Entry of virus in cells:

Introduction:

The interactions between viruses and cells are complex, in spite of their simple structure and components. Viruses utilize a number of cellular processes which contains cellular proteins to enter into the cells. Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms. Generally viruses are attached to the cell surface of proteins, carbohydrates and lipids. The interaction of viruses receptors are specific and require at least 3 point for interaction, which results in activation of cellular signalling pathways. The viruses enter the cell by endocytic mechanism. After the viruses go in the lumen of endosomes or the endoplasmic reticulum, they obtain signals which are in the form of being exposed to low pH, proteolytic cleavage, and the initiation of viral proteins, which results in modifications in the viral proteins, and then they are able to penetrate the vacuolar membrane. After they penetrate the vacuolar membrane, they pass the viral genome, the caspid, or the viral particle that is kept together into the cytosol. Afterwards, the majority of RNA viruses replicate at a variety of positions within the cytosol. In contrast, most DNA viruses continue through their passage towards the nucleus.

Viral entry into the cell:

Viruses enter the cell, which is covered by a phospholipid bilayer and acts as a cells natural barrier to its surrounding. The process by which this barrier is crossed depends upon the type of virus. There are 4 types of viral entry into the cell:

1. Attachment or Viral Adsorption: The viral receptors attached to the complementary receptors on the cell membrane.
2. Membrane Fusion: The cell membrane is punctured and later attached with the unfolding viral envelope.
3. Entry via Pore formation: An opening is established for the entry of viral particles.

Viral Penetration: The viral capsid or genome is injected into the host cells cytoplasm directly.**Entry through endocytosis:**

The type of entry through endocytosis is carried out by bacteriophages infection that form coliphages T4 and T2 and are hypodermic syringes with a tail that is capable of contracting. Endocytic vesicles carry viruses from the outer edges to the perninuclear area of the host cell, where the conditions for infection are changed and the distance is minimized towards the nucleus. Also the maturation of endosomes has slowly changing conditions, like lowering of pH or the switching of a redox environment, which enables viruses to detect their position within a cell and the passage and allows the endosomes to utilize this information to put a time of penetration and uncoating.

Viruses as Endocytic Carriers:

This type of viral entry is mostly observed in animal viruses. When these viruses attach to cells, they do not become disfigured. Rather the plasma membrane changes shape according to the shape of the virus. The outer layer of viruses is coverer proteins that attaches to receptors and formed an icosaherdral grid or as spike glycoprotein that span the entire viral envelope. The single interactions with the receptors are weak, but interaction with many different receptors increases the activity and results the binding to cells almost impossible to reverse. Lipid raft functions to control the signalling, fluidity and receptor functions on the membrane. These are rich in cholesterol and sphingolipids.

Viruses that use the Caveolar/Raft-dependent pathways form primary endocytic vesicles are dependent on cholesterol, lipid rafts, and complex signalling pathways.

Attachment Factors and Receptors:

Some viruses bind to defined endocytic receptor, like transferrin and low-density lipoprotein receptors, but viruses attach to the majority of other molecules like carbohydrate moieties with different functions like cell to cell recognition, ion transportation and attachment to the extracellular matrix. The difference between attachment factors and virus receptor is that the attachment factor simply attached to the viruses and hence focused on the top area of the cell, while virus receptors modify the viruses, promote cellular signalling or activate penetration. The situation of attachment receptor is common in influenza and polyomaviruses, where the attachment is very specific and deals with lectin domains. The other example is HIV-1 because two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to assist fusion and encourage endocytosis.

Entry by macropinocytosis:

Viruses belonging to vaccinia, adeno, picorna and other virus families enter by macropinocytosis, an endocytic mechanism involved in fluid uptake. The virus particles first activate signalling pathways that trigger actin-mediated membrane ruffling and blebbing. This is followed by the formation of large vacuoles (macropinosomes) at the plasma membrane, internalization of virus particles and penetration by the viruses or their capsids into the cytosol through the limiting membrane of the macropinosomes.

Examples for entry of viruses into the cells on the basis of types of viruses:

1. Bacterial virus entry:

Bacteriophages attack bacterial cells and inject their genomes through specific receptor sites which include lipopolysaccharides, cell wall proteins, teichoic acid or flagellar or pilus proteins and also contain specific attachment proteins on the bacteria. The phage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC. And then the base plate then settles down onto the surface and binds tightly to it which results in conformational changes in the short tail fibres, which then later contracts, pushing the tail core through the cell wall, in an ATP-driven process and is aided by a lysozyme activity associated with the tip of the tail tube.

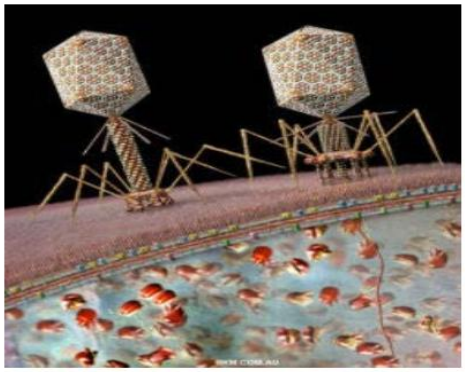

Figure 1: Enterobacteria phage T4 or viruses with 34-170 kbp dsDNA genomes, isometric heads and contractile tails - infects the gram-negative bacterium E coli

Phage lambda or enterobacteria phage λ is a tailed phage with an isometric head which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip. Although the tail is non-contractile, a DNA injection mechanism allows entry of DNA into the cell, via a sugar transport protein (PtsG) in the inner membrane, leaving the capsid behind.

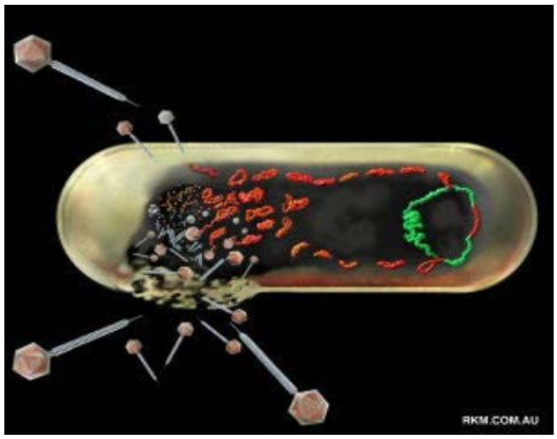

Figure 2: Lambda phage infecting *E.coli*

MS2 phage is an isometric single-stranded RNA-containing virus which infects *E.coli*. And the phage attaches to the F pili of *E.coli* via its single attachment or a protein. The A protein is covalently linked to the 5'-end of the genomic RNA; binding pilin causes cleavage of protein and releases it from the capsid. Thus, when the pilus is retracted into the cell, protein and RNA are pulled with it, leaving the empty capsid outside.

2. Animal cell entry:

Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle. Direct membrane fusion involves direct attachment to the cell surface via **binding to a specific receptor.** Here the virion membrane fuses with the cell membrane, and the virion nucleoprotein complex is delivered into the cell cytoplasm directly. An example is HIV entry process. Here the virion attachment protein **gp120** attaches initially to the **CD4** protein on a helper T-cell. The gp120 then undergoes conformational change due to binding and binds the **accessory receptor CCR-5,** a **chemokine**. **gp41**, a cleavage product of a **gp160** precursor, and a part of the **"spike protein"** of the viral membrane is then able to bind into the cell membrane, via a hydrophobic domain. A condensation of the gp41 structure results in formation of a "6 helix bundle" and causes close juxtaposition of cell and viral membranes, which promotes membrane fusion and nucleoprotein entry into the cell.

Fusion Protein mode of entry: All enveloped viruses follow fusion mode of entry, whether they fuse with the cell membrane directly or with the membrane via an internalised vesicle. This is mediated by three identified classes of envelope glycoproteins:

Class I fusion proteins

These fusion proteins are most common in retroviruses, myxoviruses, coronaviruses and paramyxoviruses. The "spikes" are composed of three identical protein subunits, largely

alpha-helical in structure and assembled as trimers which are later on cleaved into two pieces. The carboxy-terminus of one piece is anchored to the viral membrane and the new amino terminus has a characteristic stretch of 20 hydrophobic amino acids.

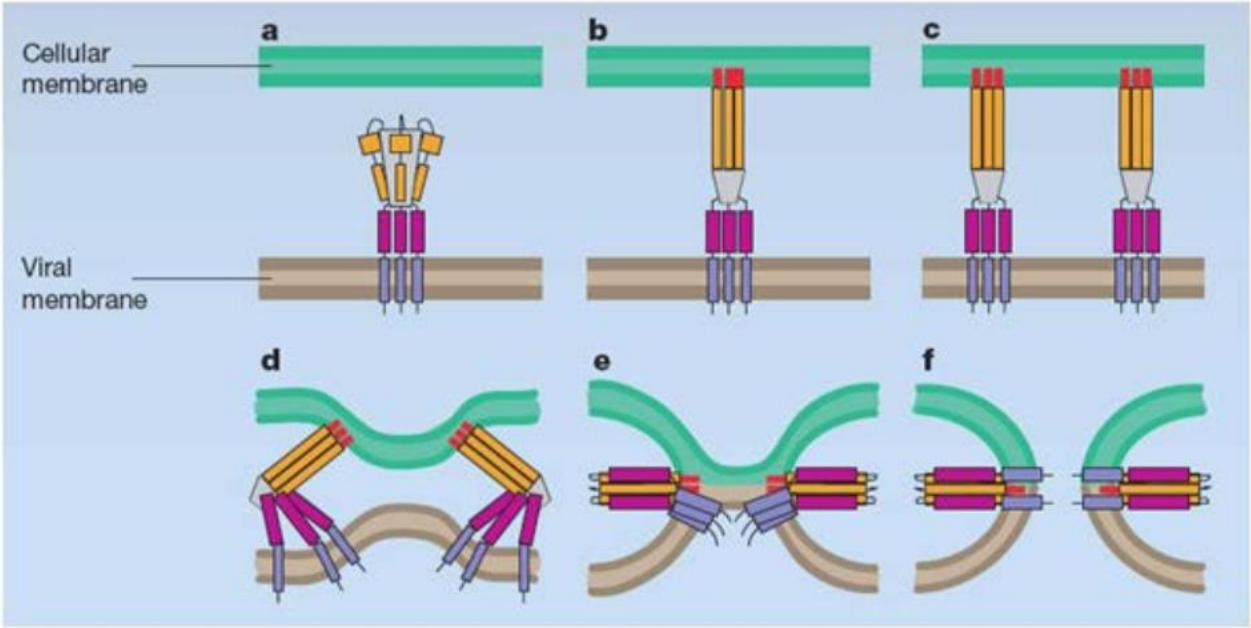

Figure 3: Proposed mechanism for membrane fusion by class I fusion proteins. (a)The metastable conformation of a trimeric fusion protein: with helical domain in orange, helical domain B in pink and the transmembrane domain in purple. (b)After binding to a receptor on the cellular membrane or an exposure to low pH found in intracellular compartment, the protein forms an extended conformation and hydrophobic fusion peptide (red) inserts into the target membrane. (c)Several trimers thought to be involved in this mechanism. (d)Protein refolding begins. The free energy thereby released causes the membrane to bend towards each other. (e)Formation of restricted hemifusion stalk allows the lipids in the outer leaflets of membrane to mix. (f)Protein refolding completes, forming the most stable form of fusion protein with fusion peptide and transmembrane domain anti-parallel to each other but in the same membrane.

Class II fusion proteins

They are found in dengue, tick-borne encephalitis, yellow fever and other flaviviruses, and Semliki Forest virus. This class of fusion proteins have a β-sheet-type structure and are not cleaved during biosynthesis. The proteins have three principal domains: Domain I begin at the amino terminus, domain II contains the internal fusion loop and domain III is at the carboxy terminus.

The dimeric protein binds to few cellular receptors for virus internalisation. The acidic pH inside endosomes causes domain II to swing upward, allowing monomers to rearrange laterally. The fusion loop inserts into the host-cell membrane, enabling trimer formation of the viral glycoprotein. Domain III shifts and rotates to create contacts, bending the membrane. The formation of further contacts leads to unrestricted hemifusion and the most stable form of the protein.

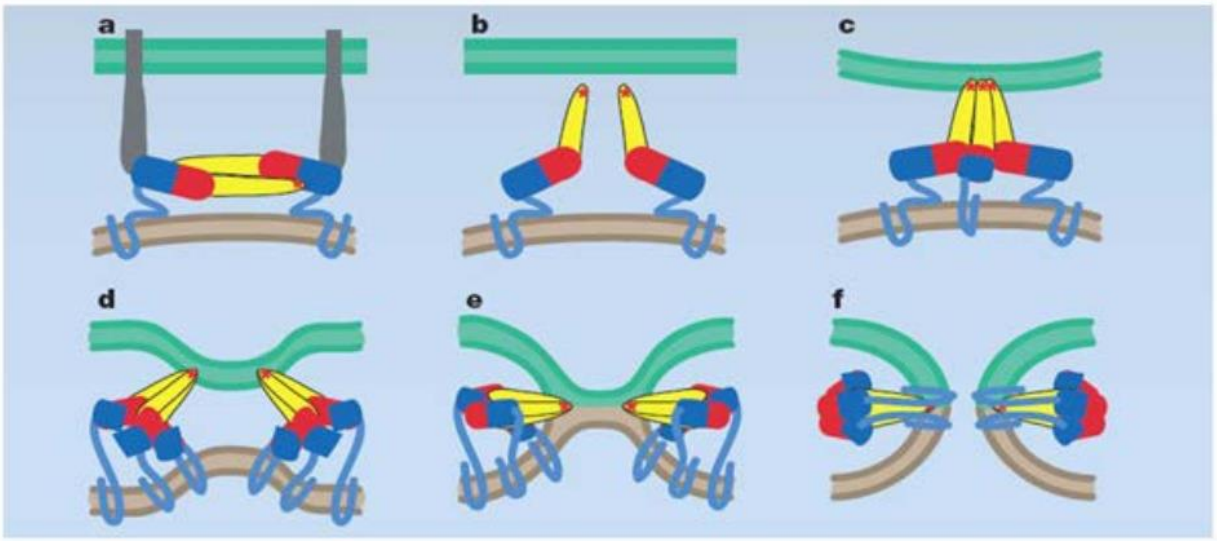

Figure 4: Proposed mechanism for fusion by class II proteins. (a)The dimeric E protein binds to a cellular receptor (grey) and the virus is internalized to endosomes. Membrane fusion release the virus into the cell body, takes place within endosomes. Domain I is in red, domain II in yellow and domain III in light and dark blue. (b)The acididc pH inside the endosomes cause domain II to swing upward and permit E monomers to rearrange laterally. (c)The fusion loop (red dot) inserts into the outer leaftlet of the host-cell membrane, enabling trimer formation. (d)The formation of trimer contacts extends from the top of the molecule. Domain III shifts and rotates to create contacts, bending the membrane. (e)The formation of further contacts leads to unrestricted hemifusion. (f)The final most stable form of the protein.

Class III fusion proteins

These proteins are characteristic of rhabdoviruses and vesicular stomatitis viruses. They form trimers of hairpins as a fusion structure by combining two structural elements. The post-fusion trimer has a central α-helical trimeric core; however, the fusion domains have two fusion loops at the tip of an elongated β-sheet. Most **non-enveloped viruses**, such as dsDNA adenoviruses and ssRNA picornaviruses, enter cells via vesicles. The former appear to enter via an endocytotic vesicle. In later, the capsid becomes rearranged as receptor binding induced structural transitions, whereby the VP4 internal protein is externalised and the virion surface becomes more lipophilic, and interacts with a vesicle membrane to form a pore so as to allow exit of the RNA into the cytoplasm.

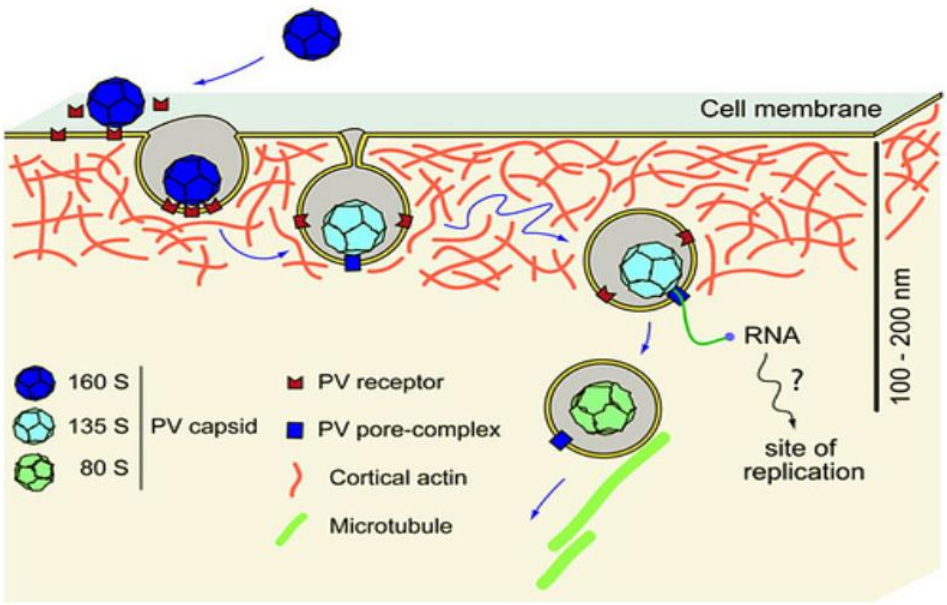

Figure 5: Poliovirus entry into live cells

Nuclear targeting:

A final result in the infection is that DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses. These are the sites where the respective viruses may be expected to replicate their genomes.

Adenoviruses are transported by means of the hexon protein of the partially degraded naked capsid which is released into the cytoplasm. This reaches a nuclear pore and allows escape of viral DNA plus certain viral polypeptides into the nucleus.

Parvoviruses enter host cells by receptor-mediated endocytosis, escape from endosomal vesicles to the cytoplasm, and then replicate their DNA in the nucleus.

Herpesviruses generally enter by fusion with the cell membrane by a process that involves several envelope glycoproteins acting together and the core particles migrate to nuclear pores, and release DNA there.

Poxviruses have "intracellular" single-enveloped and "extracellular" double-enveloped forms which enter by **direct cell fusion** (pH-independent) or **lysosomal vesicle fusion** (pH-dependent). Once core virions are in the cytoplasm, they uncoat further to expose a nucleoprotein complex which is first transcriptionally and later, replicationally, active.

3. Plant cell entry:

Every cell in plant is separated from every other cell by thick cell walls, whose dimensions are far larger than the size of the average virion. So plant cells are inaccessible to viruses. These plant cells interconnect only via specific discontinuities in the cellulose walls. These act as gated intercellular channel, which limit the passage of both molecules and virions between cells. Plant viruses possess ssRNA + ve **sense, non-enveloped** and **do not specifically interact with host cell membranes.** The mechanisms employed to enter cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.

The mode of transmission of viruses affects their concentration and localisation in plants. For example, mechanically transmitted viruses (eg: bromoviruses, tobamoviruses) tend to reach very high concentrations in most tissues by non-specific means. Whereas viruses which are introduced into plants via insect vectors with piercing mouthparts tend to be limited in their multiplication to phloem elements, which are preferred target tissues for insect feeding. Consequently, these viruses (eg: luteoviruses, geminiviruses) reach only very low concentrations in whole plants.

Entry of virus on the basis of morphological structure:

The naked virus enters either via translocation (i.e. crosses cell membrane intact directly); genome injection (attachment to the cell surface and releases its genome which penetrates the cytoplasm via a pore in the plasma membrane) or receptor mediated endocytosis. Whereas the enveloped virus enters the cell via receptor mediated endocytosis or membrane fusion.

Interesting facts:

- Some viruses are able to cross plasma membrane into cytosol by endocytic uptake, vesicular transport via cytoplasm, and transport to the endosomes and other intercellular organelles. These processes are associated with clathrin-

mediated endocytosis, macropinocytosis, caveolar/lipid raft-mediated endocytosis, or other mechanisms.

- HIV-1 uses two receptors Adenoviruses 2 and 5 are necessary to promote conformational modifications to promote the fusion and encourage endocytosis.
- The bacteriophage tail fibres are the attachment sites and bind reversibly to lipopolysaccharides and outer membrane protein OmpC.
- Phage lambda is a tailed phage which attaches to the maltose receptor on the surface of the *E.coli* cell via the J protein in the tail tip.
- Animal viruses enter into the cell by direct cell membrane fusion and entry via endocytotic or other vesicle.
- Neuclear targeting is the result of final destination in the infection of viruses. In some, DNA genomes end up in the nucleus except for Poxviridae, Phycoviridae

and some Baculoviridae and that RNA genomes end up in the cytoplasm except for myxoviruses.

- The mechanisms employed to enter virus into the plant cells appear to be passive carriage through breaches in the cell wall, followed by cell-to-cell spread in a plant by means of specifically-evolved "movement" functions, and then spread via conductive tissue as whole virions.
- Entry of the dengue virus to mammalian cells can occur via receptor-mediated endocytosis in clathrin coated pits.

Questions:

1. How do viruses gain entry to a host cell?
   a. by dissolving a piece of the host cell membrane
   b. by binding to a receptor site on the host cell
   c. by binding to an antibody site on the host cells
   d. all of the above

2. How the dengue viruses enter the cells?
    a. Receptor-mediated endocytosis
    b. Binding to the cell surface receptor
    c. Phagocytosis
    d. Pinocytosis
3. Which receptor plays an important role for the entry of retoviruses?
    a. Glycoproteins
    b. Integrins
    c. Hexon
    d. Chemokine
4. Entry of HIV is an example of
    a. Macropinocytosis
    b. Endocytosis
    c. Attachment factors and receptors
    d. Vesicles

5. Describe the different modes of entry of viruses across the plasma membrane of the cells. Explain with an example.
6. What is fusion protein mode of entry?
7. What is nuclear targeting? Explain with an example.