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Review

The three lives of viral fusion peptides

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ABSTRACT

Fusion peptides comprise conserved hydrophobic domains absolutely required for the fusogenic activity of glycoproteins from divergent virus families. After 30 years of intensive research efforts, the structures and functions underlying their high degree of sequence conservation are not fully elucidated. The long-hydrophobic viral fusion peptide (VFP) sequences are structurally constrained to access three successive states after biogenesis. Firstly, the VFP sequence must fulfill the set of native interactions required for (meta) stable folding within the globular ectodomains of glycoprotein complexes. Secondly, at the onset of the fusion process, they get transferred into the target cell membrane and adopt specific conformations therein. According to commonly accepted mechanistic models, membrane-bound states of the VFP might promote the lipid bilayer remodeling required for virus-cell membrane merger. Finally, at least in some instances, several VFPs co-assemble with transmembrane anchors into membrane integral helical bundles, following a locking movement hypothetically coupled to fusion-pore expansion. Here we review different aspects of the three major states of the VFPs, including the functional assistance by other membrane-transferring glycoprotein regions, and discuss briefly their potential as targets for clinical intervention.

Contents

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1. Introduction: viral glycoprotein-induced membrane fusion

Membrane fusion, i.e., the merging of two initially separate and apposed lipid bilayers with the result of the mixing of two initially distinct aqueous compartments, is ubiquitous to cell life. This unfavorable event typically advances at the expense of protein refolding energy, and under the control of mechanisms that ensure its evolvement at defined cell locations and physiological stages. Despite the variety of physiological conditions involving membrane fusion events, the same basic principles underlying lipid bilayer remodeling seem to apply to all the expressions of this phenomenon (see Chernomordik and Kozlov, 2003; Cohen and Melikyan, 2004; Frolov and Zimmerberg, 2010; Kozlov et al., 2010; Zimmerberg et al., 1993) and references therein for comprehensive reviews on this issue).

The protein-assisted membrane fusion reaction has been co-opted by lipid-enveloped pathogens, including enveloped viruses (White, 1992; White et al., 2008). Thus, enveloped viruses comprising highly relevant human pathogens such as Influenza virus (IFV), human immunodeficiency virus (HIV) or Ebola virus (EBOV), make use of membrane glycoproteins for selecting host cells, induce membrane fusion and gain access to internal compartments, a sequence of processes collectively known as "viral entry"(White et al., 2008). Given the dynamic nature of viral genomes and the vast number of replication cycles taking place during infection of a single host, viral entry machineries are subject to intense molecular evolution. Thus, the themes of glycoprotein structure–function common to all enveloped viruses are highly significant for the understanding of the general mechanism of protein-mediated fusion (Blumenthal et al., 2012; Chernomordik and Kozlov, 2003; Cohen and Melikyan, 2004; Harrison, 2008; Hernandez et al., 1996; Kozlov et al., 2010; Lentz et al., 2000; Melikyan, 2008). In addition, the conserved elements of these machineries, transiently or permanently exposed on the virion surface, provide clinical targets for development of inhibitors (antivirals) and immunogens (vaccines) (Blumenthal and Dimitrov, 2007; Doms and Moore, 2000; Eckert and Kim, 2001; Forssmann et al., 2010; Huarte et al., 2011; Munch et al., 2007).

Fig. 1A displays a generally accepted mechanism of membrane fusion induced by prototypical Class I viral glycoproteins, such as those of IFV, HIV or EBOV. The model is supported by the available structural and functional evidence (reviewed in references Blumenthal et al., 2012; Eckert and Kim, 2001; Harrison, 2008; Melikyan, 2008; Skehel and Wiley, 2000; Wiley and Skehel, 1987). The mature envelope glycoproteins in those viruses are organized as trimers of non-covalently associated heterodimers. Each heterodimer is composed of a surface and a trans-membrane subunit, which mediate receptor binding and virus-cell fusion, respectively (Karlsson Hedestam et al., 2008; Roux and Taylor, 2007; Skehel and Wiley, 2000; Wiley and Skehel, 1987). Upon fusion activation, the ectodomain of the membrane-anchored subunit undergoes a series of conformational changes conducive to membrane merger. First a "pre-hairpin" intermediate forms to anchor viral and cell membranes through the transmembrane (TMD) and fusion peptide (FP) domains, respectively. The presence of the FP within the ectodomain exposed to the aqueous phase constitutes a feature shared by all viral fusion proteins, and represents an absolute requirement for their fusogenic function (previously reviewed in: Durell et al., 1997; Epand, 2003; Nieva and Agirre, 2003; Tamm et al., 2002). These hydrophobic and conserved sequences usually located at the N-terminal end of the fusogenic subunit, or close to it (Table 1), are thought to be involved in driving the initial partitioning of the fusion protein into the target membrane (Nieva and Suarez, 2000). In this first stage, formation (IFV hemagglutinin (HA)) or completion (HIV-Envelope protein (Env)) of an extended coiled coil (red rods in Fig. 1A) by N-terminal helices (or N-terminal heptad repeats, NHRs) would be the propelling force that brings about exposition and translocation of the initially cryptic FP into close vicinity of the target membrane.

The filamentous structure of the "pre-hairpin" subsequently collapses into the low-energy trimeric "hairpin". In the trimeric hairpin structure, the ectodomain amino- and carboxy-termini are placed at the same end of the molecule (Eckert and Kim, 2001; Weissenhorn et al., 1997). In several instances, this second step might ensue due to the fact that the extended N-terminal coiled coil creates highly conserved grooves into which C-terminal helices (or C-terminal heptad repeats, CHRs) may hydrophobically pack in an antiparallel orientation (yellow rods in Fig. 1A). It is assumed that the conformational energy released during the trimeric hairpin formation can be used to pull membranes together and induce their merger, while its completion upon full zippering of the CHR would stabilize an open state of the fusion pore and likely contribute to its expansion (Melikyan, 2008).

Hence, insertion of the hydrophobic FP into the target membrane and formation of the low-energy 6-HB structure are common themes to all fusogenic Class I glycoproteins, which otherwise may vary in receptor specificity, size, sequence and activation pathways (Melikyan, 2008; White et al., 2008). A great deal of experimental work produced in the course of the last 25 years supports this view. In one hand, convincing evidence for the insertion of the VFPs into target membranes was early provided by Brunner's group using hydrophobic photolabeling approaches (Durrer et al., 1996; Harter et al., 1989; Tsurudome et al., 1992; Weber et al., 1994). The IFV glycoprotein HA is synthesized as a single polypeptide, which is posttranslationally cleaved into two disulfide-linked chains, HA1 and HA2. The hydrophobic N terminus of HA2 generated after cleavage embodies the IFV-FP. Brunner and co-workers demonstrated that, upon incubation at conditions that activate IFV fusion (i.e., pH 5.0 and 37 °C), the hydrophobic interactions of isolated BHA2 (the bromelain-solubilized form of hemagglutinin) or IFV virions were mediated solely by the N-terminal segment of the HA2 subunit, which corresponds to the FP (Harter et al., 1989; Tsurudome et al., 1992). They found that the predominant sites of labeling within this segment were spaced in average 3–4-residues, thereby suggesting that the FP inserted into the target membrane adopting a helical structure with an amphiphilic character (see also Fig. 3B below). From asymmetric hydrophobic photolabeling of membranes, evidence was obtained indicating that HA2-FP penetrates only the external leaflet of the bilayer in the fusion pH conformation (Brunner, 1989). In addition these authors provided evidence to sustain a mechanism of IFV inactivation at acidic pH, according to which the HA2 FP irreversibly inserted into the viral membrane (Weber et al., 1994).

On the other hand, crystallographic evidence for the formation of low-energy trimeric hairpins by divergent virus glycoproteins has been accumulated during the last two decades (Eckert and Kim, 2001; Harrison, 2008; Weissenhorn et al., 1997; White et al., 2008; Yin et al., 2006). The fact that Class II glycoproteins make use of trimeric hairpins to induce fusion underpins the functional relevance of these structures (Harrison, 2008; Vaney and Rey, 2011). However, in contrast to the helical domains implied in Class I fusion, the Class II glycoprotein subunits employ articulated β-barrel domains to assemble membrane-pulling trimeric hairpins (Harrison, 2008; Vaney and Rey, 2011; White et al., 2008). Yet another class of fusion glycoproteins combine structural features of Class I and Class II fusion proteins, and have been designated as Class III (Backovic and Jardetzky, 2009; Baquero et al., 2013; Weissenhorn et al., 2007; White et al., 2008). Fusogenic subunits of Class II and Class III proteins possess conserved hydrophobic loops that connect extended strands at the tip of elongated β-barrel domains. These "fusion loops" (FLs) are thought to insert into the target membrane and function during fusion in a way reminiscent