Virology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Virology

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

Physiological and molecular triggers for SARS-CoV membrane fusion and entry into host cells

Jean Kaoru Millet, Gary R. Whittaker*

Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, United States

ARTICLE INFO

Keywords:
Coronavirus
SARS
Spike protein
Virus entry
Endosomes
Calcium
Fusion peptide

ABSTRACT

During viral entry, enveloped viruses require the fusion of their lipid envelope with host cell membranes. For coronaviruses, this critical step is governed by the virally-encoded spike (S) protein, a class I viral fusion protein that has several unique features. Coronavirus entry is unusual in that it is often biphasic in nature, and can occur at or near the cell surface or in late endosomes. Recent advances in structural, biochemical and molecular biology of the coronavirus S protein has shed light on the intricacies of coronavirus entry, in particular the molecular triggers of coronavirus S-mediated membrane fusion. Furthermore, characterization of the coronavirus fusion peptide (FP), the segment of the fusion protein that inserts to a target lipid bilayer during membrane fusion, has revealed its particular attributes which imparts some of the unusual properties of the S protein, such as Ca^{2+}-dependency. These unusual characteristics can explain at least in part the biphasic nature of coronavirus entry. In this review, using severe acute respiratory syndrome coronavirus (SARS-CoV) as model virus, we give an overview of advances in research on the coronavirus fusion peptide with an emphasis on its role and properties within the biological context of host cell entry.

1. Introduction

Coronaviruses are a diverse group of single-stranded plus-sense RNA viruses belonging to the *Coronaviridae* family and *Nidovirales* order (de Groot et al., 2012). They infect a wide array of mammalian and avian species, including bats, and have a propensity for interspecies jumping and zoonotic transmission as exemplified by severe acute respiratory syndrome coronavirus (SARS-CoV) and more recently by Middle East respiratory syndrome coronavirus (MERS-CoV) (Graham et al., 2013; Woo et al., 2009). As coronaviruses possess an envelope, membrane fusion with host cell membranes is a required and critical step in the replication cycle allowing for delivery of genomic RNA into the cytoplasm, which eventually leads to the start of replication. This critical early step is being viewed as an attractive target for therapeutic interventions (White et al., 2008).

Virus entry constitutes a series of interactions between a virion and its host cell occurring early in the viral life cycle (Boulant et al., 2015; Grove and Marsh, 2011; Hofmann and Pöhlmann, 2004; Marsh and Helenius, 2006). For an enveloped virus, these steps allow the virus to (i) bind to a target host cell, typically via interactions with cellular receptors, (ii) fuse its envelope with a cellular membrane, either at the plasma membrane or through the endocytic pathway, and (iii) deliver its genetic material inside the cell. Virus entry is a finely regulated process, often requiring a specific sequence of interactions (binding to receptors and/or co-receptors), triggers or cues (pH, proteolytic activation), and cellular processes such as endocytosis for successful delivery of viral genomic nucleic acids (White and Whittaker, 2016). Interestingly, coronaviruses display a large degree of plasticity regarding the entry pathways they use, which can occur at the plasma membrane or through the endocytic pathway (Belouzard et al., 2012; Matsuyama et al., 2005; Nash and Buchmeier, 1997). The mechanisms by which coronaviruses enter cells depends on the strain and species considered, along with tissue and cell-type specificities (e.g. receptor and protease availability, local microenvironment).

For enveloped viruses, a critical player in the entry process is the viral fusion protein as it mediates the membrane fusion reaction (Chernomordik and Kozlov, 2008; Colman and Lawrence, 2003; Harrison, 2008; White et al., 2008; White and Whittaker, 2016). While the process of merging two distinct lipid bilayers into a single one is a thermodynamically favorable reaction, it is associated with a high kinetic barrier (Harrison, 2008). As such, and because viral fusion proteins facilitate this process, they can be viewed as catalysts for the membrane fusion reaction. This has been very well studied both structurally and functionally with the influenza hemagglutinin (HA) fusion protein (Harrison, 2015). After attachment of HA to sialic acids cellular receptors the virion is internalized through the endocytic

* Correspondence to: Department of Microbiology & Immunology, C4-127 VMC, Cornell University, Ithaca, NY 14853, United States.
E-mail address: gary.whittaker@cornell.edu (G.R. Whittaker).

https://doi.org/10.1016/j.virol.2017.12.015
Received 31 October 2017; Received in revised form 13 December 2017; Accepted 15 December 2017

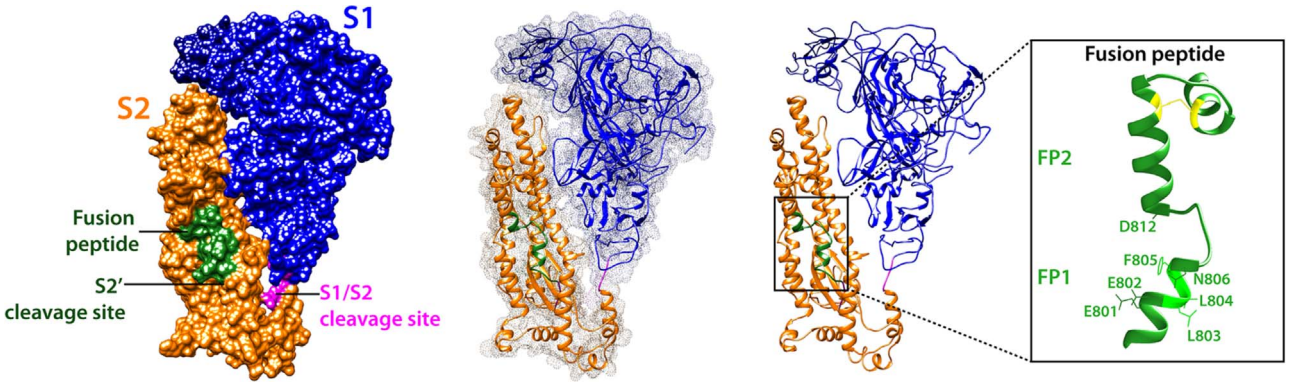

Fig. 1. Structural model of the SARS-CoV spike in pre-fusion uncleaved and monomeric form, based on pdb 3JCL (MHV spike). SARS-CoV S is colored with the S1 domain in blue and the S2 domain in orange, and is shown in in three different representations: space-filling, surface mesh and cartoon. The fusion peptide/predicted neutralizing epitope is shown green. The two proteolytic cleavage sites are shown in magenta, with S1/S2 exposed and the fusion peptide-proximal S2′ site protected. An enlarged cartoon depiction of the fusion peptide region is shown with key negatively charged and hydrophobic residues indicated.

pathway. Because of endosomal acidification, increased H^+ ion concentration within the endosome forces HA to undergo major conformational changes, which allows exposure of the fusion peptide and its insertion into target cellular membrane. This brings the viral and endosomal membrane in close proximity. Further conformational changes of several HA trimers allow merging of the outermost lipid leaflets forming an intermediate structure called the hemifusion stalk. This transient structure collapses into an expanding fusion pore allowing release of viral genetic material in the cytoplasm.

In the case of coronaviruses, viral entry into target cells is performed by the spike (S) envelope glycoprotein, which mediates both host cell receptor binding and membrane fusion. The S protein is classified as a class I viral fusion protein (Bosch et al., 2003), which includes the prototypical influenza virus hemagglutinin (HA) and retrovirus envelope (env) proteins (White et al., 2008). Class I viral fusion proteins form trimers and each monomer can often be divided into two domains, a receptor-binding domain (e.g. HA1 and gp120 for influenza virus and HIV, respectively), and a fusion domain (HA2 and gp40). Fusion domains are enriched in alpha-helices and contain regions called heptad repeats (HR) which are repetitive heptapeptides containing some hydrophobic residues and which are involved in the refolding process and coiling of central helices during membrane fusion. After membrane fusion has occurred, class I fusion proteins adopt a compact conformation, with a well-defined coiled-coil structure called a 6-helix bundle or 6HB (Belouzard et al., 2012; White et al., 2008). Importantly, the fusion domain contains a short segment, the fusion peptide (Epand, 2003; Lai et al., 2005; Tamm and Han, 2000; Tamm et al., 2002), typically composed of 15–25-amino acids, generally hydrophobic in nature, which becomes anchored to a target membrane when the fusion protein adopts the pre-hairpin conformation. The fusion peptide plays an essential role in mediating the membrane fusion reaction as it directly interacts with lipid bilayers, enabling to disrupt and connect two apposing membranes. Class I fusion proteins are often proteolytically activated or primed for fusion by host cell proteases at a specific cleavage site that usually forms the boundary between the receptor-binding and fusion domains (White and Whittaker, 2016). In the case of influenza HA and HIV env proteins, the cleavage event releases the fusion peptide as it is located at the N-terminal end of the fusion domain. While coronavirus S proteins possess salient features of class I fusion proteins, such as being a type I membrane proteins organized in trimers, possessing heptad repeats regions (HR1 and HR2), and requiring proteolytic cleavage for activation, they differ in several key aspects. The S proteins form substantially larger trimers, with S monomer size in the range of 1200–1400 amino acids (~180–200 kDa) compared to ~500 aa for influenza HA and ~800 aa for HIV. Until recently structural data on the coronavirus S was limited, due mostly to the difficulty of obtaining X-ray crystallographic structures of intact ectodomains of S. However, recent advances in cryo-electron microscopy (cryo-EM) have allowed the determination of the structure covering the majority of the ectodomain of several coronavirus S proteins in their pre-fusion conformation such as those of the murine hepatitis virus (MHV) (Walls et al., 2016a), human coronavirus HCoV-NL63 (Walls et al., 2016b), HCoV-HKU1 (Kirchdoerfer et al., 2016), MERS-CoV and SARS-CoV (Gui et al., 2017; Yuan et al., 2017). These efforts and studies constitute a huge step forward in the field as they uncovered the complexity of coronavirus S proteins and their highly glycosylated nature. The S protein can be divided into the S1 receptor-binding subunit and S2 fusion domain, usually separated by a cleavage site (S1/S2). However, coronavirus S proteins possess an additional cleavage site located within S2 and called (Belouzard et al., 2009, 2012; Millet and Whittaker, 2014). Not only are coronaviruses S proteins unusual for harboring multiple cleavage sites, they are also activated by a wide variety of host cell proteases (Millet and Whittaker, 2015), spanning different families such as cathepsins (Simmons et al., 2005), trypsin-like serine proteases such as members of the transmembrane serine protease (TTSP) family (Bertram et al., 2013; Gierer et al., 2013; Glowacka et al., 2011; Matsuyama et al., 2010), and the furin-like proprotein convertases (PCs) (Burkard et al., 2014; Millet and Whittaker, 2014). While the location of the fusion peptide has been debated, most recent data appears to argue that the segment immediately downstream of S2′ cleavage site is the *bona fide* fusion peptide. In the cryo-electron microscopy structures of coronavirus S, the fusion peptide segment appears to be exposed at the surface of the protein in the pre-fusion state (Fig. 1), another unique characteristic setting S proteins apart from other class I viral fusion proteins like the influenza virus HA. While the fusion peptides of coronaviruses are not as well characterized as the ones from other prototypical class I fusion proteins like influenza HA or HIV env, evidence has been steadily accumulating for the identification of the *bona fide* fusion peptide.

In this review, we use SARS-CoV as model for studying the mechanism of coronavirus-membrane fusion. We aim to give an overview of the entry pathway of SARS-CoV and connect this to recent advances in our understanding of the coronavirus spike fusion peptide, highlighting its unique features as well as putting these findings back to their biological context and the apparently biphasic nature of coronavirus entry. An underlying theme that emerges from these studies is that the flexible characteristic of coronavirus entry pathways is in some ways imparted by the unique and special features of the coronavirus fusion peptide.

2. SARS-CoV mediated entry into cells and the activation of membrane fusion

As with many viruses, SARS-CoV entry into cells is dictated by the