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Membrane Fusion

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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 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 membrane fusion.

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The Fusion Loops

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Summary

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.

Stalk Two membranes getting into contact by mixing the lipids of their outer leaflets.

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

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to protonation (key histidine residues have been 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, which is associated with a significant energy barrier (Figure 1(a)). This process involves local membrane bending creating a first site of contact (Figure 1(b)). 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 (Figure 1(c) and 1(d)). 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 (Figure 1(e)). The hemifusion diaphragms that have been observed by electron microscopy and X-ray analysis of crystalline lipid phases provide evidence for stalk-like structures. Because the hemifusion diaphragm is a stable long-lived structure its formation could alternatively signify a dead end reaction. Fusion pore formation (Figure 1(f)), 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 stalk or hemifusion state. Finally the fusion pore extends laterally until both membranes form a new extended lipid bilayer.

The applicability of the stalk model to viral membrane fusion processes is supported by a number of observations. Labeling techniques applied to in vitro liposome-based fusion assays allow discrimination between merging of lipid bilayers and content mixing thus visualizing subsequent steps in membrane fusion. Distinct steps in the fusion pathway can be further manipulated by lipids. 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. Further support for the stalk model derives from electron microscopy images of influenza virus particles fusing with liposomes, which reveal structures resembling stalk intermediates.

These observations are consistent with the hypothesis that viral fusion proteins generate initial contacts between two opposing membranes and that their extensive refolding regulates and facilitates fusion via lipidic intermediate states by lowering the energy to form stalk-like intermediate structures that drive fusion.

### Table 1: Structure based classification of viral fusion proteins

| Virus family       | Genus               | Virus, fusion protein class | PDB code<sup>a</sup> | PDB code<sup>b</sup> |
|--------------------|---------------------|----------------------------|-----------------------|-----------------------|
| Orthomyxoviridae   | Influenza           | Class I                    | Native<sup>a</sup>    | Post fusion<sup>b</sup> |
|                    |                     |                            | 3HMG, 1HA0            | 1HTM, 1QU1            |
|                    |                     |                            | 1FLC                  |                       |
| Paramyxoviridae    | Rubulavirus         |                            |                       |                       |
|                    | Simian Parainfluenza virus | 5F                 | 1SVF                  |                       |
|                    | Human Parainfluenza virus F | 2B9B, 1ZTM       |                       |                       |
| Avulavirus         | Newcastle disease virus F |                    |                       |                       |
| Pneumovirus        | Respiratory syncytial virus F |                |                       |                       |
| Filoviridae        | Ebolavirus          |                            | 4JHW                  | 1GSC, 3RRT            |
| Arenaviridae       | Arenavirus          |                            | 1EBO                  |                       |
| Retroviridae       | gammaretrovirus      |                            | 3CSY                  |                       |
|                    | Moloney Murine leukemia virus, TM |          | 1MFO                  |                       |
|                    | Human endogenous retrovirus-FR, syncytin-2 |        | 1Y4M                  |                       |
| Lentivirus         | Human immunodeficiency virus 1 Env, gp140, gp41 | 4NCO             | 1ENV, 1AIK            |
|                    | Simian immunodeficiency virus gp41 |            | 2SIV, 2EZO            |
|                    | Visna virus TM       |                            | 1JEK                  |                       |
| Coronaviridae      | Betacoronavirus      |                            | 1WDG                  | 2BEQ, 1WYY            |
|                    | Mouse hepatitis virus S2 |                |                       |                       |
|                    | Sars corona virus E2 |                            |                       |                       |
|                    | Class II             |                            |                       |                       |
| Flaviviridae       | Flavivirus           |                            | 1SVB                  | 1URZ                  |
|                    | Tick-borne encephalitis virus E |          |                       |                       |
|                    | Dengue virus E       |                            | 10AN                  | 10K8                  |
|                    | Japanese encephalitis virus E |          |                       |                       |
|                    | West Nile virus E    |                            | 3P54                  |                       |
|                    | 2I69                 |                            |                       |                       |
| Togaviridae        | Alphavirus           |                            | 1J9W                  | 1RER                  |
|                    | Semliki forest virus E1 |                    |                       |                       |
|                    | Sindbis virus E1-E2  |                            | 3MUU (intermediate?)  |                       |
|                    | Chikungunya virus p62-E, E1-E2-E3 |        |                       |                       |
| Rubivirus          | Rubella virus E1     |                            | 3N44, 3N40            |                       |
|                    | Class III            |                            | 4ADJ                  |                       |
| Rhabdoviridae      | Rhabdoviridae        |                            | 2J6J                  | 2CMZ                  |
| Herpesviridae      | Herpesviridae        |                            | 2GUM                  | 3FVC                  |
| Baculoviridae      | Baculoviridae        |                            | 2DUZ                  |                       |
|                    | Autographa californica GP64 |                |                       |                       |

<sup>a</sup>PDB codes can be found at [http://www.rcsb.org](http://www.rcsb.org). Note that only the most representative PDB codes are listed.

<sup>b</sup>Native conformation, post fusion conformation.
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, Simian parainfluenza virus 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. The Ebola virus glycoprotein is not only processed by furin, but its glycoprotein subunit 1 (GP1) needs to be additionally cleaved by cathepsin proteases in the late endosome to activate its fusion potential during entry. Proteolytic glycoprotein cleavage generates in most cases a metastable glycoprotein structure that can switch into a more stable structure upon cellular receptor interaction or 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 publication of the first crystal structure of Influenza virus hemagglutinin (HA) by Wiley and co-workers in 1981, HA has served as the prototype of a class I fusion protein. The HA\textsubscript{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 with relatively low millimolar affinity (Figure 2). Productive virus attachment to host target cells requires multivalency of binding to achieve sufficient affinity that triggers endocytosis and entry via the endosomal compartment. The type of sialic acid recognized by hemagglutinin is host specific. Human viruses generally interact with α\textsubscript{2}-6-linked receptors and avian viruses, specific for α\textsubscript{2}-3-linked glycans, need to adapt to humanlike receptors to overcome the species barrier. HA\textsubscript{1} also interacts with the stem of the fusion domain HA\textsubscript{2} in an extended conformation via its N- and C-termini. HA\textsubscript{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 anti-parallel helix, which extends towards the N-terminal fusion peptide that is buried within the trimer interface (Figure 2).

Structure of the Precursor Influenza Virus Hemagglutinin HA\textsubscript{0}

The structure of uncleaved Influenza virus HA\textsubscript{0} shows that only 19 residues around the cleavage site are in a conformation different from the one observed in the native cleaved HA structure. This difference entails an outwards projection of the last residues of HA\textsubscript{1} (323–328) and the N-terminal residues of HA\textsubscript{2} (1–12), resulting in the exposure of the proteolytic cleavage site (Figure 2). Upon cleavage, the fusion peptide HA\textsubscript{2} residues 1–10 become sequestered within the trimeric structure and fill a negatively charged cavity adjacent to the cleavage site (Figure 2).

Structure of the Low pH Activated Conformation of Hemagglutinin HA\textsubscript{2}

Low pH such as the pH in endosomes, destabilizes the HA\textsubscript{1} trimer contacts, which causes the globular head domains to dissociate. This movement facilitates two major conformational changes. (i) 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 Å away from its buried position in native HA. (ii) Another dramatic change occurs towards the end of the central triple-stranded coiled coil, where a short fragment unfolds to form a reverse turn which positions a short helix anti-parallel 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\textsubscript{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 (Table 1) and a common picture emerged for their mode of action. The characteristic of all class I fusion protein core structures 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 anti-parallel layers that are either mostly helical or adopt extended conformations. The hallmark of the helical hairpin structure is the juxtaposition of the fusion peptide and of the transmembrane region at the same end of the rod-like structure. The post fusion structures are considered to be the product of conformational rearrangements in the native structure, as in case of influenza virus HA and Respiratory syncytia virus F (Figures 3 and 4). The fusion potential of most class I fusion proteins is generally activated by low pH or by direct interaction with cellular receptors. In contrast, most members of the Paramyxoviridae family require the concerted action of two glycoproteins. The receptor-binding protein, hemagglutinin-neuraminidase (HN), H or G binds to its cellular receptor and activates the fusion protein F via the stalk region as shown for Parainfluenza virus 5 receptor-binding protein HN.
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 (middle panel, *Simian parafluivirus* SF, pdb codes 2B9B and 1SVF) and left panel,
Class I Mediated Membrane Fusion

The HIV-1 gp41 core structure provided the first structural evidence for the positioning of the N- and C-terminal ends, the fusion peptide and the transmembrane region, at the same end of an elongated structure, which 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 glycoprotein that lead to the exposure of the fusion peptide and its interactions with the target membrane (Figure 5(a) and 5(b)). Exposure of the fusion peptide seems to be the rate limiting step in the process. This generates a prehairpin intermediate structure that is, at least in case of HIV-1 and influenza virus fusion, long-lived. Consequently this extended intermediate conformation can be targeted by fusion inhibitors such as the HIV-1 specific T-20 peptide (Figure 5(b)). (3) Cooperativity of two or three glycoproteins is important at this stage, because it is thought to facilitate the refolding that leads to the apposition of the two membranes leading to lipid mixing. Fusion protein refolding occurs most likely fast and involves zipping up of the C-terminal region against the N-terminal coiled coil domain, ultimately forming the hairpin structure (Figure 5(c) and 5(d)). Refolding 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 a potential hemifusion diaphragm, followed by fusion pore opening and expansion (Figure 5(e)). It is 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 may produce stable intermediate structures, as suggested by the two low pH structures of influenza virus HA2. 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 lower membrane that anchors native HA (left panel)), 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). Such HA intermediates in fusion have been detected experimentally and stepwise refolding may lock the fusion process at distinct steps. The two membrane anchors, which are not present in any class I fusion glycoprotein structure, also play an active role in the fusion reaction. Replacement of the transmembrane region by a glycosylphosphatidylinositol anchor arrests influenza virus HA-driven fusion as well as HIV-1 envelope glycoprotein driven fusion at the hemifusion stage. Furthermore, the membrane proximal external region (MPER) of the fusion protein, which in case of HIV-1 gp41 is conserved and mostly hydrophobic, may insert into the viral membrane during the fusion process. Although some studies suggest that class I fusion proteins maintain their trimeric structure throughout the fusion process, others indicate that the trimer symmetry is first broken and later on reassembled from monomers that themselves may interact with membranes via their hydrophobic surfaces.

human Respiratory syncytial virus, pdb codes 4JHW and 3RRT). 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 native structures of HA and F differ quite substantially, the conformational changes result in similar core hairpin structures (upper panel) orienting both membrane anchors towards the target cell membrane. The membrane orientations of the TM region and the fusion peptide are indicated by orange arrows and yellow triangles, respectively. The complete postfusion conformation of Respiratory syncytial virus F reveals that the top domain stays intimately associated with the fusion protein core (upper right panel). In contrast in case of Influenza virus HA, HA1 detaches from HA2 during fusion.
Fusion Peptide

Class I fusion peptide sequences vary between virus families and are often hydrophobic and glycine rich. Although most fusion peptides (FP) 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 structure and the Ebola virus Gp2 fusion loop was shown to adopt a fixed conformation, both of which are required for membrane fusion activity. It has been suggested that fusion peptides in general may not only anchor the glycoprotein to the target cell membrane, but may oligmerize at the target cell membrane and thereby destabilize the lipid bilayer and/or induce membrane curvature.

Class II Fusion Proteins

Biosynthesis of Fusion Proteins

Positive-strand RNA viruses employ class II fusion proteins for entry. These include the Togaviridae family with the genus alphaviruses (Sindbis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, Venezuelan equine encephalitis virus, Ross River virus, O’nyong’nyong virus, Chikungunya virus, Semiliki Forest virus) and the genus Rubivirus (Rubella virus) as well as the Flaviviridae family with the genera flavivirus (West Nile virus, dengue virus, tick-borne encephalitis virus, yellow fever virus), hepacivirus (Hepatitis C virus) and pestivirus (bovine viral diarrhea virus) (Table 1). Flaviviruses express the glycoprotein E that associates with a second precursor glycoprotein prM, while alphaviruses express two glycoproteins, the fusion protein E1 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 Flaviviridae E and Togaviridae E1 fusion proteins.

Structure of the Native Fusion Protein

The native conformations of the Flaviviridae E glycoproteins and that of the Togaviridae E1 glycoproteins (Table 1) 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 anti-parallel flavivirus E glycoprotein homodimer. In analogy, the fusion loop is probably sequestered within the Semiliki forest virus E1-E2 heterodimer and is sequestered in the Chikungunya virus E1-E2-E3 hetrotrimer (Figure 6, lower left panel; E1-E2-E3 is generated by furin cleavage activation of the heterodimer E1-p62). Dimeric E-E and E1-E2 interactions keep the glycoproteins in an inactive, membrane-parallel conformation that covers the viral membrane. Semiliki forest virus E1-E2 heterodimers form an icosahedral scaffold with \( T = 4 \) symmetry. Similarly, flavivirus

![Figure 6](image.png)
E homodimers completely cover the viral membrane surface. In summary, the conformation and domain organization of class II fusion glycoproteins is completely different from class I glycoprotein spikes, which do not form a specific symmetrical protein coat.

Structure of the Activated Fusion Protein

Flaviviridae E and Togaviridae E2 interact with cellular receptors, which direct the virions to the endocytotic pathway. There are only minimal changes in secondary structure during the low pH induced rearrangement of *Tick-borne encephalitis virus* E and *Semliki forest virus* E1 in endosomes. 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 accommodate the segment, which connects to the transmembrane anchor and thus positions the fusion loops next to the membrane anchors. Rearrangements of the C-terminal regions leading up the transmembrane domain have been elucidated in several structures. One significant difference between the *Tick-borne encephalitis virus* E and *Semliki forest virus* E1 low pH conformations are the orientations of the fusion loops. TBE E fusion loops undergo homo-trimer interactions, while *Semliki forest virus* 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 hetero-dimers dissociate from the membrane-parallel conformation (Figure 7(a)) in a reversible manner assuming monomeric conformations that likely expose their fusion loop to the target membrane (Figure 7(a')). The existence of this monomeric state of the glycoprotein is different from class I mediated fusion, although monomeric intermediates of class I fusion proteins have been debated. Fusion loop membrane interaction of monomers then triggers trimerization leading to an extended conformation. Trimerization is irreversible and tethers the fusion protein to the target membrane (Figure 7(b)). This structure is functionally comparable to the postulated prehairpin structure of class I fusion proteins such as HIV-1 gp41 (Figure 4(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 *Tick-borne encephalitis virus*), to block further glycoprotein refolding and thus membrane fusion. Subsequent additional refolding, namely the reorientation of domain I, pulls the two membranes into closer apposition that ultimately leads to the formation of a hemifusion stalk-like structure (Figure 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).

Fusion Peptide

The native and low pH induced crystal structures of the TBE virus E, *Dengue fever virus* E and SFV E1 proteins indicate that the conformation of the fusion loop changes upon acidification. The low pH structures suggest that hydrophobic side chains of the loop may 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, was suggested to induce local membrane deformation as postulated in the stalk model (Figures 1(c) and 7(c)). Such fusion loop oligomerization is supported by fusion loop interactions observed in the low pH structure of *Semliki forest virus* E1. Therefore, this E1 conformation may represent an intermediate fusion state preceding the suggested postfusion conformation of flavivirus E trimers with homo-trimeric fusion loop interactions. *In vivo,*
the latter conformation may be induced by the final refolding of the C-terminal membrane proximal region and thus determining ‘open’ and ‘closed’ conformations of Semliki forest virus E1 trimers and Tick-borne encephalitis virus E trimers, respectively (Figure 6).

**Fusion Protein Cooperativity in Membrane Fusion**

Homo- or heterodimeric class II fusion proteins form a protein shell covering the complete viral membrane in the native state. Upon activation in vitro, both, soluble Semliki forest virus 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 indicates that formation of a distinct fusion protein lattice may exert a cooperative effect on the fusion process.

**Class III Fusion Proteins**

**Biosynthesis of Fusion Proteins**

The glycoprotein G from Vesicular stomatitis virus (VSV), a member of the negative strand RNA virus family, the Rhabdoviridae (e.g., VSV and Rabies virus) constitutes the prototype member of class III fusion proteins, with structural similarity to both class I and II fusion proteins. Unlike class I and II envelope proteins both, VSV G is not expressed as a precursor protein that requires proteolytic activation.

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 II fusion machines, the conformational changes induced by low pH are reversible. Changes in pH can easily revert between 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.

A second member of class III mediated fusion is Herpes virus a member of the double-stranded DNA virus Herpesviridae family. However, Herpes 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. The third member of class III mediated fusion is the baculovirus (Autographa californica GP64), which behaves similar to Vesicular stomatitis virus G and enters via the endosomal pathway (Table 1).

**Structure of the Native VSV Glycoprotein**

VSV G adopts a tripod-like structure composed of three domains. The N-terminal central part contains a β sheet rich region, the lateral domain DI that connects the C-terminus to the transmembrane region and harbors a helical domain that mediates trimerization (domain DII). This is followed by the top domain containing a pleckstrin homology (PH) domain (domain DIII), which has the fusion domain inserted (domain DIV). The fusion loops are exposed at one end of the fusion domain and point towards the viral membrane. Overall the organization of the fusion domain is similar to the class II fusion domain. The central part, DI and DII that mediates trimerization, packs against the top domain and the linker of the fusion domain. The C-terminal end present in the structure interacts with the β sheet of the fusion domain (Figure 8).

**Structure of the Low pH Activated VSV Glycoprotein**

Upon acidification, Vesicular stomatitis virus G undergoes a substantial rearrangement of its domains with little secondary structure change in the fusion domain DIV and domain DIII containing the PH domain. In contrast DII rearranges to form a helical hairpin structure that mediates trimerization at one end and resembles the hairpin structures characteristic for class I fusion proteins (Figure 8). The following conformational rearrangements have been suggested, based on the comparison of the native and the post fusion conformations. Considering the position of DIII as invariant, low pH projects the fusion domain DIV towards the target membrane by two movements: a 94° rotation around a hinge region present between DIV and DIII. This arrangement could bridge both viral and target cell membranes. The second movement, the formation of the six-helical bundle structure (DIII) with the chain reversal of the C-terminus will project D1 at one end of the rod positioned laterally of DII and at the opposite end of the fusion domain DIV. This will position the C-terminus connecting to the transmembrane region next to the fusion loops. The fusion loops which are splayed apart in the native structure are centered at one end of the elongated structure and the fusion domain DIV contributes to trimerization. Overall G contains elements of class II (fusion domain, DIV) and class I (helical hairpin in the post fusion conformation, DII) fusion proteins. The overall domain rearrangements are similar to those of class II fusion proteins and the secondary structure changes leading to the formation of the helical hairpin (DII) resembles class I fusion proteins (Figure 8). Interestingly, the conformational changes of Vesicular stomatitis virus G are reversible, which underscores an important difference from class I and class II fusion proteins. It was suggested that Vesicular stomatitis virus G exists in equilibrium between trimers and monomers at the viral surface under physiological conditions. At a slightly acidic pH (pH 6.7) the monomer extends into an
Figure 8  Ribbon diagrams of the class III fusion glycoproteins from VSV G at physiological (lower panel, pdb code 2J6J) and at low pH (upper panel, pdb codes 2CMZ). The individual domains are colored as follows: domain I in red, domain II in blue, domain III in orange and domain IV in yellow. The orientations of the fusion loops (yellow arrow) and the transmembrane regions (blue arrow) are indicated. It is assumed that fusion loops that point towards the membrane in the native state do not interact with the membrane (lower panel). In contrast, the pro fus ion conformation implies that the fusion loops interact with the bilayer.
elongated intermediate, which refolds into the post fusion structure as a monomer which subsequently trimerizes. Alternatively, the extended monomeric intermediate trimerizes and subsequently refolds into the postfusion conformation as a trimer. Notably, the pH-regulated conformations of baculovirus gp64 are also reversible and further underline that these fusion proteins may derive from a common ancestor.

The Fusion Loops

The fusion loops extending from the stem-like domain of VSV G are similar to those observed in class II fusion proteins. The architecture is such that it was proposed that only few hydrophobic side chains intercalate into one lipid bilayer leaflet, potentially up to 8.5 Å. This membrane intercalation of side chains may induce curvature of the outer leaflet with respect to the inner leaflet, which would satisfy the stalk model. The role of lipodic intermediate states, including the hemifusion state, has been confirmed experimentally by analyzing Rabies virus G catalyzed fusion reactions.

Role of Cholesterol in Membrane Fusion

The concentration of cholesterol present in the target cell membrane has been shown to be critical for entry of many enveloped viruses, including class I, II and III fusion protein members. Notably, artificial depletion of cholesterol from cells inhibits membrane fusion. Consistent with the role of cholesterol in membrane fusion, cholesterol-25-hydroxylase an innate immune factor that is upregulated by type I interferons, converts cholesterol to a soluble antiviral factor, 25-hydroxycholesterol and broadly inhibits membrane fusion of many enveloped viruses tested so far (class I: HIV, Influenza A virus, Ebola virus; class II: Rift Valley Fever Virus, Russian Spring-Summer Encephalitis virus and class III: Vesicular stomatitis virus, Herpes simplex virus, Murine Gammaherpesvirus 68, Murine Cytomegalovirus).

Summary

Structural biology has contributed considerably to understand the principles of protein driven membrane fusion reactions. A common principle is the refolding of the fusion protein conformation which leads to the apposition of viral and cellular membranes by placing their membrane anchors, the fusion peptide or loop and the transmembrane region at the same end of a rod-like structure upon completion of fusion. Bringing two membranes into close contact is energetically unfavorable and the role of the fusion protein is to overcome this energy barrier to facilitate lipid mixing, fusion pore opening and expansion. Therefore the fusion protein structures and their conformational transitions predict that the principles of membrane fusion are very similar for class I, II and III fusion proteins. Differences exist with regard to the conformations of the postulated intermediate fusion protein structures, their oligomeric states, the kinetics of refolding and the principles of fusion protein cooperativity, which require further work.

Further Reading

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