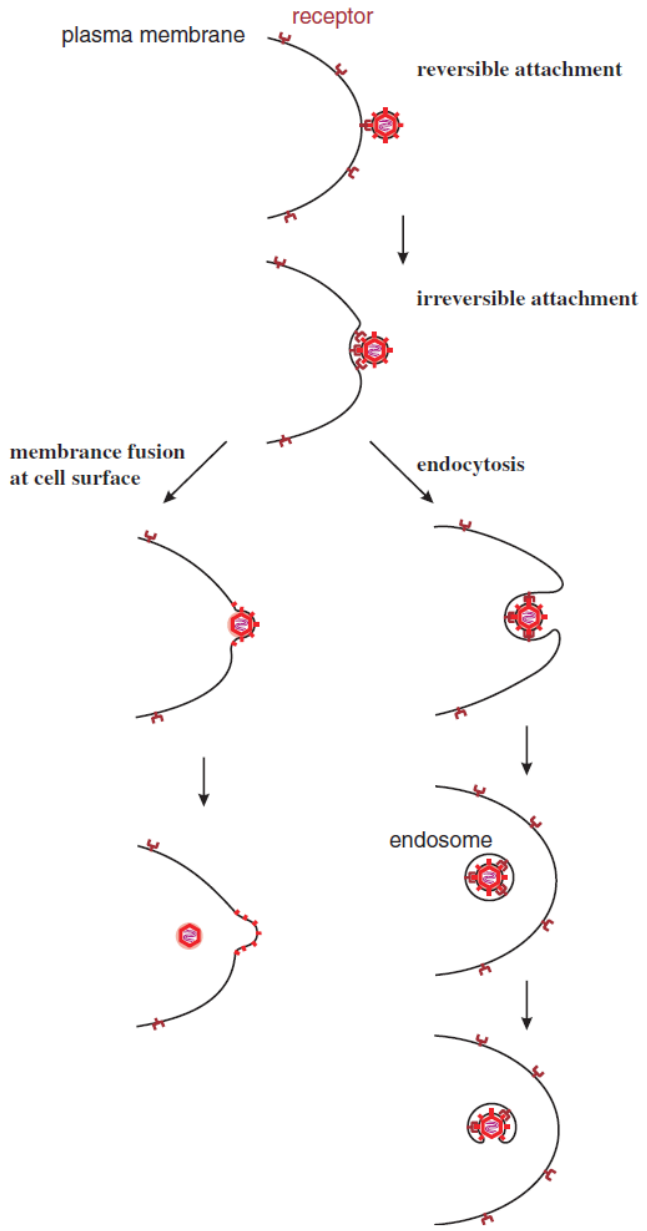


Figure 5.3 *Attachment and entry of an enveloped virion.* Some enveloped viruses can enter a host cell both by membrane fusion at the cell surface and by endocytosis. Others can enter only by endocytosis.

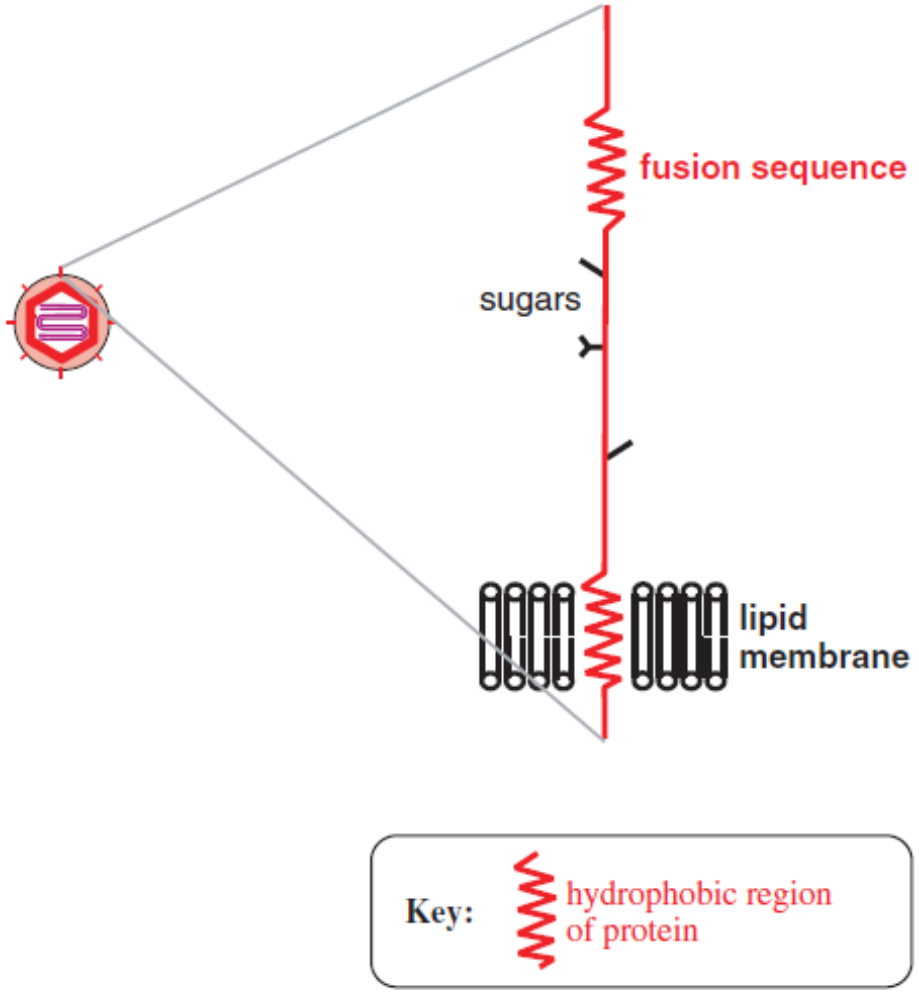


Figure 5.4 *Characteristics of fusion proteins of enveloped viruses.* Fusion proteins are envelope glycoproteins that have an external hydrophobic sequence that can be inserted into the target membrane.

Many fusion proteins are synthesized as part of a larger protein, which is cleaved after synthesis. The cleavage products remain linked, either through non-covalent bonds (e.g. retroviruses) or through a disulphide bond (e.g. influenza viruses), and are found in the virion envelope as dimers or trimers, depending on the protein. Each monomer has at least two hydrophobic sequences: a transmembrane sequence and a fusion sequence that is responsible for membrane fusion by inserting into a cell membrane.

The fusion sequence normally lies hidden and must become exposed in order for fusion to take place. Conformational changes to the fusion protein that expose the fusion sequence may come about either as a result of the virus binding to a receptor or they may be induced by the low pH within an endosome. If the fusion sequence becomes exposed while the virion is at the cell surface, then infection could occur either by fusion with the plasma membrane or by endocytosis. If, however, exposure to low pH is required to expose the fusion sequence, then endocytosis is the only option. There are therefore two categories of membrane fusion:

- **pH-independent fusion, e.g. herpesviruses, HIV**
- **acid-triggered fusion, e.g. influenza viruses, rhabdoviruses.**

Once the fusion sequence is exposed it can insert into the target membrane (plasma membrane or endosome membrane). A further conformational change in the fusion protein pulls the two membranes together and then mediates their fusion. This process involves a release of energy from the fusion protein, which irreversibly changes shape.

Intracellular transport

Once in the cell the virus, or at least its genome, may have to be delivered to a particular location, such as the nucleus. For some viruses the destination is reached using one of the transport systems of the cell, such as the microtubules.

Most RNA viruses of eukaryotes replicate in the cytoplasm; the majority encode all the enzymes for replication of their genomes and they have no requirement for the enzymes of the nucleus. The influenza viruses, however, are exceptions as they require the cell splicing machinery, so their genomes must be delivered into the nucleus.

Retroviruses too are RNA viruses that replicate their genomes in the nucleus. They copy their genomes to DNA in the cytoplasm, then most retroviruses must wait in the cytoplasm until mitosis begins. During mitosis the nuclear envelope is temporarily broken down and the virus DNA (with associated proteins) is able to enter the nuclear compartment. These viruses therefore can replicate only in cells that are dividing. The DNA (with associated proteins) of a group of retroviruses, however, can be transported into an intact nucleus. This group (the lentiviruses, which includes HIV) can therefore replicate in non-dividing cells.

Some DNA viruses, such as iridoviruses and poxviruses, replicate in the cytoplasm of eukaryotic cells, but most DNA viruses replicate in the nucleus. For these viruses (and the influenza viruses and the lentiviruses) the virus genome must be transported to the nuclear envelope and then across it. The structural proteins of some of these viruses have sequences that allow them to attach to microtubules.

Microtubules are components of the cytoskeleton, providing support for various components of the cell and acting as tracks for the transport of materials, such as certain organelles, to particular sites in the cell. Microtubules are hollow cylinders, 25 nm in diameter, and are composed of the proteins α- and β-tubulin. The ends of each microtubule are designated as plus and minus. In most animal cells the plus ends are located near the plasma membrane, while the minus ends are attached to a structure called the centrosome (also referred to as the microtubule organizing center) near the nucleus (Figure 5.5).

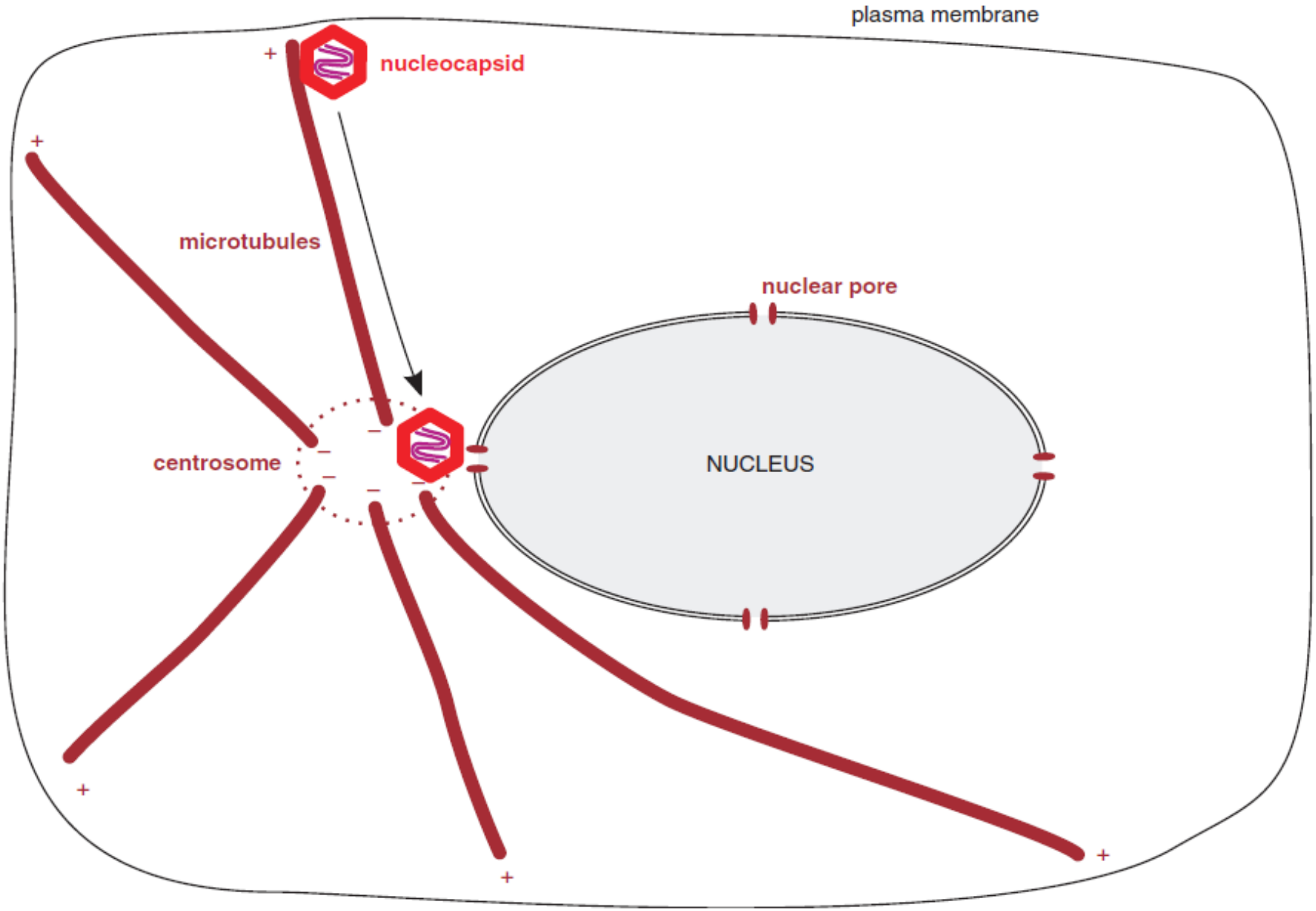


Figure 5.5 *Transport of a nucleocapsid along a microtubule.* The nucleocapsid is transported from the plus end of a microtubule near the plasma membrane to the centrosome, which is located close to the nucleus.

Genome uncoating

Uncoating can be defined as the complete or partial removal of the capsid to release the virus genome. Depending on the virus, the process can take place

- **at the cell surface, the capsid remaining on the exterior surface of the cell;**
- **within the cytoplasm;**
- **at a nuclear pore;**
- **within the nucleus.**

It should be noted that successful entry of a virion into a cell is not always followed by virus replication. The host's intracellular defences, such as lysosomal enzymes, may inactivate infectivity before or after uncoating. In some cases the virus genome may initiate a latent infection rather than a complete replication cycle.

Entry of bacteriophages into cells

Like animal viruses, phages bind specifically to cell surface molecules that function as receptors and coreceptors. For many phages these molecules are on the surface of the host cell wall, but for some phages they are on the surface of other structures (pili, flagella or capsules).

Many of the virus attachment sites are on particular virion structures, such as the tail fibres of phage T4. As with animal viruses, the attachment of a phage to its host is initially reversible, and becomes irreversible after attachment to further receptors and/or co-receptors.

Infection of a cell with a phage involves entry into the cell of the virus genome and perhaps a few associated proteins, the capsid and any associated appendages remaining at the cell surface. This is in contrast to most animal and plant viruses, where the entire virion, or at least the nucleocapsid, enters the host cell. Unless the host is a mycoplasma (mycoplasmas do not have cell walls), delivery of a phage genome into a cell requires penetration of a cell wall and perhaps also a slime layer or capsule; the virions of many phages carry enzymes, such as lysozymes, to aid this process.