# Membrane Fusion

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## Glossary

**Fusion pore** Small opening at the site of two merged lipid bilayers, which allows the exchange of fluids. Fusion pores expand gradually to complete membrane fusion.

**Hemifusion** Membrane fusion intermediate state with the two proximal leaflets of two opposed bilayers merged to one.

**Lipid raft** Small membrane microdomain enriched in cholesterol and glycosphingolipids. These domains are resistant to solubilization by Triton X-100.

**Type 1 TM protein** A glycoprotein composed of an N-terminal external domain and a single transmembrane region followed by a cytoplasmic domain.

## Introduction

Enveloped viruses contain a lipid bilayer that serves as an anchor for viral glycoproteins and protects the nucleocapsid containing the genetic information from the environment. The lipid bilayer is derived from host cell membranes during the process of virus assembly and budding. Consequently, infection of host cells requires that enveloped viruses fuse their membrane with cellular membranes to release the nucleocapsid and accessory proteins into the host cell in order to establish a new infectious cycle. Glycoproteins from enveloped viruses evolved to combine two main features. Firstly, they contain a receptor-binding function, which attaches the virus to the host cell. Secondly, they include a fusion protein function that can be activated to mediate fusion of viral and cellular membranes. Both tasks can be encoded by a single glycoprotein or by separate glycoproteins, which act in concert.

Three different classes of viral fusion proteins have been identified to date based on common structural motifs. These include class I fusion proteins, characterized by trimers of hairpins containing a central alpha-helical coiled-coil structure, class II fusion proteins, characterized by trimers of hairpins composed of beta structures, and class III proteins, forming trimers of hairpins by combining structural elements of both class I and class II fusion proteins (**Table 1**).

Viral glycoproteins interact with distinct cellular receptors by initiating conformational changes in the

fusion protein leading to membrane fusion. Fusion occurs either at the plasma membrane, where receptor binding triggers conformational changes in the glycoprotein, or in endosomes upon virus uptake by endocytosis. In the latter case the low pH environment of the endosome leads to protonation (key histidine residues have been specifically implicated in the process), which induces conformational changes that lead to fusion of viral and cellular membranes.

The biophysics of membrane fusion is dominated by the stalk hypothesis. According to this view, fusion of two lipid bilayers in an aqueous environment requires that they come into close contact associated with a significant energy barrier. This process involves local membrane bending creating a first site of contact. Complete dehydration of the initial contact site induces monolayer rupture that allows mixing of lipids from the two outer leaflets, resulting in a hemifusion stalk. In a next step, the model predicts that radial expansion of the stalk leads to either direct fusion pore opening or to the formation of another intermediate, the hemifusion diaphragm, an extended bilayer connecting both membranes. The hemifusion diaphragm may also expand into a fusion pore. Fusion pore formation, which is characterized by an initial opening and closing ('flickering') of the pore may be mediated by several factors such as lateral tension in the hemifusion stalk or bilayer and the curvature at the edges of the hemifusion state. Finally the fusion pore extends laterally until both membranes form a new extended lipid bilayer (**Figure 1**).

The applicability of the stalk model to viral membrane fusion processes is supported by a number of observations. Labeling techniques allow to distinguish between merging of lipid bilayers and content mixing thus visualizing intermediate steps in membrane fusion. This has been applied to several liposome fusion systems demonstrating that membrane fusion steps can be arrested at different stages. Furthermore, certain lipids such as inverted cone-shaped lysophospholipids induce spontaneous positive bilayer curvature and inhibit hemifusion, while cone-shaped phosphatidylethanolamines induce negative curvature and promote hemifusion. In contrast, the lipid effect on the opening of the fusion pore is the opposite. Finally, electron microscopy images of influenza virus particles fused with liposomes reveal structures resembling stalk intermediates.

These observations are consistent with the hypothesis that viral fusion proteins generate initial contacts between two opposing membranes and their extensive refolding

**Table 1**    Crystal structures of viral fusion proteins

| Virus family | Virus species | PDB code |
| --- | --- | --- |
| Class I | | |
| Orthomyxoviridae | Influenza A virus HA | 1HA0, 3HMG, *1HTM, 1QU1* |
| | Influenza C virus HEF | 1FLC |
| Paramyxoviridae | Simian parainfluenza virus 5 F | 2B9B,*1SVF* |
| | Human Parainfluenza virus F | 1ZTM |
| | Newcastle disease virus F | *1G5G* |
| | Respiratory syncytial F | *1G2C* |
| Filoviridae | Ebola virus gp2 | 1EBO, 2EBO |
| Retroviridae | Moloney Murine leukemia virus TM | 1AOL |
| | Human immunodeficiency virus 1 gp41 | 1ENV, 1AIK |
| | Simian immunodeficiency virus gp41 | 2SIV, 2EZO |
| | Human T cell leukemia virus 1 gp21 | 1MG1 |
| | Human syncytin-2 TM | 1Y4M |
| | Visna virus TM | 1JEK |
| Coronaviridae | Mouse hepatitis virus S2 | 1WDG |
| | Sars corona virus E2 | 2BEQ, 1WYY |
| Class II | | |
| Flaviviridae | Tick-borne encephalitis virus E | *1URZ,* 1SVB |
| | Dengue 2, and 3 virus E | 1OK8 IUZG, 10AN, 1TG8 |
| Togaviridae | Semliki forest virus E1 | 1E9W *1RER* |
| Class III | | |
| Rhabdoviridae | Vesicular stomatitis virus G | 2GUM |
| Herpesviridae | Herpes simplex virus gB | *2CMZ* |

regulates and facilitates fusion via lipidic intermediate states by lowering the energy to form stalk-like intermediate structures.

## Class I Fusion Glycoproteins

### Biosynthesis of Fusion Proteins

Class I fusion proteins are expressed as trimeric precursor glycoproteins that are activated by proteolytic cleavage with subtilisin-like enzymes such as furin. This produces a receptor-binding subunit that is either covalently or non-covalently attached to the membrane fusion protein subunit, which anchors the heterotrimer to the viral membrane. The endoproteolytic cleavage positions a hydrophobic fusion peptide at or close to the N-terminus of the fusion domain. Subtilisin-like proteases recognize a conserved multibasic recognition sequence R-X-K/R-R or a monobasic cleavage site present in various glycoproteins. The nature of the cleavage site and its efficient cleavage (e.g., influenza virus hemagglutinin) has been associated with pathogenicity. The multibasic recognition sequences present in influenza virus HA, *SV5* F protein, *HIV*-1 gp160, and Ebola virus GP lead to mostly intracellular processing, whereas monobasic cleavage sites in Sendai virus F protein or influenza virus HA are efficiently cleaved extracellularly, resulting in a more tissue-restricted distribution of these viruses.

Cleavage activates the fusion potential of the viral - glycoproteins and is required for most class I glycoprotein-mediated fusion events. Although some evidence suggests Ebola virus processing by furin is not required for entry, it still requires the activity of endosomal cysteine proteases for efficient entry. Proteolytic cleavage thus generates in most cases a metastable glycoprotein structure that can switch into a more stable structure upon cellular receptor interaction including proton binding in the acidic environment of endosomes. This metastability was first recognized to play an important role in influenza virus hemagglutinin-mediated entry and has since been associated with all class I glycoproteins.

### Structure of Native Influenza Virus Hemagglutinin

Since the structure solution of influenza virus hemagglutinin (HA), HA has served as the prototype of a class I fusion protein. The $HA_1$ domain, which contains the receptor-binding domain, folds into a beta structure that binds sialic acid-containing cellular receptors at the top of the molecule. In addition, both N- and C-termini of $HA_1$ interact with the stem of fusion domain $HA_2$ in an extended conformation. $HA_2$ anchors hemagglutinin to the viral membrane and folds into a central triple-stranded coiled-coil structure that is followed by a loop region and an antiparallel helix, which extends towards the N-terminal fusion peptide that is buried within the trimer interface (**Figure 2**).
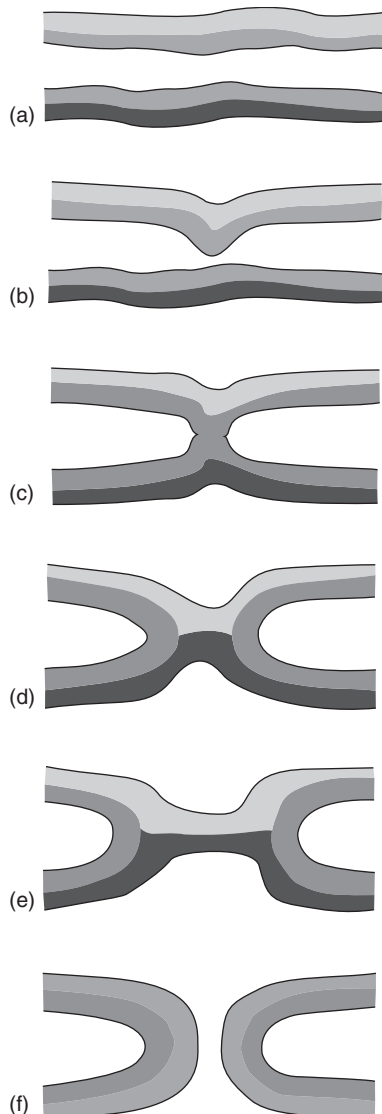
**Figure 1** Fusion of two lipid bilayers. (a) Two parallel lipid bilayers do not approach closely. (b) Close contact mediated by local membrane bending. Hemifusion stalks with contact of outer leaflets (c) and inner leaflets (d). Fusion pore opening (f) may proceed directly from the stalk structure (d) or via a hemifusion diaphragm (e).
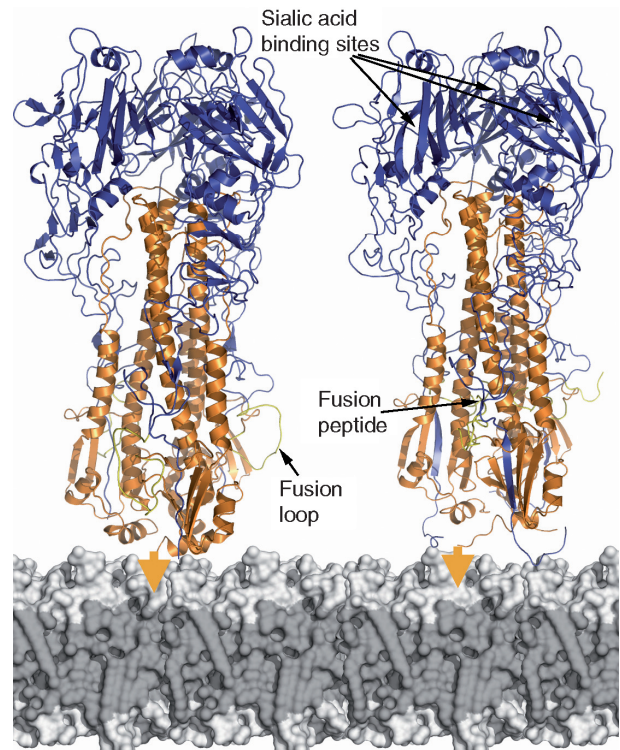


**Figure 2** Ribbon diagram of trimeric conformations of influenza A virus hemagglutinin before (left, pdb code 1HA0) and after (right, pdb code 3HMG) proteolytic processing. The $HA_1$ receptor-binding domain is shown in blue and the positions of the sialic acid-binding sites are indicated. The fusion protein subunit $HA_2$ is shown in orange. The orientation towards the lipid bilayer is indicated by the orange triangle.

## Structure of the Precursor Influenza Virus Hemagglutinin $HA_0$

The structure of uncleaved influenza virus $HA_0$ shows that only 19 residues around the cleavage site are in a conformation, which is different from the one seen in the native cleaved HA structure. This difference entails an outward projection of the last residues of $HA_1$ (323–328) and the N-terminal residues of $HA_2$ (1–12), resulting in the exposure of the proteolytic cleavage site (**Figure 2**). Upon cleavage, $HA_2$ residues 1–10 fill a mostly negatively charged cavity adjacent to the cleavage site, which leads to the sequestering of the fusion peptide within the trimeric structure (**Figure 2**).

## Structure of the Low pH-Activated Conformation of Hemagglutinin $HA_2$

Low pH destabilizes the $HA_1$ trimer contacts, which causes the globular head domains to dissociate. This movement facilitates two major conformational changes. (1) A loop region (residues 55–76) refolds into a helix (segment B in HA) and extends the central triple-stranded coiled-coil in a process that projects the fusion peptide approximately $100 \, \text{Å}$ away from its buried position in native HA. (2) Another dramatic change occurs toward the end of the central triple-stranded coiled-coil, where a short fragment unfolds to form a reverse turn which positions a short helix antiparallel against the central core. This chain reversal also repositions a β-hairpin and the extended conformation that leads to the transmembrane region (**Figure 3**). Although its orientation changes, the core structure of the receptor-binding domain $HA_1$ does not change upon acidification.

Since both the neutral pH structure and the core of the low pH structure from hemagglutinin have been solved, a number of class I fusion protein structures have been determined and a common picture has emerged for their mode of action (**Table 1**). The characteristic of all class I
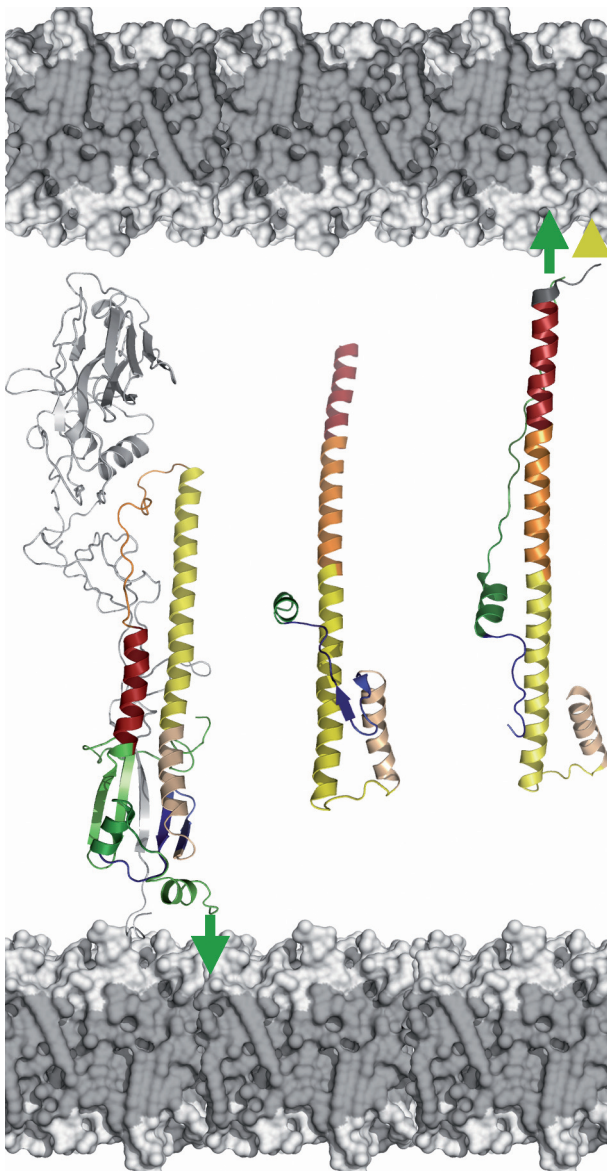
assumed that they all represent the postfusion conformation. Although there is only structural evidence for extensive conformational rearrangement of the fusion protein subunit in case of hemagglutinin HA and the paramyxovirus F protein (**Figure 4**), it is assumed that all known class I fusion protein core structures are the product of conformational rearrangements induced by receptor binding.

## Class I-Mediated Membrane Fusion

The positioning of the N- and C-terminal ends containing the fusion peptide and the transmembrane region at the same end of a core structure, which was first established for the *HIV*-1 gp41 core structure, led to the proposal of the following general fusion model. (1) Proteolytic cleavage activation transforms the glycoprotein into a metastable conformation. (2) Receptor binding induces conformational changes in the fusion protein that exposes the fusion peptide and allows fusion peptide interactions with the target membrane (**Figures 5(a) and 5(b)**). This generates a prehairpin intermediate structure that can be targeted by fusion inhibitors such as the HIV-1-specific T-20 peptide (**Figure 5(b)**). (3) Extensive refolding of the fusion domain most likely requires the dissociation of the C-terminal regions (**Figures 5(b) and 5(c)**, indicated by blue lines) and leads to the apposition of the two membranes, concomitantly with the zipping up of the C-terminal region against the N-terminal coiled-coil domain ultimately forming the hairpin structure (**Figures 5(c) and 5(d)**). The complete refolding process is thought to pull the two membranes into close-enough proximity to concomitantly allow membrane fusion. The extensive rearrangement of the fusion protein is thought to control the formation of different intermediate bilayer structures such as the hemifusion stalk (**Figure 5(d)**), and or the hemifusion diaphragm, followed by fusion pore opening and expansion (**Figure 5(e)**). It is generally assumed that membrane fusion occurs while the helical hairpin structure is formed and the core fusion protein structures represent postfusion conformations. Refolding of the fusion protein might produce defined stable intermediate structures, as suggested by the two low pH structures of influenza virus HA$_2$. One indicates that most of the outer layer has not yet zipped up to form the hairpin structure (**Figure 3**, middle panel: the C-terminal ends could extend back to the transmembrane region), while the other one reveals the extended conformation of the outer layer which forms together with the N-terminal coiled-coil a stable N-capped structure (**Figure 3**, right panel). Stepwise refolding may thus lock the fusion process irreversibly at distinct steps in agreement with a general irreversibility of class I-mediated fusion processes. The two membrane anchors, which are not present in the fusion conformation structures, also play an active



**Figure 3** Ribbon representations of the conformational changes in HA$_2$ upon low pH exposure. Only one monomer is shown for clarity. Left panel: Native cleaved HA (pdb code 3HMG), HA$_1$ in gray, the secondary structure elements for HA$_2$ that change are shown in different colors. Middle panel: Low pH HA$_2$ (pdb code 1HTM) projecting the N-terminus leading to the fusion peptide towards the target cell membrane. Right panel: The C-terminal region has completely zipped up against the N-terminal coiled-coil domain (pdb code 1QU1). The membrane orientations of the TM region and the fusion peptide are indicated by green arrows and yellow triangles, respectively.

fusion protein cores is their high thermostability suggesting that they represent the lowest energy state of the fusion protein. Secondly, they all contain a central triple-stranded coiled-coil region with outer C-terminal antiparallel layers that are either mostly helical or adopt extended conformations, thus forming trimers of helical hairpins. Since they resemble the low pH form of HA, it is
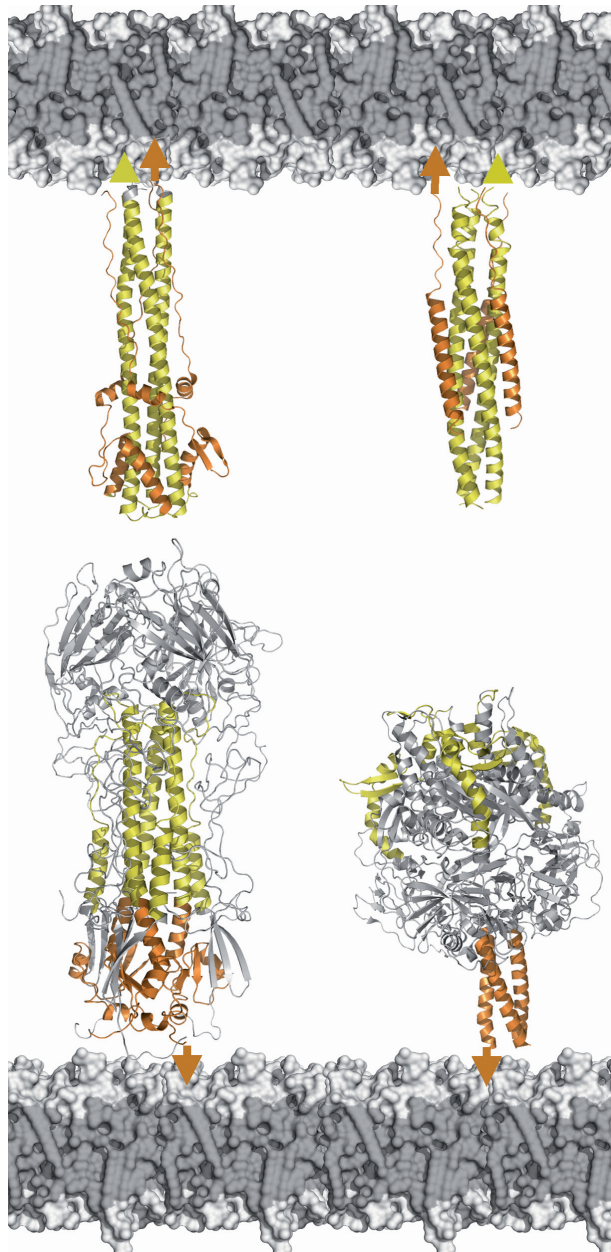
**Figure 4** Comparison of the conformational changes induced upon receptor binding of two class I fusion proteins, influenza A virus hemagglutinin (left panel, pdb codes 3HMG and 1QU1) and paramyxovirus F protein (right panel, Simian parainfluenza virus 5 F, pdb codes 2B9B and 1SVF). The lower panel shows ribbon diagrams of native HA and F with secondary structure elements that change conformation upon activation highlighted in two colors (the inner triple-stranded coiled-coil region of the postfusion conformation in yellow and the C-terminal layers in orange). Although both native structures differ quite substantially, the conformational changes result in similar hairpin structures (upper panel) orienting both membrane anchors toward the target cell membrane. The membrane orientations of the TM region and the fusion peptide are indicated by orange arrows and yellow triangles, respectively.

role in the fusion reaction. Replacement of the transmembrane region by a glycophosphatidylinositol anchor leads to a hemifusion phenotype in case of hemagglutinin-driven membrane fusion, highlighting the role of the transmembrane region. Furthermore the C-terminal membrane proximal region plays an important role in fusion as shown in the case of *HIV*-1 gp41-mediated fusion.

## Fusion Peptide

Class I fusion peptide sequences vary between virus families and are usually characterized by their hydrophobicity and a general preference for the presence of glycine residues. Although most fusion peptides of class I fusion proteins locate to the very N-terminus of the fusion protein, a few are found within internal disulfide-linked loops (e.g., filovirus Gp2 and the Avian sarcoma virus fusion proteins). NMR studies on the isolated influenza virus hemagglutinin fusion peptide revealed a kinked helical arrangement, which was suggested to insert into one lipid bilayer leaflet. This mode of bilayer interaction was proposed not only to mediate membrane attachment but also to destabilize the lipid bilayer. A further important function of fusion peptide sequences might be their specific oligomerization at the membrane contact site, which might constitute sites of initial membrane curvature.

## Cooperativity of Fusion Proteins

A number of studies suggest that more than one class I fusion protein trimer is required to promote class I-driven membrane fusion. It has been suggested that activated hemagglutinin glycoproteins interact with each other in a synchronized manner and cooperativity of refolding allows the synchronized release of free energy required for the fusion process. This implies that activated fusion proteins assemble into a protein coat-like structure that helps to induce membrane curvature, possibly also by inserting the fusion peptides into the viral membrane outside of the direct virus–cell contact site. However, it should be noted that no clearly ordered arrays of activated class I glycoproteins have yet been observed experimentally.

## Role of Lipids in Fusion

The lipid content of a viral envelope such as that of *HIV*-1 was shown to contain mostly lipids normally present in lipid raft microdomains at the plasma membrane. Lipid rafts are small ordered lipid domains that are enriched in cholesterol, sphingomyelin, and glycosphingolipids. A number of enveloped viruses (e.g., influenza virus, HIV-1, Ebola virus, measles virus) use these platforms for assembly and budding, and some evidence suggest
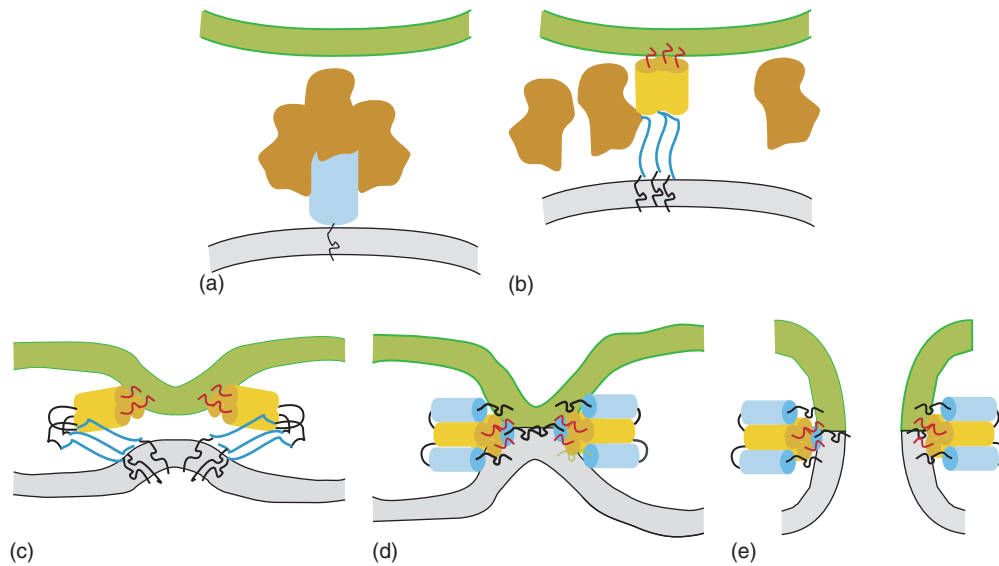
**Figure 5**   Model for class I glycoprotein-mediated membrane fusion. See text for explanation. The receptor-binding domains are indicated in brown and the fusion protein as cylinders. Note that some fusion proteins such as F from paramyxoviruses associate with an attachment protein (HN, H, or G). The latter interacts with F and cellular receptors triggering F-mediated fusion at the plasma membrane.

that raft platforms are also required for virus entry. Since the fusion activity of viral glycoproteins such as *HIV*-1 Env is affected by cellular receptor density as well as Env glycoprotein density, it has been suggested that both ligands have to be clustered efficiently to cooperatively trigger productive Env-mediated membrane fusion. This observation is consistent with the sensitivity of *HIV*-1 entry to cholesterol depletion.

## Class II Fusion Proteins

### Biosynthesis of Fusion Proteins

Class II fusion proteins comprise the fusion proteins from positive-strand RNA viruses such as the *Togaviridae* family, genus *Alphaviruses* (e.g., *Semliki Forest virus* (SFV)), and the *Flaviviridae* (e.g., *Dengue, Yellow fever,* and *Tick-borne encephalitis* virus (TBE)) (**Table 1**). Flaviviruses express the glycoprotein E that associates with a second precursor glycoprotein prM, while alphaviruses express two glycoproteins, the fusion protein El and the receptor-binding protein E2. E1 associates with the regulatory precursor protein p62. Both E-prM and E1-p62 heterodimerization are important for folding and transport of the fusion proteins. Cleavage of the fusion protein chaperones p62 and prM by the cellular protease furin in the secretory pathway is a crucial step in the activation of E and E1 fusion proteins.

### Structure of the Native Fusion Protein

The native conformations of the flavivirus E glycoproteins and that of the alphavirus E1 glycoproteins are similar and fold into three domains primarily composed of β-sheets, with a central domain I, flanked by domain III connecting to the transmembrane region on one side and domain II on the other side (**Figure 6**, lower panel). Domain II harbors the fusion loop that is stabilized by a disulfide bridge and mostly sequestered within the antiparallel flavivirus E glycoprotein homodimer. In analogy, the fusion loop might be sequestered within the *SFV* E1–E2 heterodimer. Dimeric E–E and E1–E2 interactions keep the glycoproteins in an inactive, membrane-parallel conformation that covers the viral membrane. *SFV* E1–E2 heterodimers form an icosahedral scaffold with $T = 4$ symmetry. Similarly, flavivirus E homodimers completely cover the viral membrane surface. The arrangement of the class II glycoproteins is thus completely different from the appearance of class I glycoprotein spikes, which do not form a specific symmetrical protein coat. In addition to forming the outer protein shell, flavivirus E and alphavirus E2 interact with cellular receptors, which direct the virion to the endocytotic pathway.

### Structure of the Activated Fusion Protein

There are only minimal changes in secondary structure during the low pH-induced rearrangement of *TBE* E and *SFV* E1. However, the conformational changes result in an approximate 35–40 Å movement of domain III and a rotation of domain II around the hinge axis connecting domains I and II. This rearrangement produces a hairpin-like structure with a similar functional architecture as class I fusion proteins (**Figure 6**, upper panel). The outside of the trimer reveals a groove that was suggested to
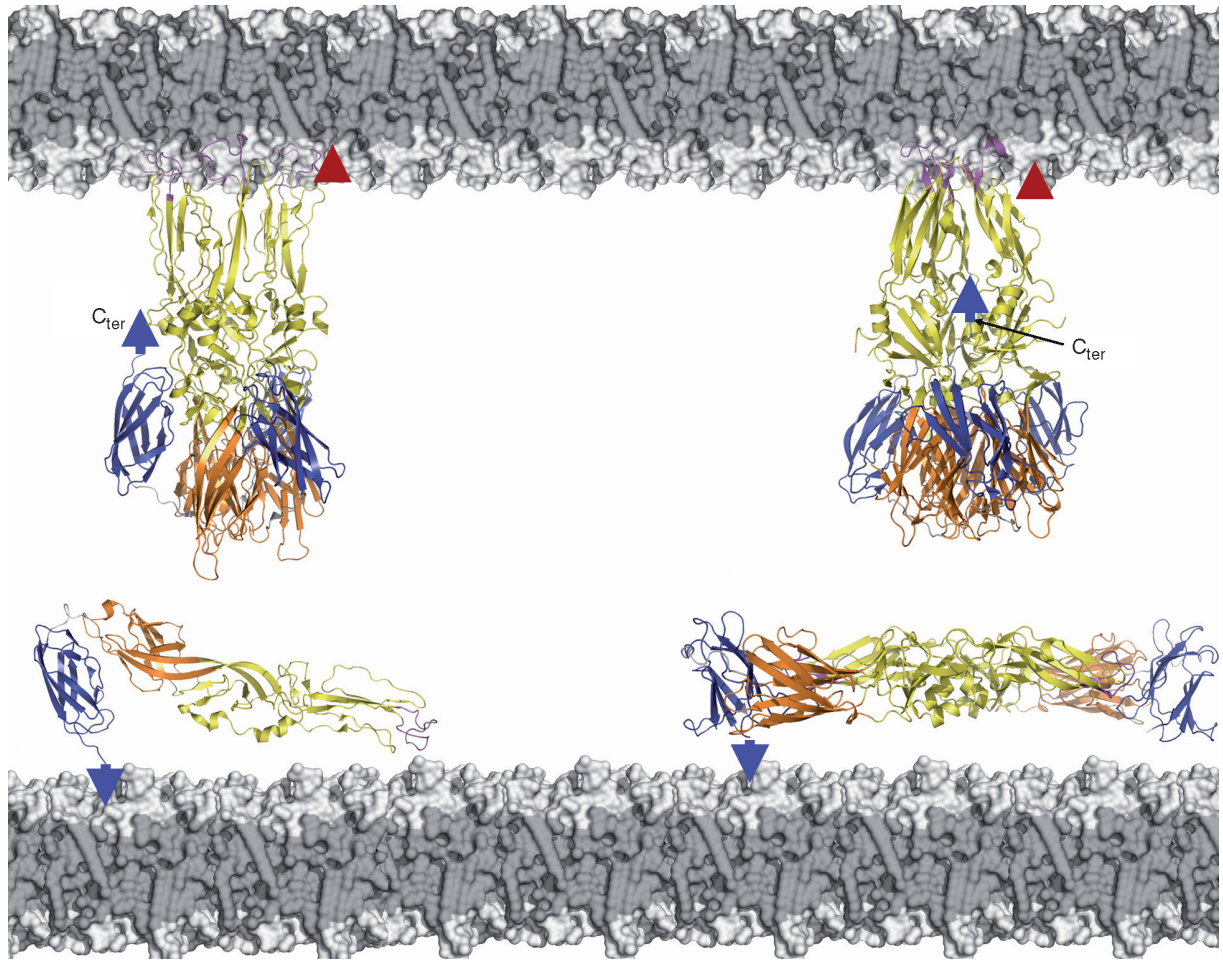
**Figure 6** Ribbon diagram of the structures of *SFV* E1 (left panel, pdb codes 2ALA and 1RER) and of the *TBE* E (right panel, pdb codes 1URZ and 1SVB) in their native dimeric state (lower panel) and low-pH-activated trimeric conformation (upper panel). The three main domains of E1 and E are colored differently: domain I in blue, domain II in orange, and domain III in yellow. In both cases, activation of the conformational changes leads to trimeric hairpin structures. The membrane orientations of the TM region and the fusion peptide are indicated by blue arrows and red triangles, respectively.

accommodate the segment, which connects to the transmembrane anchor and thus positions the fusion loops next to the membrane anchors. One significant difference between the *TBE* E and *SFV* E1 low pH conformations are the orientations of the fusion loops. *TBE* E fusion loops undergo homotrimer interactions, while *SFV* E1 fusion loops do not interact within trimers (**Figure 6**).

## Class II-Mediated Membrane Fusion

At the low pH of endosomes E and E1 undergo conformational rearrangements that involve three major steps. Firstly, the homo- or heterodimers dissociate from the membrane-parallel conformation in a reversible manner assuming monomeric fusion proteins that expose their fusion loop to the target membrane (**Figures 7(a) and 7(a′)**). This seems to be a main difference between class I and class II fusion, since trimer dissociation into monomers has not been implicated in any class I fusion pathway.

Secondly, fusion loop membrane interaction leads to the formation of homotrimers with an extended conformation. Trimerization is irreversible and tethers the fusion protein to the target membrane (**Figure 7(b)**). It is comparable to the postulated prehairpin structure of class I fusion proteins such as *HIV*-1 gp41 (**Figure 7(b)**). Notably, both fusion intermediates can be targeted by either fusion protein peptides (T-20 in case of *HIV*-1 gp41) or recombinant fusion protein domains (such as the E3 domain in case of *TBE*), to block membrane fusion. Further refolding, namely the reorientation of domain I, then pulls the two membranes into closer apposition that ultimately leads to the formation of a hemifusion stalk-like structure (**Figures 7(c) and 7(d)**). Finally complete zipping up of the C-terminal ends against the N-terminal core domains allows fusion pore opening and its expansion (**Figure 7(e)**). Similar to the case of class I fusion protein-driven fusion, refolding is thought to provide the energy for fusion (**Figure 7**).
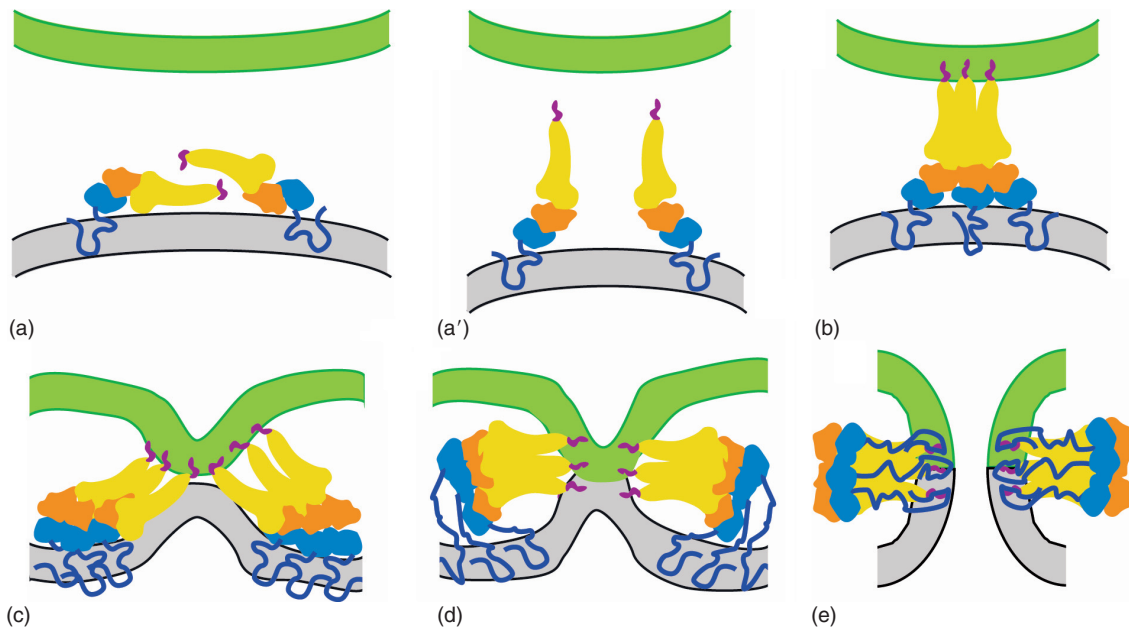
**Figure 7**  Model for membrane fusion of class II fusion proteins. See text for explanation. The three domains are colored as in **Figure 6**.

### Fusion Peptide

The native and low-pH-induced crystal structures of the *TBE* virus E, dengue fever virus E, and *SFV* E1 proteins reveal that the conformation of the fusion loop changes upon acidification. The low-pH structures indicate that only hydrophobic side chains of the loop insert into the hydrocarbon chains of the outer leaflet of a target membrane. This is sufficient to anchor the fusion protein to the host cell membrane. Further oligomerization of fusion loops, as shown in the case of the low pH form of *SFV* E1, where crystal packing analysis revealed fusion loop interactions between trimers, was suggested to induce local membrane deformation, such as induction of a nipple-like membrane deformation (**Figure 7(c)**). This has been predicted in the stalk model to play an important role in the generation of lipidic intermediates during membrane fusion. Therefore the *SFV* E1 conformation might reflect an intermediate fusion state preceding the suggested postfusion conformation of flavivirus E trimers with homotrimeric fusion loop interactions. *In vivo*, the latter conformation might be induced by the final refolding of the C-terminal membrane proximal region and thus determining 'open' and 'closed' conformations of *SFV* E1 trimers and *TBE virus* E trimers, respectively (**Figure 6**).

### Fusion Protein Cooperativity in Membrane Fusion

Homo- or heterodimeric class II fusion proteins already form a protein shell covering the complete viral membrane in the native state. Upon activation *in vitro*, both, soluble *SFV* E1 protein and flavivirus E protein insert their fusion loops into liposomes and form arrays of trimers organized in a lattice composed of rings of five or six. The E protein lattice on liposomes contains preferentially rings of five, which seems to affect the curvature of coated liposomes. In contrast rings of six form mostly flat hexagonal arrays *in vitro*. E1 pentameric rings can also be reconstructed from the crystal packing of E1 trimers. This strongly suggests that formation of a distinct fusion protein lattice might exert a cooperative effect on the fusion process.

### Role of Lipids in Fusion

Although heterodimer dissociation exposes the *SFV* E1 fusion loop, its insertion into a lipid bilayer requires low pH triggering and cholesterol, which is consistent with the observation that *SFV* fusion depends on cholesterol and sphingolipids. The lipids required for E1 activation and fusion imply indirectly that lipid raft microdomains might be targeted for fusion. Flavivirus fusion, however, seems to be less dependent on cholesterol than alphavirus fusion.

### Class III Fusion Proteins

#### Biosynthesis of Fusion Proteins

The glycoprotein G from vesicular stomatitis virus (VSV), a member of the *Rhabdoviridae* (e.g., *VSV* and *Rabies virus*), negative-strand RNA viruses, and gB from Herpesvirus,

a member of the *Herpesviridae*, double-stranded DNA viruses, constitute a third class of viral fusion proteins based on the structural similarity of the postfusion conformation of their respective glycoproteins. Unlike class I and II envelope proteins both, *VSV* G and herpesvirus gB, are neither expressed as precursor proteins nor are they proteolytically activated.

Rhabdoviruses express a single trimeric glycoprotein G, which acts as receptor-binding domain to induce endocytosis and as the fusion protein that controls fusion with endosomal membranes upon acidification. However, different from class I and class II fusion machines the conformational changes induced by low pH are reversible. Changes in pH can easily revert the three proposed conformations of G, the native conformation as detected on virions, an activated state that is required for membrane interaction, and an inactive postfusion conformation.

Herpesvirus entry and fusion is more complex since it requires four glycoproteins, namely gD, gH, gL, and gB. Glycoprotein gD contains the receptor-binding activity and associates with gB as well as gH and gL. While gB seems to constitute the main fusion protein, the others are thought to be required for activation of the fusion potential of gB, which is pH independent.

### Structure of the Low-pH-Activated VSV Glycoprotein and Herpesvirus gB

*VSV* G is composed of four domains that, interestingly, show similarities to both class I and class II fusion protein structures. It contains a β-sheet-rich lateral domain at the top, a central α-helical domain that mediates trimerization, and resembles the α-helical hairpin structure of class I fusion molecules, a neck domain containing a pleckstrin homologous (PH) domain, and the fusion loop domain that builds the trimeric stem of G. This stem-like domain exposes two loops at its very tip containing aromatic residues constituting the membrane-interacting motif of G. The stem domain resembles that of class II fusion proteins, albeit its different strand topology, which could be the result of convergent evolution. Although the complete C-terminus is not present in the structure, it points towards the tip of the fusion domain, indicating that both membrane anchors, the fusion loops and the transmembrane region, could be positioned at the same end of an elongated hairpin structure (**Figure 8**). The overall similarity of the structural organization of *VSV* G with that of herpesvirus gB indicates a strong evolutionary relationship between the rhabdovirus G and herpesvirus gB fusion proteins (**Figure 8**).

### The Fusion Loops

The fusion loops extending from the stem-like domain of *VSV* G is similar to those observed in class II fusion proteins. The architecture is such that only few hydrophobic
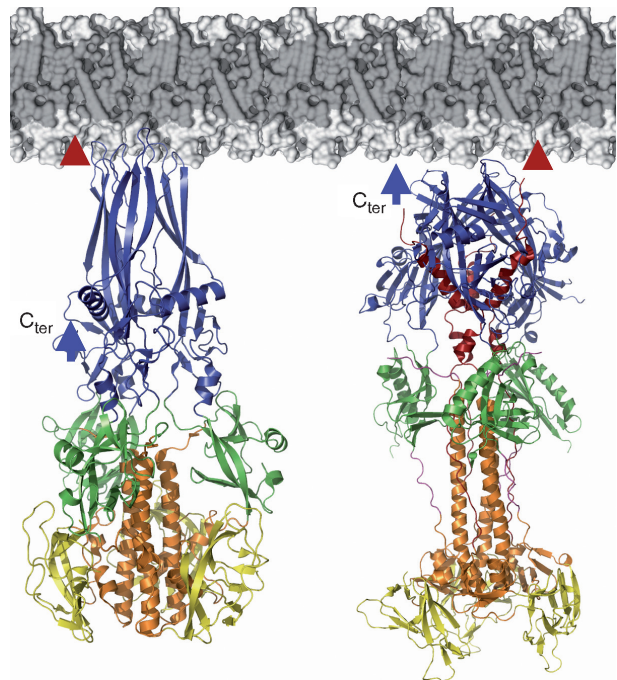


**Figure 8** Ribbon diagram of class III fusion glycoproteins from *VSV* G (left panel, pdb code 2CMZ) and Herpesvirus gB (right panel, pdb code 2GUM). The individual domains are colored as follows: domain I in yellow, domain II in orange, domain III in green, and domain IV in blue. Their orientation toward the target membrane is indicated and shows the attachment of the putative fusion loops to one leaflet of the bilayer (red triangles) and the putative orientation of the TM region toward the same end of the fusion loop region (blue arrow).

side chains can intercalate into one lipid bilayer leaflet, potentially up to 8.5 Å. Intercalation of side chains into one leaflet may induce curvature of the outer leaflet with respect to the inner leaflet, which would satisfy the stalk model. The role of lipidic intermediate states, including the hemifusion state, has been confirmed experimentally in case of rhabdovirus G-mediated fusion.

Since both *VSV* G and herpesvirus gB resemble class I and class II fusion proteins which adopt a hairpin conformation with both membrane anchors at the same end of the molecule, it is most likely that they follow very similar paths in membrane fusion as suggested for class I and class II fusion proteins.

### Summary

Although accumulating structural evidence suggests that the structural motifs used by viral fusion proteins and the mode of their extensive refolding varies substantially, the final product, namely the generation of a hairpin-like structure with two membrane anchors at the same end

of an elongated structure, is maintained in all known postfusion conformations of viral glycoproteins. Thus the overall membrane fusion process is predicted to be the same for class I, II, and III fusion proteins, although the kinetics of refolding and fusion might vary to a large extent due to the involvement of different structural motifs to solve the problem of close apposition of two membranes.

*See also:* Metaviruses.

## Further Reading

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# Metaviruses

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## Glossary

**Erranti** Comes from the Latin *errans*, meaning to wander.

**Integration** The insertion of cDNA into the genome of a host cell.

**Long terminal repeats (LTRs)** Sequence repeats on both ends of retroviruses and many retrotransposons that play a critical role in reverse transcription.

**LTR-retrotransposon** A form of transposable element that propagates by the reverse transcription of an RNA intermediate and the subsequent integration of the cDNA.

**LTR-retroelements** Include any genetic element with LTRs. They are retroviruses and LTR-retrotransposons regardless of whether they possess env or env-like proteins.

**Meta** Comes from the Greek metathesis for 'transposition'. Also to indicate uncertainty regarding whether these are true viruses.

**Semoti** Comes from the Latin *semotus*, meaning 'distant' or 'removed'. This refers to the large evolutionary distance between semotiviruses and members of the other two genera of the family *Metaviridae*.

## Introduction

The family *Metaviridae* includes a vast number of genetic elements that populate the genomes of eukaryotes. They possess two long terminal repeats (LTRs) that flank coding sequences for the capsid protein Gag, and the enzymes protease (PR), reverse transcriptase (RT), and integrase (IN) (**Figure 1**). The assignment of elements to the *Metaviridae* versus other families with the same structure is based on phylogenetic relationships. Seven regions of RT sequence with strong homology are aligned and elements with similar sequence patterns are grouped into families such as the *Metaviridae*. In addition, elements belonging to the *Metaviridae* are distinguished from those in the family *Pseudoviridae* by the order of the coding sequences for RT and IN. While any element belonging to the *Metaviridae* contains RT sequence upstream of IN (**Figure 1**), members of the family *Pseudoviridae* encode IN before RT. Before the nomenclature for viruses and retrotransposons was standardized by the ICTV, the *Metaviridae* was named after two of its founding elements, gypsy/Ty3.

It is of great interest that many members of the *Metaviridae* possess envelope (env) proteins with similar structure to retrovirus env's, known for their role in particle release and infection (**Figure 1**). This presents the possibility that many elements first thought to be retrotransposons may actually be infectious viruses. These potential