INTRODUCTORY BIOLOGY AND MICROBIOLOGY

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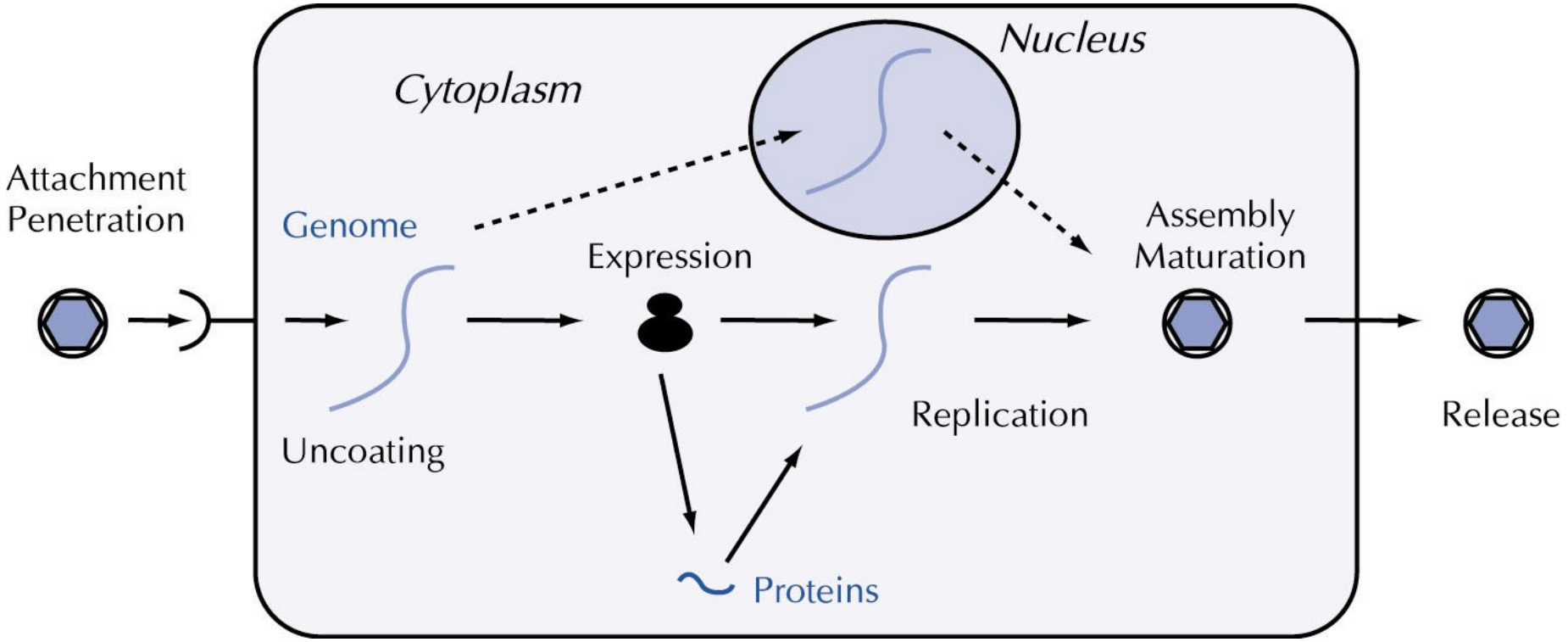
Viral Replication Cycle

Introduction

- Replication (reproduction) is divided into 3 phases namely:
  - Phases 1 - Initiation of infection which involves adsorption of virions; penetration or uptake of viral particles;
  - Phase 2 - Replication and expression of the virus genome: replication of viral nucleic acid and synthesis of viral proteins

- Phase 3 - Release of virions from the infected cell - Assembly of viral capsids and release of mature viruses.

The Replication Cycle



- Adsorption of virus onto host cell occurs through random collision of the virion with a potential host.
- Viruses have reactive sites on their surface which interact with specific **receptors** on suitable host cells.

Adsorption of virions

- This is usually a passive reaction (not requiring energy) though demonstrates tropism i.e. organism and tissue specific.
- The specificity of the reaction between viral protein and host receptor defines and limits the host species as well as the type of cell that is infected.

Virus-Receptor Interactions

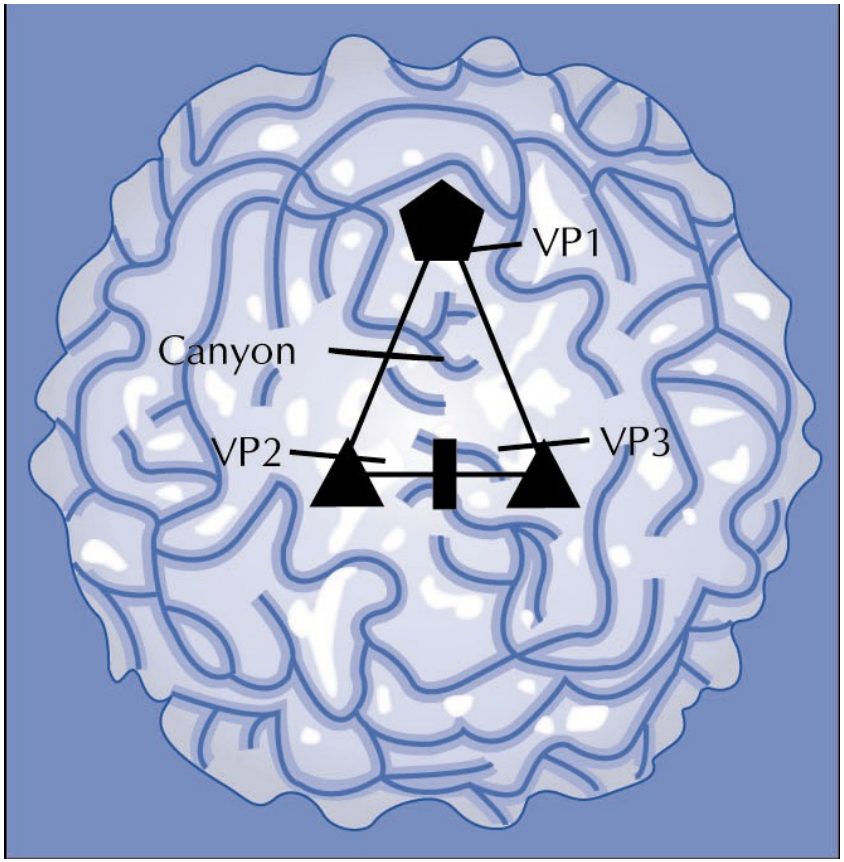
- The major human rhinovirus (HRV) receptor molecule, ICAM-1 (intercellular adhesion molecule 1), is an adhesion molecule whose normal function is to bind cells to adjacent substrates.
- ICAM-1 is regarded as a member of the immunoglobulin superfamily of proteins.

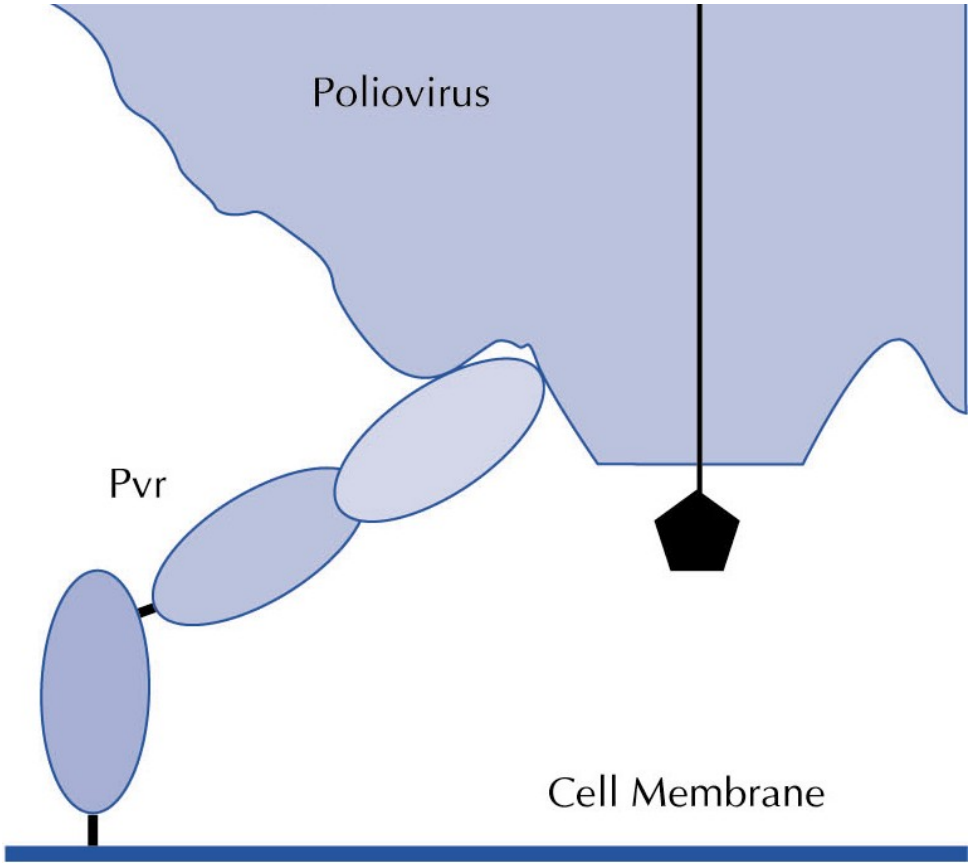
- Similarly, the poliovirus receptor is an integral membrane protein, which is also a member of this family, with one variable & two constant domains.
- In human rhinoviruses (HRVs), there is a deep cleft known as the 'canyon' in the surface of each triangular face of the icosahedral capsid, which is formed by the flanking monomers, VP1, VP2, & VP3.

- The interaction between ICAM-1 & the virus particle occurs in this canyon.
- Unlike other areas of the virus surface, the amino acid residues forming the internal surfaces of the canyon are relatively invariant.

- It is believed that these regions are protected from antigenic pressure because the antibody molecules are too large to fit into the cleft - radical changes here, although allowing the virus to escape an immune response, would disrupt receptor binding.
- In polioviruses, there is a similar canyon which runs around each fivefold vertex of the capsid.

Poliovirus Receptor Binding



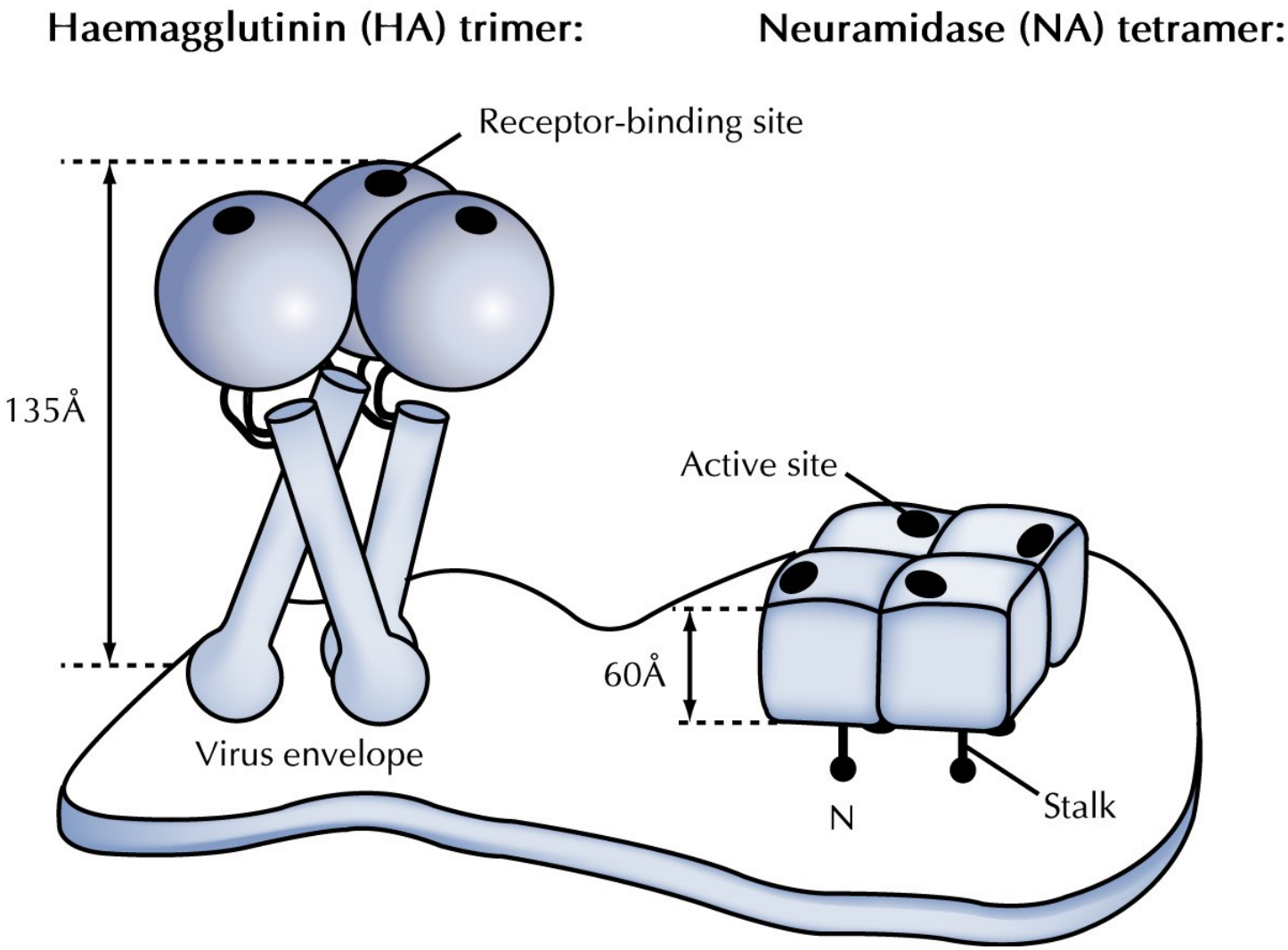


Influenza Virus Receptor Binding

- Another well studied example of virus-receptor interaction is that of influenza virus.
- The haemagglutinin protein forms one of the two types of glycoprotein spikes on the surface of influenza virus particles, the other type being formed by the neuraminidase protein.
- Each haemagglutinin spike is composed of a trimer of three molecules, while the neuraminidase spike consists of a tetramer.

- The haemagglutinin spikes are responsible for binding the influenza virus receptor, which is sialic acid (N-acetyl neuraminic acid), a sugar group commonly found on a variety of glycosylated molecules.
- As a result, there is little cell-type specificity imposed by this receptor interaction & therefore influenza viruses bind to a wide variety of different cell types (e.g. causing haemagglutination of red blood cells) in addition to the cells in which productive infection occurs.

Influenza Virus Receptor Binding



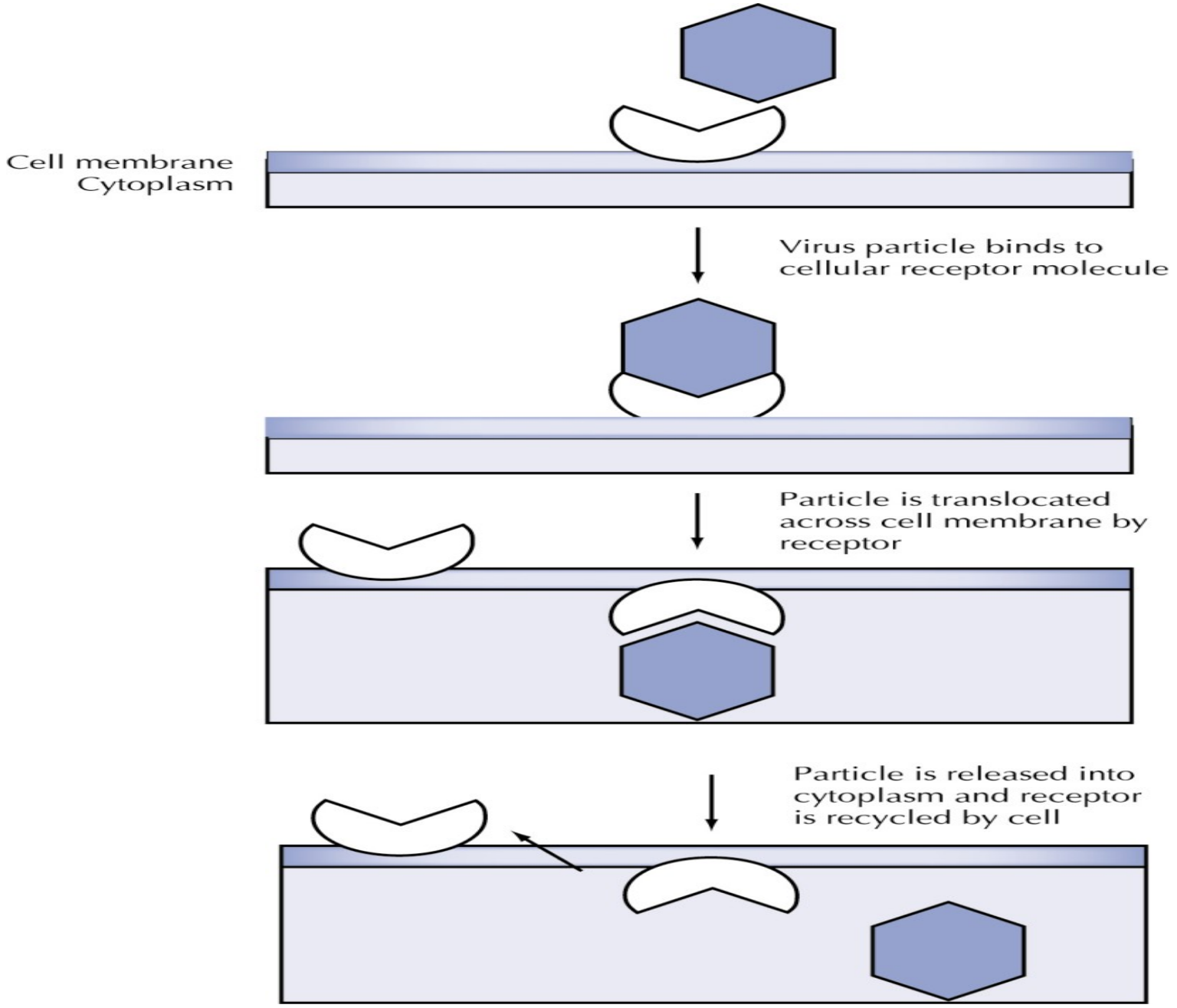
- Damage to these binding sites (eg. by disinfectants or heat), or blocking by specific antibodies (neutralizing antibodies) can render virions non-infectious.

Penetration

- Penetration of the target cell normally occurs a very short time after attachment of the virus to its receptor in the cell membrane.
- Unlike attachment, cell penetration is generally an energy-dependent process, i.e. the cell must be metabolically active for this to occur.
- Three main mechanisms are involved:

1. Translocation

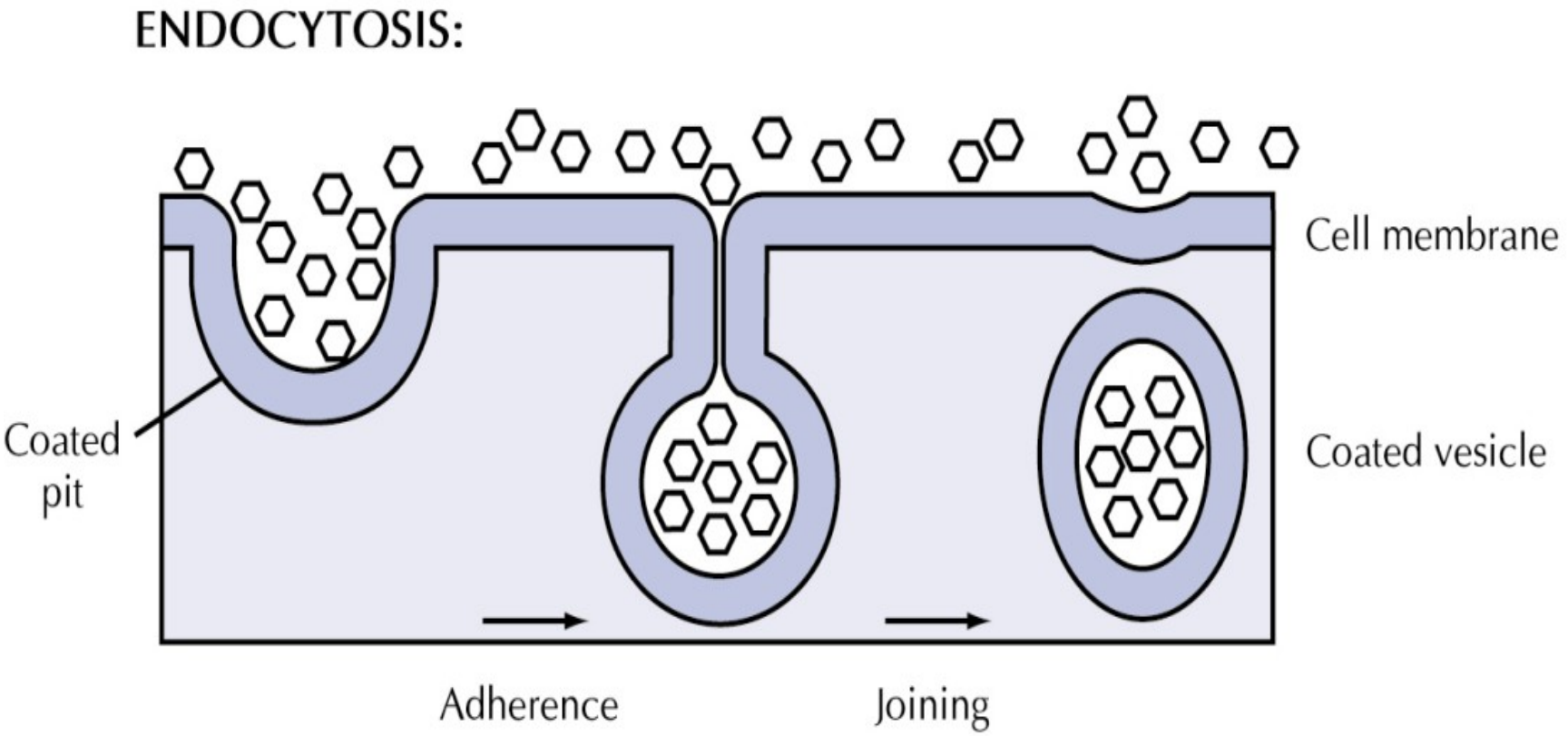
- Translocation of the entire virus particle occurs across the cytoplasmic membrane of the cell.
- This process is relatively rare among viruses & is poorly understood.
- It is mediated by proteins in the virus capsid and specific membrane receptors.


Cell membrane
Cytoplasm
Virus particle binds to cellular receptor molecule
Particle is translocated across cell membrane by receptor
Particle is released into cytoplasm and receptor is recycled by cell

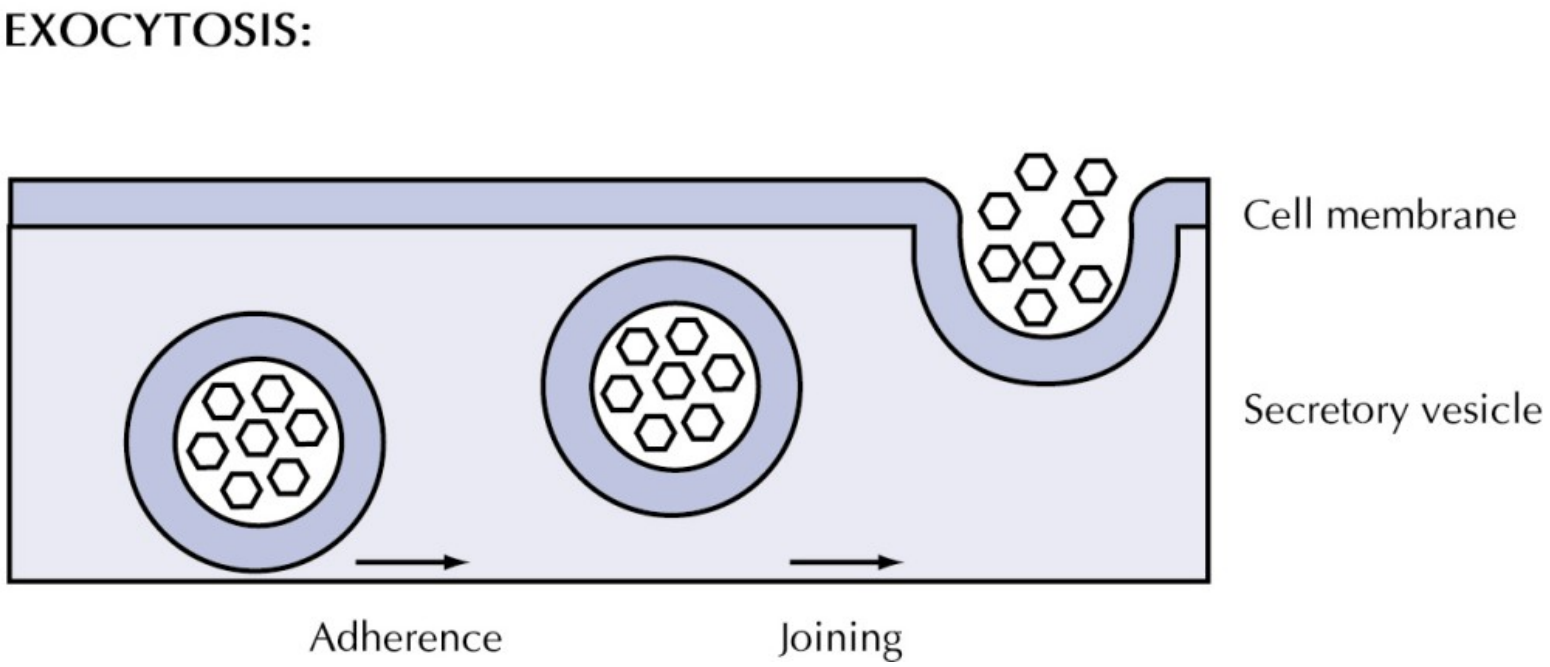
2. Endocytosis

- The virus enters into intracellular vacuoles of the cells.
- This occurs through normal formation and internalization of coated pits at the cell membrane.
- Receptor-mediated endocytosis is an efficient process for taking up & concentrating extracellular macromolecules.

Endocytosis

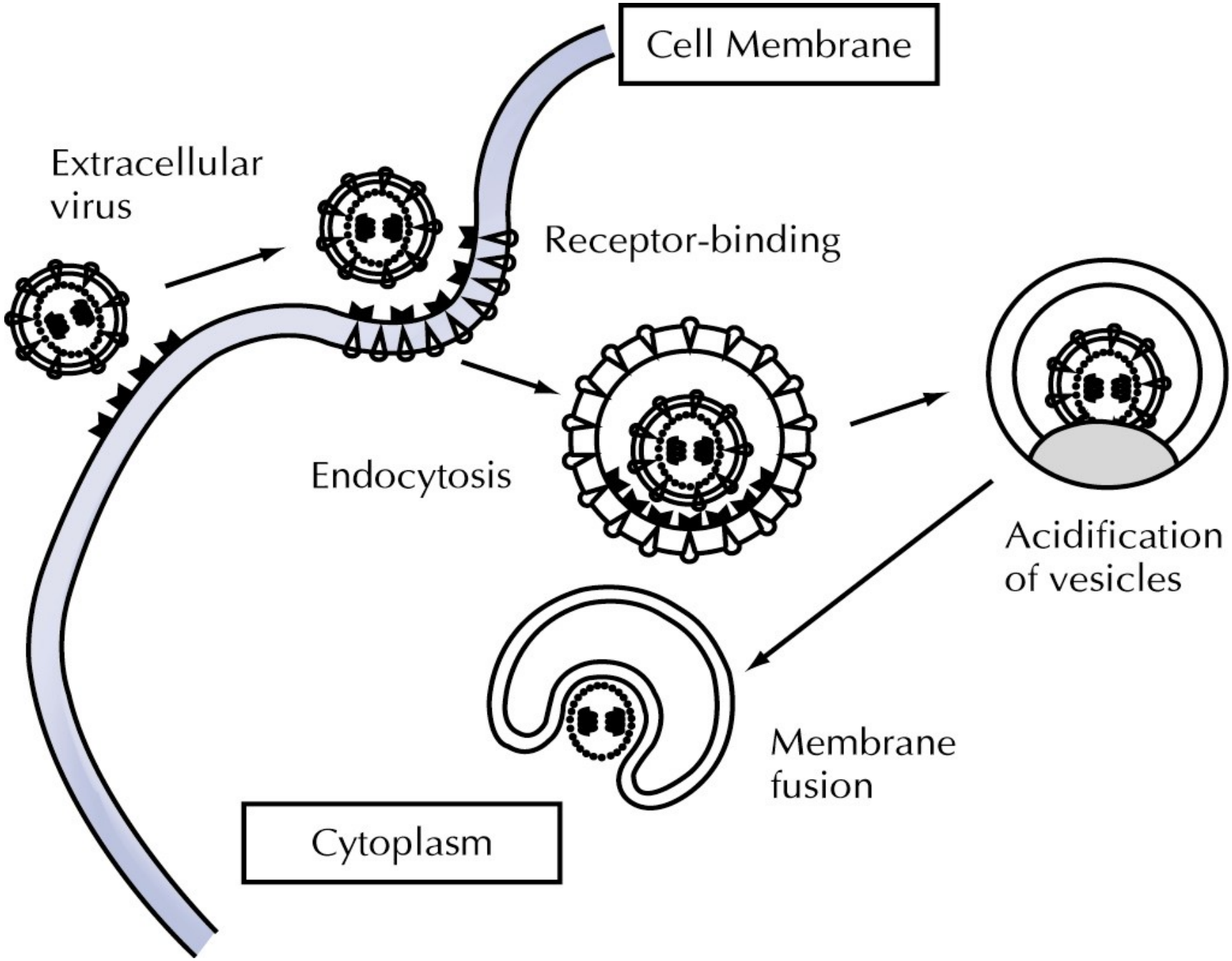


Exocytosis


EXOCYTOSIS:
Cell membrane
Secretory vesicle
Adherence
Joining

3. Fusion

- Fusion of the virus envelope (enveloped viruses only) with the cell membrane, occurs either at the cell surface or in a cytoplasmic vesicle.
- Fusion requires the presence of a specific fusion protein in the virus envelope, e.g. influenza haemagglutinin or retrovirus transmembrane (TM) glycoproteins, which promotes joining of the cellular and virus membranes resulting in a nucleocapsid being deposited directly in the cytoplasm.
- There are two types of virus-driven membrane fusion: pH-dependent & pH-independent.


Cell Membrane
Extracellular virus
Receptor-binding
Endocytosis
Acidification of vesicles
Membrane fusion
Cytoplasm

Uncoating

- During uncoating the virus capsid is completely or partially removed and the virus genome is exposed, usually in the form of a nucleoprotein complex.
- The product of uncoating depends on the structure of the virus nucleocapsid
- The structure and chemistry of the nucleocapsid determines the subsequent steps in replication.

Assembly

- Assembly involves the collection of all the components necessary for the formation of the mature virion at a particular site in the cell.
- During assembly, the basic structure of the virus particle is formed.
- The site of assembly depends on the site of replication within the cell and on the mechanism by which the virus is eventually released from the cell & varies for different viruses

- in picornaviruses, poxviruses and reoviruses assembly occurs in the cytoplasm.
- in adenoviruses, polyomaviruses and parvoviruses it occurs in the nucleus.

- As with the early stages of replication, it is not always possible to identify the assembly, maturation & release of virus particles as distinct & separate phases.

Maturation

- Maturation is the stage of the replication-cycle at which the virus becomes infectious.
- Maturation usually involves structural changes in the virus particle, which may result from specific cleavages of capsid proteins to form the mature products or conformational changes in proteins during assembly.

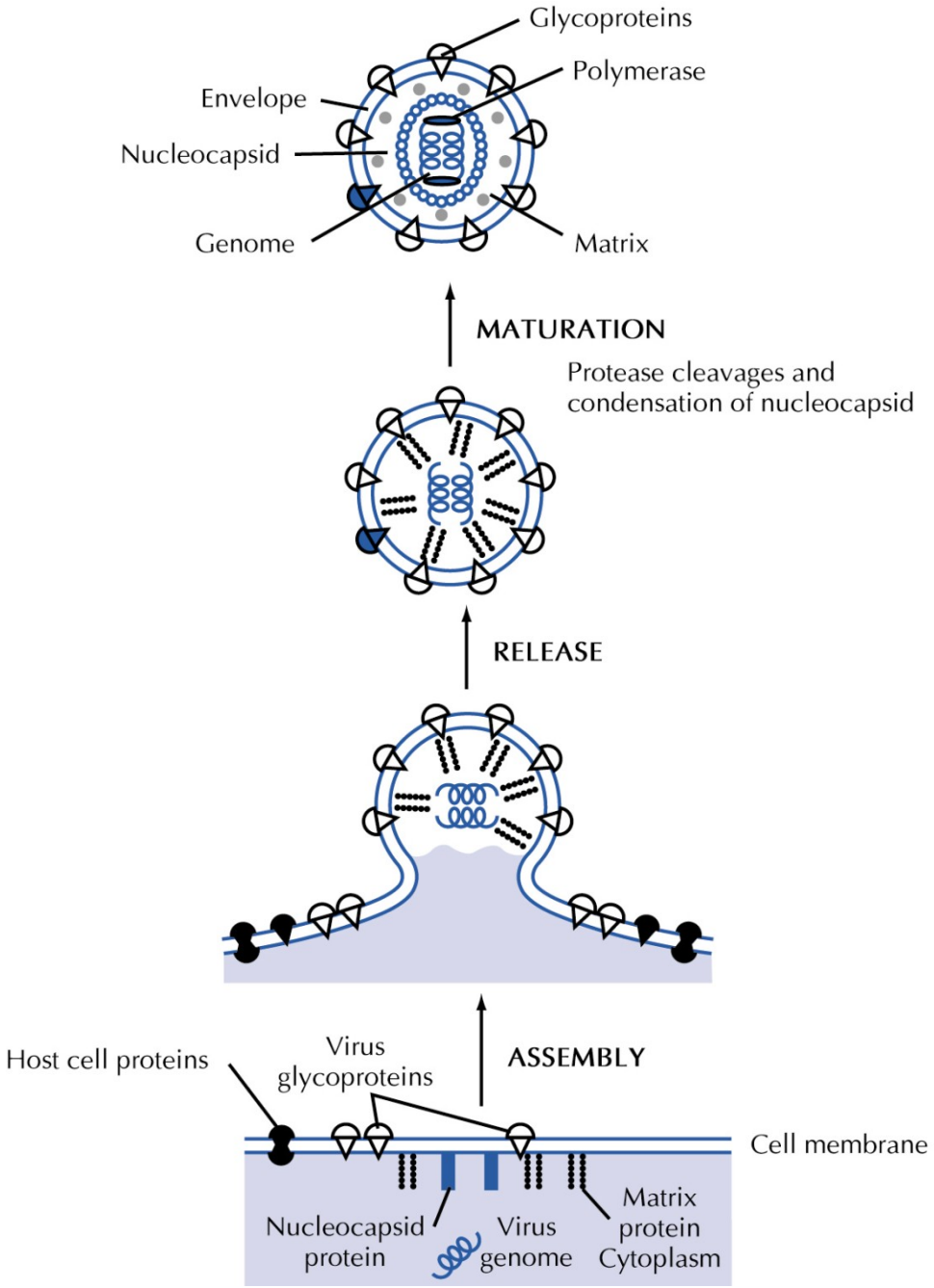
- Such events frequently lead to substantial structural changes in the capsid which may be detectable by criteria such as differences in the antigenicity of incomplete & mature virus particles or the condensation of nucleoproteins with the virus genome.
- Virus proteases are frequently involved in maturation, although cellular enzymes or a mixture of virus & cellular enzymes are used in some cases.

Release

- The viruses escape the cell by one of two mechanisms:
- For lytic viruses (most non-enveloped viruses), release is a simple process - the infected cell breaks open & releases the virus.

- Enveloped viruses acquire their lipid membrane as the virus buds out of the cell through the cell membrane or into an intracellular vesicle prior to subsequent release.
- Virion envelope proteins are picked up during this process as the virus particle is extruded - this process is known as budding.

Budding


Glycoproteins
Polymerase
Envelope
Nucleocapsid
Genome
Matrix
MATURATION
Protease cleavages and
condensation of nucleocapsid
RELEASE
Host cell proteins
Virus
glycoproteins
ASSEMBLY
Cell membrane
Nucleocapsid
protein
Virus
genome
Matrix
protein
Cytoplasm

-END-