

HUMAN CELL BIOLOGY--BIO315HF

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Influenza Virus and HIV Cell Entry

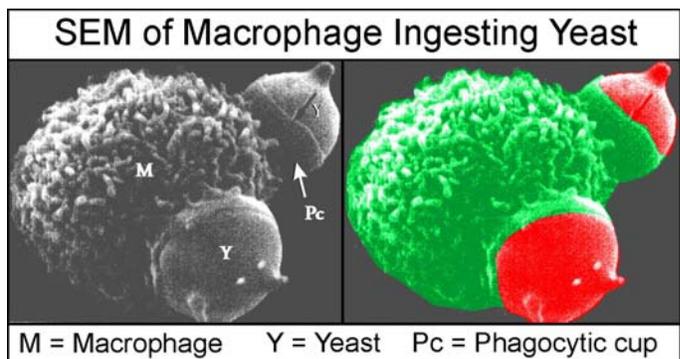
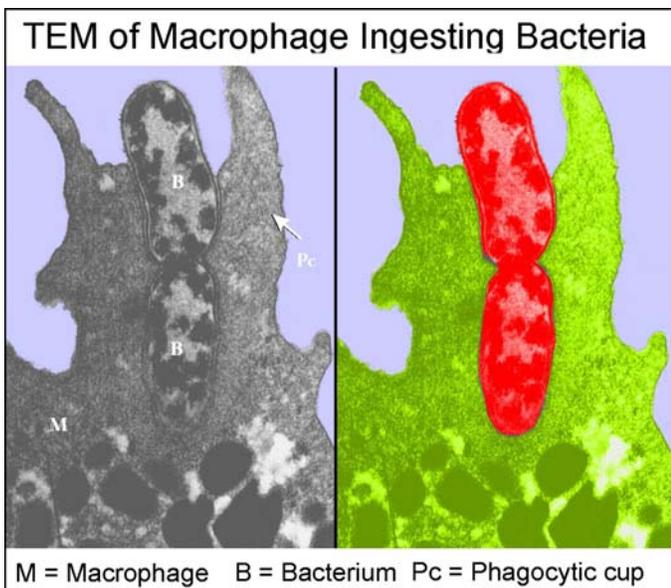
Lecture Outline

- Some Events Involving Biomembrane Fusion
- Agents That Induce Biomembrane Fusion
- Skeletal Muscle Formation: The Fusion of Myoblasts
- Retroviruses: Enveloped Viruses
- pH-Controlled Viral Protein-Mediated Fusion
- Model of Biomembrane Fusion: Influenza Virus Fusion Protein
- Human Immunodeficiency Virus Penetration
- The Proteins Involved In HIV Entry
- HIV and Immunodeficiency

Some Events Involving Biomembrane Fusion

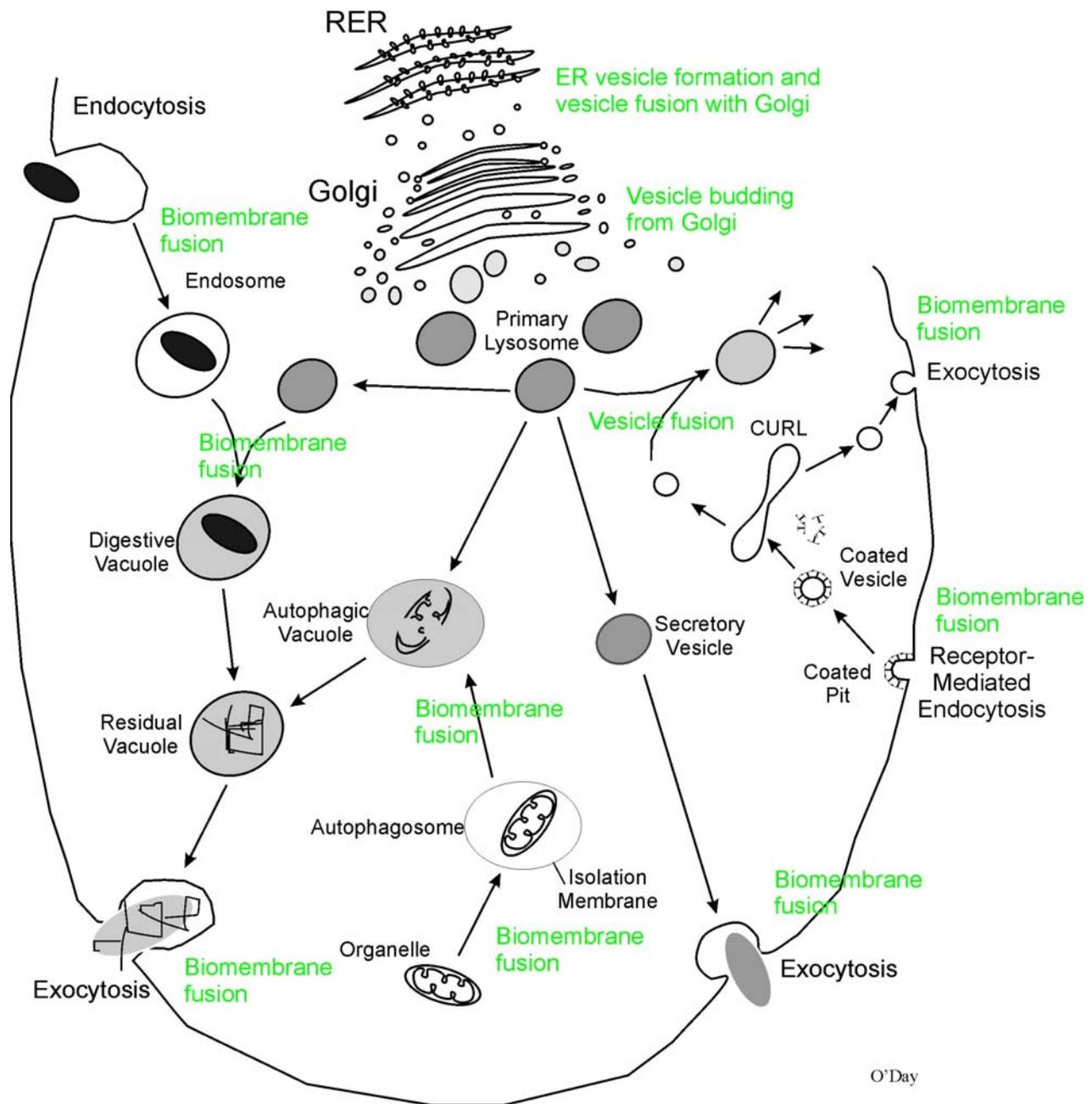
The fusion of biological membranes underlies day to day functions as well as some special events in the life of the human cell. Here are some examples:

- Cell-Cell Fusion (e.g., sperm-egg, myoblasts)
- Viral Penetration & Release
- Exocytosis (e.g., neurotransmitter release)
- Endocytosis (phagocytosis, pinocytosis, receptor mediated endocytosis covered in detail later in course)
- Cytoplasmic Vesicle Fusion (various endosomes; covered in more detail later in course)
- Cytokinesis (fusion of cell membranes to separate daughter cells after mitosis)



An examination of just some of the biomembrane events that underlie the formation and function of lysosomes verifies the on-going importance of biomembrane fusion in cells. In the following figure, biomembrane fusion events are shown in green.

Some Biomembrane Fusion Events



Agents That Induce Biomembrane Fusion

The fusion of membranes occurs naturally but can also be induced by various factors. Molecules that induce fusion are called fusogens.

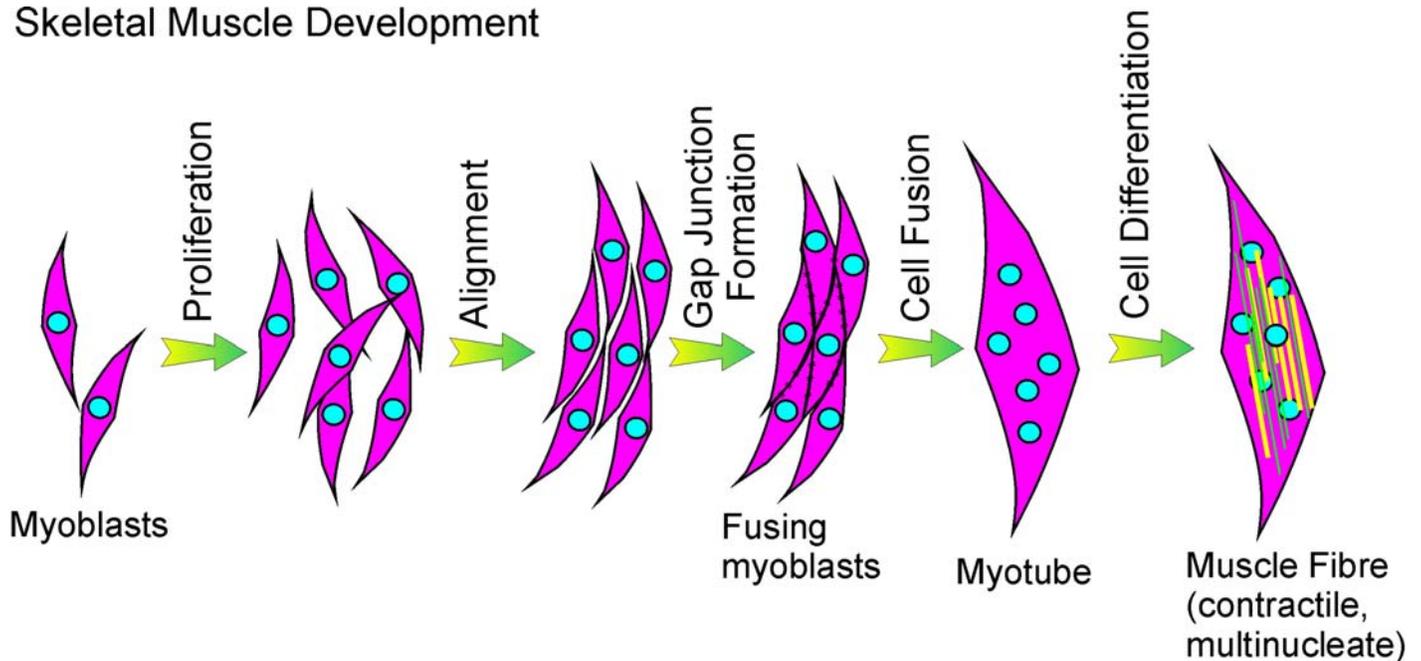
- Human Fusion Proteins (e.g., for sperm-egg fusion; myoblast fusion)
- Physical agents (e.g., electricity; polyethylene glycol (PEG) artificially induce fusion)
- Viral Fusion Proteins (e.g., for penetration of viruses)

Skeletal Muscle Formation: The Fusion of Myoblasts

Skeletal muscle is a tissue that differentiates from myotubes after the fusion of myoblasts. After they have stopped dividing, myoblasts align and form gap junctions. The coupling of the cells via gap junctions serves

to co-ordinate the subsequent event of cell fusion to form the myotubes. Differentiation of the multinucleate myotubes involves the formation of synaptic components as well as other muscle-specific molecules such as the cytoskeletal components actin and myosin and their associated proteins.

Skeletal Muscle Development



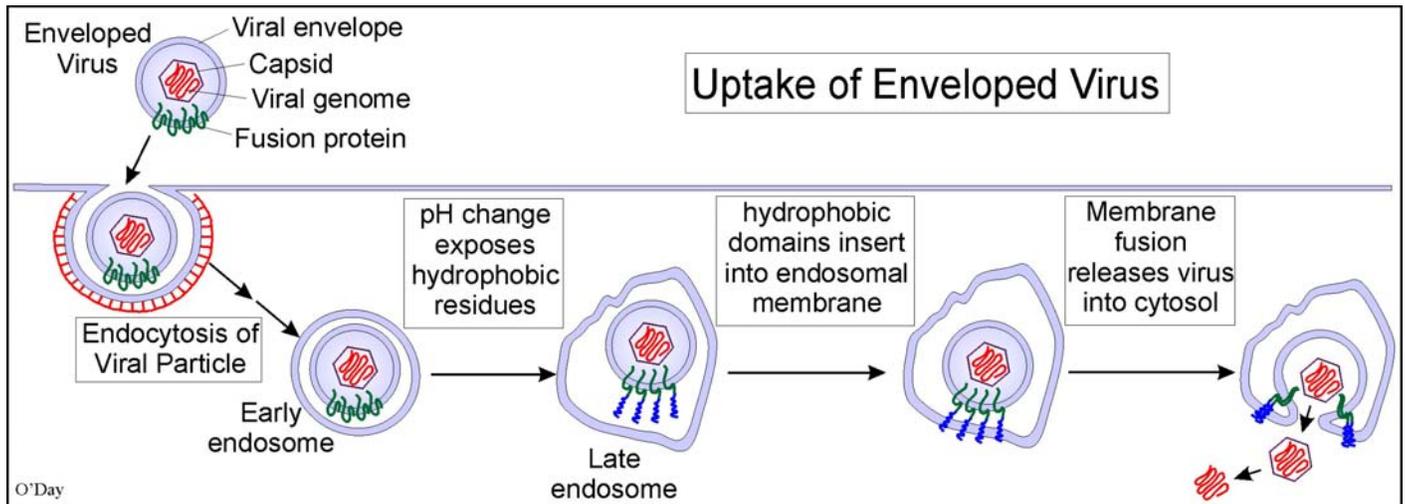
Retroviruses: Enveloped Viruses

Much of our understanding of biomembrane fusion has come from studies on viral-induced fusion. As mentioned in the lecture on membrane structure, viruses also serve as valuable tools for diverse kinds of research. However, understanding how different viruses are able to penetrate cells and to induce cell fusion may provide routes to developing anti-viral therapies. Here we will discuss retroviruses and biomembrane fusion.

- Retroviruses use RNA as genetic material
- E.g., Semliki Forest Virus; Human Immunodeficiency Viruses (HIV)
- Many tumour viruses are retroviruses
- Retroviruses are surrounded by Membrane Envelope (from host cell with viral proteins embedded)

pH-Controlled Viral Protein-Mediated Fusion

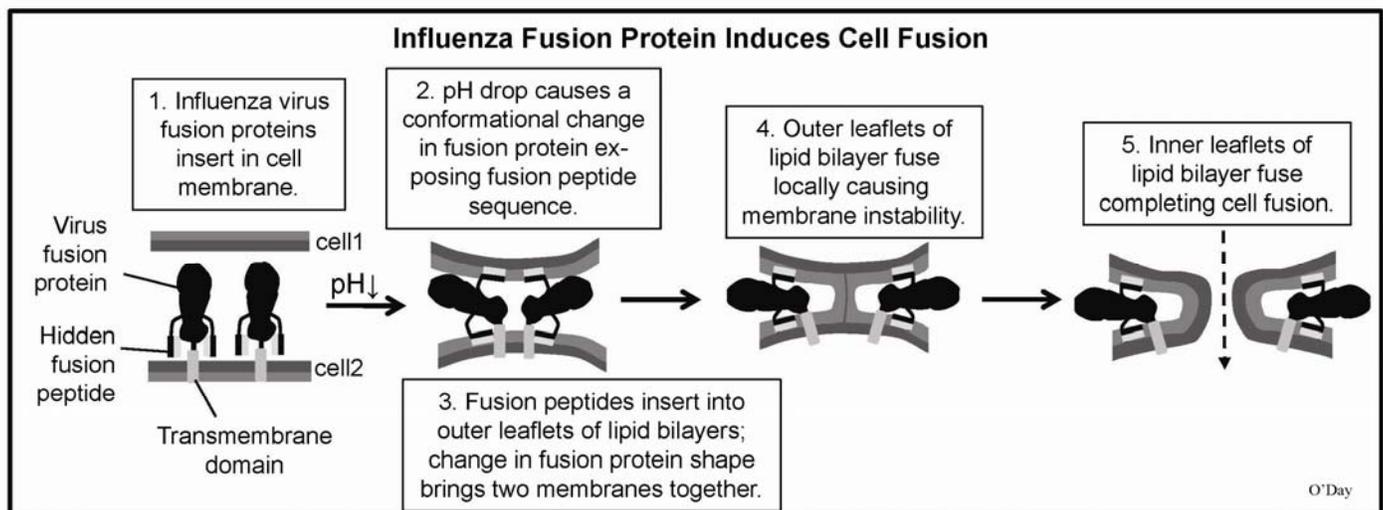
- Virus is ingested by phagocytosis
- Endosome formation occurs
- pH drops to acidic
- Change in pH leads to conformational change in fusion protein
- Hydrophobic amino acid sequence is exposed
- Hydrophobic sequences insert into endosomal membrane
- Biomembrane fusion results



Model of Biomembrane Fusion: Influenza Virus Fusion Protein

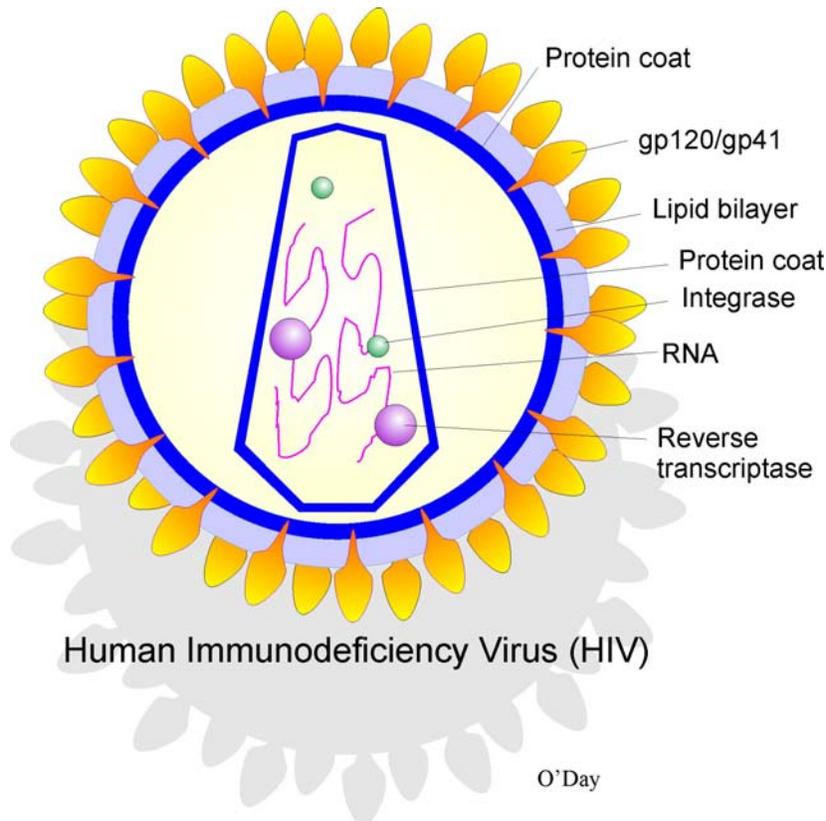
The following work was done *in vitro* allowing experimenters to adjust the pH of the culture medium in which the cells were suspended.

- Transmembrane domain localizes influenza virus fusion protein in cell membrane of cell 1
- Fusion peptide (hydrophobic) present but hidden
- A change in conformation (e.g., induced by drop in pH) exposes fusion protein
- Fusion peptide inserts into membrane of cell 2
- Outer leaflets of lipid bilayers of cells fuse
- Inner leaflets fuse completing fusion process

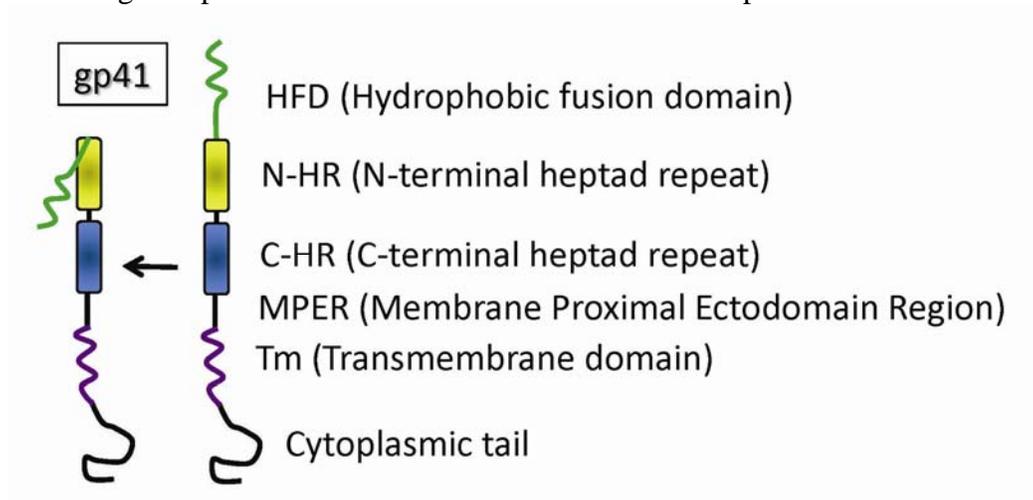


Human Immunodeficiency Virus Penetration

- HIV is a retrovirus (RNA is genetic material plus replicating enzymes contained within protein coat)
- Viral Envelope = Host cell membrane + Envelope glycoproteins (gp120/gp41)
- Glycoproteins in envelope will bind to host glycoproteins
- Fusion domains within viral envelope glycoproteins mediate fusion

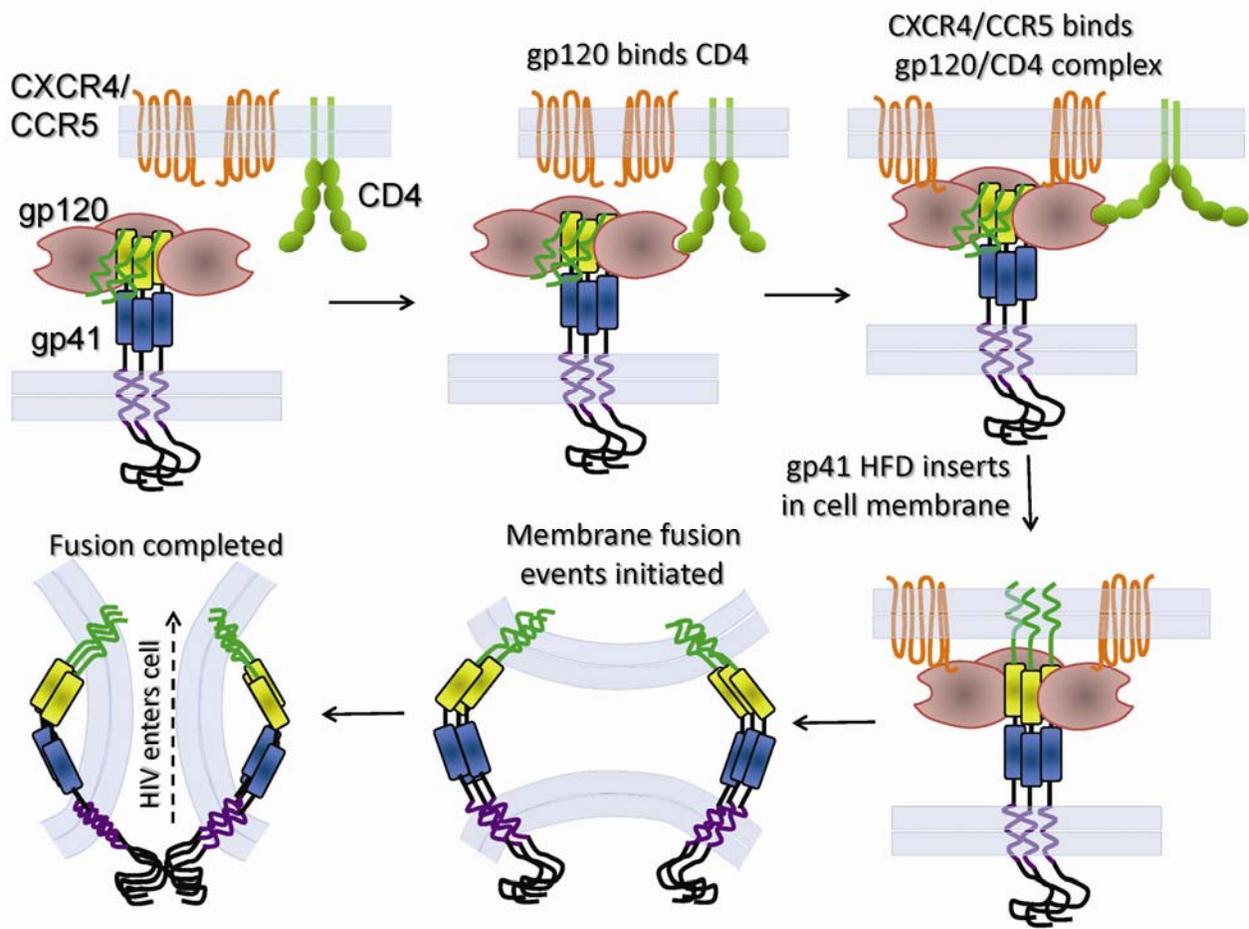


- HIV envelope protein is a heterotrimer
- Each monomer is comprised of gp41 & gp120
- gp120 (receptor binding unit) binds to CD4 and co-receptor CXCR5 (or CXCR4)
- gp41 is the transmembrane fusogenic unit; it contains a hydrophobic fusion peptide domain plus other domains HR repeats, MPER that have all been shown to be important in viral entrance
- pH change does not mediate gp41 conformational change; ligand-receptor binding and multiple conformational changes in proteins lead to biomembrane fusion/viral penetration



The Proteins Involved In HIV Entry

- First gp120 binds to CD4 on the human cell inducing a conformational change in the proteins.
- Then CXCR4 or CCR5 binds leading to a further conformational change
- This brings gp41 into close contact allowing the HFD region to penetrate the lipid bilayer
- Disruption of the bilayer allows the the viral membrane to fuse with the human cell membrane
- Protein coated viral content enters the cytoplasm



This is another active area of research with many details being elucidated regularly.

HIV and Immunodeficiency

We've seen how HIV enters cells. This step is essential so the virus can hijack the nuclear and cytoplasmic machinery of the cell to replicate itself which will then lead to the release of more virus to infect new cells. However this does not explain some of the other aspects of HIV infections that underlie full-blown AIDS which is a result of HIV compromising the immune system leading to immunodeficiency. Immunodeficiency is a result of the loss of T cells. Research has shown that the level of CD4+ T cell depletion in HIV infected persons greatly exceeds the number of infected T cells. Thus it is believed the infected cells are somehow affecting the cells around them to die (i.e., apoptosis is induced)—an event called the “bystander hypothesis”. While the viral envelope proteins (gp120/gp41) are implicated in this model, the mechanism by which infected cells affect their neighbours remains to be resolved (Garg & Blumenthal, 2008. *Cell. Mol. Life. Sci.* 65: 3134-3144)

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