Virology 479-480 (2015) 498–507

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Viral membrane fusion

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ARTICLE INFO

Article history:
Received 30 January 2015
Returned to author for revisions
23 February 2015
Accepted 10 March 2015
Available online 10 April 2015

Keywords:
Virus entry
Fusion mechanism
Fusion protein

ABSTRACT

Membrane fusion is an essential step when enveloped viruses enter cells. Lipid bilayer fusion requires catalysis to overcome a high kinetic barrier; viral fusion proteins are the agents that fulfill this catalytic function. Despite a variety of molecular architectures, these proteins facilitate fusion by essentially the same generic mechanism. Stimulated by a signal associated with arrival at the cell to be infected (e.g., receptor or co-receptor binding, proton binding in an endosome), they undergo a series of conformational changes. A hydrophobic segment (a "fusion loop" or "fusion peptide") engages the target-cell membrane and collapse of the bridging intermediate thus formed draws the two membranes (virus and cell) together. We know of three structural classes for viral fusion proteins. Structures for both pre- and postfusion conformations of illustrate the beginning and end points of a process that can be probed by single-virion measurements of fusion kinetics.

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Introduction

Enveloped viruses require membrane fusion to enter a cell. They expose on their surface many copies of a fusion protein, held in a "prefusion conformation" by constraints that come either from another part of the same protein or from a different viral protein. Two events lead to a fusogenic conformational transition. One ("priming") makes the transition possible, often by virtue of a proteolytic cleavage; the other ("triggering") initiates the transition, usually as a result of ligand binding. The ligand can be a proton, in the case of low-pH induced conformational changes (example: influenza virus); it can be a co-receptor on the cell surface or in an internal compartment to which the entering virus traffics (example: HIV); or it can be a distinct protein on the virion surface, itself triggered to signal fusion, often by interaction with the cell-surface receptor for the virus in question (example: paramyxoviruses). Viral fusion proteins are "suicide enzymes", because they undergo an irreversible priming step and act only

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http://dx.doi.org/10.1016/j.virol.2015.03.043
0042-6822/

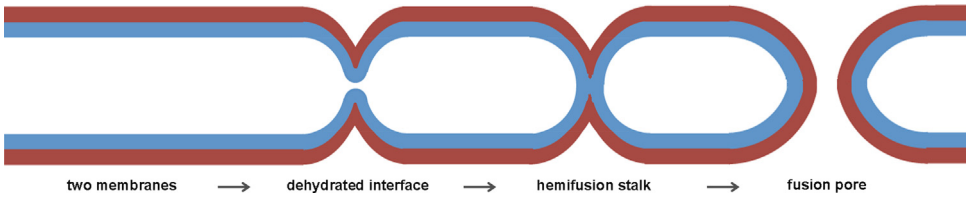

Fig. 1. Steps in fusion of two lipid bilayers. Apposed leaflets in blue; distal leaflets in brown.

once; intracellular fusion proteins, such as the well-known SNAREs, recycle, through the agency of an ATP-dependent protein such as NSF, and priming is therefore reversible.

Why is catalysis of fusion necessary? Although thermodynamically favorable, fusion of two membranes must overcome a kinetic barrier, due to a repulsive "hydration force", which increases steeply as the distance between the surfaces of the two bilayers falls below 20 Å (Parsegian et al., 1979; Rand and Parsegian, 1984). Because of this barrier, the two membranes require a source of free energy other than thermal fluctuation to bring them closer together than the 20 Å spacing at which the hydration force becomes very strong. Bilayer fusion proceeds through a so-called "hemifusion intermediate", in which apposed leaflets have merged, but not yet distal ones (Kuzmin et al., 2001; Yang and Huang, 2002; Lee, 2010). Considerable evidence supports the picture shown in Fig. 1, for the hemifusion transition and for subsequent fusion pore formation. The productive, hemifused state is simply a narrow stalk, minimizing the area of close contact and hence minimizing the work done to overcome hydration force repulsion. Widening of the stalk into a "hemifusion diaphragm" is probably a kinetic dead end, at least if the diaphragm is more than a few lipid molecules wide (Diao et al., 2012).

Viral fusion proteins fall into a small number of structural classes – three reasonably well characterized ones at the time of this review (Harrison, 2008). The first of these three includes many of the best studied human pathogens, such as influenza virus (Skehel and Wiley, 2000) and HIV-1 (Chan and Kim, 1998). The proteins are trimers of a single-chain precursor, which requires a proteolytic cleavage to make it fusogenic. The cleavage, which may simply eliminate a single peptide bond, generates two fragments. The N-terminal fragment, in many cases a receptor-binding domain (e.g., the HA_1 fragment of influenza virus hemagglutinin or the gp120 fragment of HIV-1 envelope protein), constrains the C-terminal, fusogenic fragment (e.g., HA_2 or gp41), until triggered to release it. The latter bears a hydrophobic "fusion peptide" at or near its newly generated N-terminus and a transmembrane anchor, which holds it in the viral membrane, near its C-terminus.

Most members of the second structural class of fusion proteins – those found on flaviviruses, alphaviruses, and bunyaviruses – are in an icosahedrally symmetric array on the mature virion (von Bonsdorff and Harrison, 1975; von Bonsdorff and Pettersson, 1975; Lescar et al., 2001; Zhang et al., 2002; Kuhn et al., 2002). Although the virion of rubella virus is less regular that those of the closely related alphaviruses (Battisti et al., 2012), its fusion protein has a characteristic class II structure (DuBois et al., 2013). For flaviviruses and alphaviruses, the priming event is cleavage of a second viral surface protein, which is, in effect, a "chaperone" that blocks any response to conditions of triggering (Lobigs and Garoff, 1990; Guirakhoo et al., 1991). When cleavage has inactivated the chaperone, triggering (exposure to reduced pH in case of both flavi- and alphaviruses) induces a reorgnization of the surface lattice and trimerization of the fusion protein. The hydrophobic segment that engages the target membrane during the fusogenic conformational change is an internal "fusion loop".

Members of the third class of fusion proteins, found on rhabdoviruses (G protein), herpesviruses (gB), and group 1 alphabaculoviruses (gp64) combine certain features of the first two (Backovic and Jardetzky, 2009). Herpesvirus gB is part of a larger fusion complex that includes several other proteins; the rhabdovirus G proteins are the sole surface proteins of those viruses. There is no obvious priming event, and most of the conformational transition in G that induces rhabdovirus fusion is reversible (Roche and Gaudin, 2002). The proteins, which have two spatially adjacent, hydrophobic fusion loops on each subunit, are trimeric in both pre- and postfusion conformations, and they do not form a regular array on the virion surface.

Stuctures and fusogenic structural transitions

Class I: priming by cleavage of trimeric, single-chain precursor

The classic, and still best characterized, example is influenza virus hemagglutinin (HA) (Skehel and Wiley, 2000; Wilson et al., 1981). Fig. 2a shows the pre- and post-fusion ectodomains of HA_1:HA_2, joined schematically to their transmembrane anchors; Fig. 3 shows the presumed sequence of events that links the two conformations. A crucial stage of the interpolated transition is an extended intermediate, in which the fusion peptide at the N-terminus of HA_2 has engaged the target membrane, creating a bridge between the two bilayers destined to fuse. Evidence for this intermediate is strong, but indirect. Studies on other class I fusion proteins leave little doubt that a moderately long-lived, extended, so-called "prehairpin" intermediate is a general, on-pathway state.

To get from this intermediate to the observed postfusion state, the long central helix breaks, and the segment between the break and the membrane reconfigures so that it runs back along the central coiled-coil, ultimately drawing together the fusion peptide and the C-terminal transmembrane anchor – along with the two membranes in which they reside (Bullough et al., 1994; Chen et al., 1999). An important characteristic, emphasized originally in a model for HIV gp41-mediated fusion, is that the zipping up of the three C-terminal "outer-layer" segments is asymmetric (Weissenhorn et al., 1997). In none of the known postfusion structures do these segments interact with each other around the outside of the postfusion trimer, consistent with an asymmetric collapse. Full threefold symmetry is regained at the end of the transition, when a fusion pore has opened and the membrane-proximal parts of the structure have clicked into place (Chen et al., 1999). The full transition turns HA_2 "inside out", in the sense that most of the central coiled-coil in the postfusion structure comes from parts of the polypeptide chain that were on the outside of the trimer in the prefusion structure, while the outer part of the overall HA_2 postfusion hairpin comes from parts of the polypeptide chain that were on the inside of the trimer before the transition.

Other class I fusion proteins appear to conform, with some variation, to the scheme shown in Fig. 3 for influenza virus HA. Recently determined structures for a prefusion conformation HIV-1 gp120:gp41 envelope protein confirm earlier proposals that its fusogenic conformational change would follow an HA-like sequence (Bartesaghi et al., 2013; Lyumkis et al., 2013; Julien et al., 2013; Pancera et al., 2014), but the triggering events are more complex. The