Paramyxovirus Glycoproteins and the Membrane Fusion Process

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Abstract

The family *Paramyxoviridae* includes many viruses that significantly affect human and animal health. An essential step in the paramyxovirus life cycle is viral entry into host cells, mediated by virus-cell membrane fusion. Upon viral entry, infection results in expression of the paramyxoviral glycoproteins on the infected cell surface. This can lead to cell-cell fusion (syncytia formation), often linked to pathogenesis. Thus membrane fusion is essential for both viral entry and cell-cell fusion and an attractive target for therapeutic development. While there are important differences between viral-cell and cell-cell membrane fusion, many aspects are conserved. The paramyxoviruses generally utilize two envelope glycoproteins to orchestrate membrane fusion. Here, we discuss the roles of these glycoproteins in distinct steps of the membrane fusion process. These findings can offer insights into evolutionary relationships among *Paramyxoviridae* genera and offer future targets for prophylactic and therapeutic development.

Keywords

Paramyxoviridae; Paramyxovirus; attachment glycoprotein; fusion glycoprotein; viral receptors; viral entry; F-triggering; fusion cascade; membrane fusion; association model; dissociation model; fusion; attachment; fusion model; syncytia; prefusion; postfusion; prehairpin intermediate; hexamer of trimers; fusion pore formation; Nipah; Hendra; Measles; RSV; NDV; Mumps; hMPV; Hemifusion

Introduction

The *Paramyxoviridae* family of viruses contains many important pathogens that infect a wide variety of hosts including pandas, hyenas, whales, anacondas, bats, dogs, rats, avians, and humans. Members of this virus family include measles virus (MeV), respiratory syncytial virus (RSV), human metapneumovirus (hMPV), human parainfluenza viruses (e.g. hPIV3, PIV5), mumps virus (MuV), canine distemper virus (CDV), and Newcastle disease virus (NDV), as well as the deadlier zoonotic Hendra and Nipah viruses (HeV and NiV, respectively) (1–3) (Fig. 1). For example, NiV infection causes 40–90% mortality rates in humans (4–9). The *Paramyxoviridae* family already includes an extensive repertoire, however recent discoveries support the existence of over 60 additional paramyxoviruses in bats from South America and Africa, for example Ghana virus (GhV) (10–13).

The *Paramyxoviridae* family is further subdivided into two sub-families: *Paramyxovirinae* and *Pneumovirinae*. *Paramyxovirinae* contains multiple genera: *Avulavirus*, *Rubulavirus*, *Respirovirus*, *Morbillivirus*, *Henipavirus*, *Aquaparamyxovirus* and *Ferlavirus* (3, 14–16). Pneumoviruses, on the other hand, include only two genera: *Metapneumovirus* and *Pneumovirus*. Furthermore, recently discovered viruses, such as Beilong virus (BeV), Tailam virus (TaV) and J paramyxovirus (JPV), have yet to be classified into sub-families (15, 17, 18). The evolutionary relationships between representative paramyxoviruses, as based on their relatively highly-conserved polymerase protein sequences, are outlined in Fig. 1.

Paramyxoviruses are membrane enveloped with receptor-binding (or attachment) glycoproteins HN, H, or G, and fusion glycoproteins,F, anchored in their viral membranes. The designation of the attachment glycoprotein as hemagglutinin-neuraminidase (HN), hemagglutinin (H), or glycoprotein (G) depends on protein function and differs between genera (19–26). Specifically, the HN attachment glycoprotein is characteristic of the *Avulavirus*, *Respirovirus*, and *Rubulavirus* genera (2, 19, 20, 26–33). HN consists of "H," *hemagglutinin* activity, which mediates binding to sialic acid, and "N," *neuraminidase* activity, which cleaves sialic acid. This activity assists budding virions in detaching from the infected cell and reduces re-entry of virions into other infected cells. In comparison, the H attachment glycoprotein is found in members of the *Morbillivirus* genus and only has hemagglutinin activity (34, 35). The G attachment glycoprotein is utilized by viruses in the genus *Henipavirus* and the *Pneumovirinae* subfamily and does not have either hemagglutinin or neuraminidase activities. Instead, the G attachment glycoproteins bind protein receptors.

For the paramyxoviruses, interactions between the viral attachment glycoprotein and the host cell receptor is generally sufficient to trigger the fusion protein, allowing virus entry. The genera *Avulavirus, Rubulavirus*, and *Respirovirus* HN proteins (e.g. those of PIV5, NDV, and hPIV1–3) utilize sialic acid as a receptor. *Morbillivirus* H glycoproteins (e.g. those of MeV, PPRV and CDV) can utilize CD46/MCP, CD150/SLAM, or nectin 4/PVRL4 as receptors (34, 36–43). The G glycoproteins of pneumoviruses (e.g. RSV or hMPV) can utilize nucleolin, ICAM1, heparan sulfate or other glycosaminoglycans (19, 22, 44–49). *Henipavirus* (e.g. NiV or HeV) G glycoproteins utilize protein receptors such as ephrinB2 and/or ephrinB3 (23, 50–57). Interestingly, there are some viruses that do not require the

attachment protein to trigger fusion, such as the pneumoviruses, hMPV and RSV (20, 22, 48, 58–60). In the cases of parainfluenza virus 5 (PIV5) and Sendai virus (SeV), the fusion protein can be triggered, though less efficiently, without the presence of the attachment glycoprotein (33, 61).

Most paramyxoviruses are thought to enter host cells via direct fusion between the host cell plasma membrane and the viral membrane. Unlike other viruses, such as Ebola or Influenza viruses, paramyxoviruses are generally believed to not require endocytosis and low-pH to initiate viral and host cell membrane fusion (62–64). Once a cell is infected, the attachment and fusion glycoproteins are expressed at the cell surface (65–70). Thus, cells infected with paramyxoviruses can fuse with naïve receptor-containing cells, forming multi-nucleated cells (syncytia) (5, 7, 59, 71–75). This is considered a mode of viral spread from infected to naïve cells.

During cell infection, other viral proteins are synthesized and the genome is replicated. Paramyxoviruses contain non-segmented, single-stranded RNA genomes with negative-sense polarity (-ssRNA). Genome sizes vary among different paramyxoviruses, ranging between near 15kbp to near 19kbp. Since paramyxoviruses are -ssRNA viruses, virions must package their own RNA-dependent-RNA-polymerase (large protein), encoded by the L gene, to allow for transcription and replication. The genome also encodes for the nucleoprotein (N) that coats the RNA genome and protects it from degradation, forming ribonucleoprotein (RNP) complexes, and assists viral genome replication and virion packaging (76–80). Additionally, RNP complexes also contain the viral phosphoprotein (P) (76). The phosphoprotein complexes with the large protein to form a functioning RNA-dependent-RNA-polymerase. The P gene transcript can undergo further processing to form the V and W proteins, while C is translated via frame shift. These proteins play important roles in blocking innate immune responses, enhancing virulence, and managing viral RNA synthesis in many paramyxoviruses (81–88). Finally, the matrix protein (M) is generally required for complete viral assembly and budding (69, 79, 89–95). The presence of the N, P, L, M proteins and the surface glycoproteins is conserved across the *Paramyxoviridae* family.

The glycoproteins

The attachment glycoproteins of paramyxoviruses form tetramers, specifically dimers of dimers (21, 24–26, 34, 35, 53). Each monomer is capable of binding a receptor molecule. Once bound by the appropriate receptor, conformational changes in the attachment glycoprotein are generally thought to expose the stalk region, triggering the fusion glycoprotein to undergo the membrane fusion cascade (21, 96–104) (Fig. 2A).

The fusion glycoprotein of paramyxoviruses is classified as a trimeric Class I fusion protein (105–111). Class I fusion proteins exhibit high alpha-helical secondary structural content, and typically require processing to expose the intramolecular hydrophobic fusion peptide (FP). Before the fusion protein is biologically active, it is made as a precursor (F_0) that is transported to the cell surface and then endocytosed. In the endosomal compartment, the precursor glycoprotein (F_0) is cleaved by either cathepsins (e.g. for HeV or NiV) or furin-like proteases (e.g. for RSV, NDV) (112–115). The processed precursor is composed of F_1

and F_2 subunits that are disulfide linked and transported back to the plasma membrane as a fusion-active unit (Fig. 2B).

The fusion core of an F trimer consists of the heptad repeat one and two (HR1 and HR2) domains (105, 116, 117). The HR1 domain is located proximal to the N-terminus of the F_1 subunit and is designated as HR1, HRA, or HRN. Conversely, the HR2 domain is proximal to the C-terminus and designated as HR2, HRB, or HRC (105, 116, 117). Importantly, an additional helical region is located upstream from the fusion peptide, as a part of F_2, and designated as HR3 or HRC (111, 118). As can be seen from these designations, two completely distinct regions can be called HRC. Therefore, to avoid confusion, we will use the nomenclature HR1, HR2, and HR3 to refer to these 3 helical regions (Fig. 2B).

The HR1 and HR2 alpha-helical domains have a high propensity to bind each other and form antiparallel helical bundles (105, 106, 116, 117). The HR1 domain, adjacent to the FP is located towards the head of the fusion protein, is relatively hydrophobic, and is shielded in the metastable, pre-fusion conformation. On the other hand, the amphipathic HR2 region is exposed to aqueous solutions and exists in a "stalk-like" conformation. The binding of HR1 and HR2 provides the energy for viral and cellular membrane merging. The HR3 domain has been shown to not interact with the HR1 or HR2 domains in the case of RSV, suggesting other structural roles (106, 111, 118, 119). For PIV5, an analogous domain in the F_2 subunit affects the fusion capabilities of F (120). The role of the HR3 domain remains a knowledge gap for most paramyxoviruses.

Structural information for the H, HN, and G Glycoproteins

Crystal structures for the extracellular, globular domains of the H, HN, and G glycoproteins have been solved for a substantial portion of the paramyxoviruses, including at least one virus from each genus within the *Paramyxovirinae* sub-family (26, 28, 53, 121, 122). While there are some differences between the receptor attachment proteins of different paramyxoviruses, considerable structural similarities stand out. First, paramyxovirus receptor attachment proteins are transmembrane type II proteins comprised of an N-terminal cytoplasmic tail followed by a transmembrane domain, a stalk, and a globular head. Additionally, attachment glycoproteins tetramerize into dimers of dimers (67). Until somewhat recently, the structures of paramyxoviral attachment protein stalks were unknown; however, recent studies with Newcastle disease virus have shown that the helical stalks of each monomer cluster to form a four-helix bundle (4HB) (25). Beyond the stalk, the C-terminal globular head of each monomer contains a β-propeller with six blades, each with four β-sheets running anti-parallel to each other (1). Close structural analysis of the monomers in complex reveals that the interactions in the stalk and across the β-1 and β-6 blades are critical to the formation of the tetramer (53, 67). The geometries of HN, H, or G glycoproteins are highly similar. Relatively lower similarity between the structures of G and H, as well as the existence of an enlarged, nonfunctional sialic acid binding motif within the MeV H protein, supports a model where G and H proteins diverged from HN towards binding proteinaceous receptors (67, 123).

Evidence for specific attachment-fusion protein interaction sites

For both HN and G glycoproteins, receptor interactions have been shown to occur within binding pockets in the head. Two examples of this are shown in Fig. 3 (26, 52). This is contrasted with findings that the receptors for H bind to the side of the head, as reviewed in (123, 124). While it has not always been the case, current understanding is that upon receptor binding, the attachment protein undergoes conformational changes that, in turn, trigger the fusion protein to undergo its own series of conformational changes, ultimately leading to membrane fusion executed by F (53). Examples of active site changes upon receptor binding have been seen for the NiV G, NDV HN, and PIV3 HN proteins (25, 53, 122). In the case of NiV G, structural and biochemical assays support two specific and sequential conformational changes in the head domain that act to reveal the stalk for F-triggering (97). Similarly, MeV H heads are suggested to shift during F-triggering (125). At least one study has identified F-HN sites of interaction for NDV (126). While the interactions between the fusion and attachment glycoproteins are not well understood, studies have shown the importance of the attachment protein stalk domain in such interactions (21, 97). For some paramyxoviruses, the globular head may also be involved in interactions with F, specifically during F-triggering (127, 128). Models of structural interactions between F and H/HN/G exist, yet the absence of any co-crystallization of these proteins remains a significant objective for the field.

Conserved structural patterns in paramyxovirus fusion proteins

There are several knowledge gaps concerning the structures of the paramyxovirus fusion protein. Obstacles include its transition through several extremely different conformations, protease-dependent processing that occurs during fusion protein maturation, and the transient nature of the pre-hairpin intermediate conformation (PHI). In contrast to the attachment glycoproteins, fusion glycoproteins are type I transmembrane proteins with their extracellular regions at the N-terminus and their transmembrane (TM) and cytoplasmic tail (CT) domains at the C-terminus (Fig. 2).

Structures have been solved for fusion proteins from most of the genera in the *Paramyxoviridae* family. Specifically, pre-fusion structures are available for HeV, NDV, NiV, and PIV5 (107, 109–111, 119, 129–133). Work for PIV5 has demonstrated that the F pre-fusion conformational structure does not significantly change after cleavage (107). While no paramyxovirus fusion protein has been effectively crystallized at the PHI, its existence is supported by many biochemical studies and by recent findings using transmission electron microscopy (107). The post-fusion six-helix bundle (6HB) conformation has been resolved for RSV, NDV, and hPIV3 (119, 131, 132). Examples for the pre- and post-fusion conformations of F are shown in Fig. 4 (119, 131). The least understood conformation of F is the transient PHI. Determination of this structure through traditional crystallographic means is unlikely to occur. Thus the field must find alternative methods to study this conformation.

The paramyxovirus membrane fusion process

Several steps are recognized as important during paramyxoviral viral-cell or cell-cell membrane fusion. Early steps include: binding of the attachment protein HN/H/G to its cognate cell-surface receptor, the resulting activation of the HN/H/G protein to undergo its own set of conformational rearrangements, and the subsequent triggering of the F protein to undergo extensive conformational rearrangements that result in membrane merging. Later steps in the membrane fusion process include fusion pore formation and expansion. Noteworthy, although these are very well recognized membrane fusion steps, additional steps in this complex process may remain to be discovered (108, 134, 135).

Early steps in the paramyxovirus membrane fusion process

Triggering of F by the attachment protein HN, H, or G

Despite the lack of structural data for paramyxoviral attachment-fusion glycoprotein interactions, all functional data available points to such interactions being essential for members of the *Paramyxovirinae* subfamily, and for some members of the *Pneumovirinae* subfamily. Our knowledge of both the spatial and temporal nature of these interactions is relatively poor. However, we know that these interactions can occur at different times for different paramyxoviruses. This has given rise to the Association vs. Dissociation models of attachment-fusion protein interactions (31).

Association vs. dissociation models

At least two models can explain the differences in the timing of the attachment-fusion glycoprotein interactions. The HN glycoproteins of paramyxoviruses that bind sialic acid, such as PIV5, hPIV3, NDV, etc., do not appear to bind their homologous fusion glycoproteins until after HN has bound its respective receptor, appearing to follow the association or provocateur model. In contrast, the H or G glycoproteins that attach to membrane protein receptors, such as MeV or CDV H, which can bind SLAM or CD46, or NiV or HeV, which can bind ephrinB2 and/or ephrinB3, appear to interact with the F protein prior to receptor binding. Then, upon receptor binding the glycoproteins relatively dissociate, following the dissociation or clamp model. These models have been previously reviewed at length (27, 31, 32). Multiple publications have shown strong evidence to support either the association model for the sialic acid binding paramyxoviruses, such as NDV (103), or to support the dissociation model for protein-binding viruses, such as MeV, NiV, or HeV (127, 136–140).

Five models of F activation

We know a substantial amount regarding how Class I fusion proteins execute membrane fusion, from studying not only paramyxoviral F proteins, but also other RNA viral glycoproteins such as influenza virus HA and HIV gp41. However, we know relatively little regarding how the paramyxoviral F protein receives the signal from G, and how G couples receptor binding with F-triggering. There are at least five proposed modes of F-triggering by G. Namely, the sliding, stalk-exposure, safety-catch, bi-dentate, and oligomerization models (Fig. 5).

The _sliding model_ proposes that upon receptor binding, the heads of the attachment glycoprotein slide laterally from a planar to a more staggered configuration (Fig. 5A). In turn the stalk of the protein would move from a compact closed 4HB to a partially dissociated conformation, leading to disengagement from and "freedom" of F. This model infers that the heads, upon receptor binding, generate specific signals that travel down to the stalks for F activation. Since head/stalk hybrid attachment proteins have been reported to preserve F-triggering activity for example for MeV, and mutants that assume the activated attachment protein conformation still trigger membrane fusion, this model may at best not fit all paramyxoviruses (37, 141).

The _stalk-exposure/induce fit model_ proposes that receptor binding may induce conformational changes of the attachment protein from a four-heads-down conformation to a (at least) some-heads-up conformation. Such heads-up conformation would free a stalk domain in the attachment protein to interact with F (Fig. 5B). Both the latest structural and mechanistic data for NDV and PIV5 HN proteins (25, 100, 142), as well as the fact that the headless attachment proteins of PIV5, MeV, and NiV can trigger F, are consistent with the stalk-exposure model (97, 101, 102). It is important to note, however, that for several viruses, including MeV, CDV, hPIV3, and NiV, attachment protein-F interactions exist prior to receptor binding, so variations of this model are likely.

The _safety-catch model_ proposes that the attachment protein heads, for example in the four-heads-down conformation would fold into an auto-repressed conformation, as in "locking" the ability of their stalks to trigger F (Fig. 5C). The "safety lock" has been proposed to be provided by head-stalk connecting domains or spacers, which can also be found solely in the stalk domains (98, 102, 143). This safety lock would be removed by movement of the heads upon receptor binding. This model suggests that F can interact with the attachment protein prior to receptor binding without premature triggering because of the auto-repressed conformation of the attachment protein. This model may be seen as a variation of the stalk-exposure/induce fit model just described above.

The _bi-dentate model_ has been originally suggested for NiV. This model proposes that F interacts with both the head and stalk of the attachment protein, and that receptor binding switches the major interactions with the fusion protein from the head to the stalk of the attachment protein (Fig. 5D). Evidence to support this model include the strong interactions of G and F prior to receptor binding (127, 136, 137), the interactions of G and F when N-glycans were introduced to the stalk (144), and the three receptor-induced conformational changes observed in G, two in the head and one in the stalk, prior to F-triggering (97).

The _Oligomerization model_ proposes that receptor binding may lead to increased attachment protein oligomerization, which in turn triggers F (Fig. 5E). This model has been suggested based on the fact that the heads-up conformation may not be sufficient for F-triggering for hPIV3 (145). However, there is no direct evidence for the higher-order formation of oligomers upon receptor binding. Noteworthy, NiV F has been recently reported to form hexamers of trimers, and such oligomeric formations appear to have a role in membrane fusion modulation (110). The role of G oligomerization in the activation of such F oligomeric complexes remains to be determined.

It is important to note that these models are not necessarily mutually exclusive. There is a level of redundancy among the models, and two or more of them may apply to a single paramyxovirus. More structural and functional data, particularly for attachment fusion protein interactions, will likely help further describe and corroborate these models. Cryo-TEM and single particle 3D reconstruction from electron micrographs using direct electron detection (DED) technology might be helpful for this endeavor.

Late steps in the paramyxovirus membrane fusion process

Fusion of membrane bilayers is not spontaneous or trivial. Only by expending the energy stored in the metastable pre-fusion F trimer (Fig. 6A), through irreversible conformational changes, can the paramyxoviruses gain access to its host cell machinery (146). Additionally, this process may even require several F proteins undergoing synchronized conformational changes to overcome the high-energy barrier of membrane fusion, with the henipaviruses potentially using six F trimers to execute membrane fusion (33, 110). F mediated membrane fusion typically occurs at a neutral pH, shown for PIV5 (147); although low pH does not inhibit entry in PIV5, RSV, or NDV (147–149). SER virus, as well as certain hMPV strains, are known exceptions and do have low pH requirements (150, 151). Most *Paramyxoviridae* virions likely directly fuse with their target cell membrane, but there is evidence that endocytic processes may occur for NiV, NDV, and RSV (150, 152–155). MuV has been shown to be sensitive to calcium concentrations and a calcium-channel agonist has shown inhibition, while SeV is unaffected by either (156, 157). Other requirements may also exist.

A recent crystal structure of pre-fusion NiV F trimers displays a hexamer-of-trimer architecture. Using this structure to establish a hypothetical, hexameric model for F, keeping in mind that evidence for this is NiV-specific, individual trimers could be interacting with a part of HR1, the FP, and a part of F_2. Two monomers in each F trimer appear to be aligned for interactions with adjacent trimers, while the third monomer faces away and remains exposed. This exposed monomer could be the site where G initiates triggering (110). With FPs and their surrounding regions interacting with neighboring F trimers, and with dramatic conformational changes occurring as F transitions into the PHI, the transition, itself, of F into a PHI could facilitate triggering of neighboring trimers. Destabilization of a neighbor's pre-fusion conformation by "pulling" up the FP could result in a triggering event. The signal would then continue trimer to trimer within the hexamer. This scheme of cooperative triggering provides an intermolecular basis for how a single triggered F may kinetically relay a signal from the attachment protein to other F proteins and may address the need for multiple F proteins to be involved in membrane fusion by spatiotemporally synchronizing multiple (six?) fusion cascades.

Although it has not been formally shown to exist for all members of the *Paramyxoviridae* family, the PHI is known to form after F triggering (Fig. 6B). To reach this conformation, HR2 melts, losing its coiled-coil structure, forming an open stalk. HR1 refolds into an elongated helix and harpoons outwards towards the target membrane forming a new, trimeric coiled-coil stalk. The FP, attached to HR1, is projected towards and inserted into the target membrane. Hydrophobicity anchors the FP within the target membrane as a helix and the FP may further oligomerize, as PIV5 FPs have shown the capacity to form hexameric bundles

(158). While the PHI's existence has been most demonstrated for PIV5, there are significant similarities in membrane fusion across the family (130).

After reaching the PHI, it has been stated that the globular head of F undergoes conformational changes aligning HR1 and HR2 antiparallel to one another. We speculate that conformational changes in the globular head of F pull the N-terminal end of the open stalk, amphipathic HR2 to the C-terminal end of the newly formed trimeric HR1 stalk (Fig. 6C). These two domains have a high avidity for one another, so the initial meeting of HR1 and HR2 may lead to a "zippering" event along the grooves in the HR1 stalk. As HR2 zippers down the HR1 stalk, it regains structure, being pulled back into a helix, as seen in post-fusion crystal structures. Zippering progresses and the membranes bound proximally to each HR are then brought closer together. Once HR1 and HR2 have fully bound, the membranes have been pressed together and the outer leaflets fuse. The instability of a strong positive curvature brought on by pulling the membranes together and with continued force from HR1:HR2 interactions, may cause lipids in the outer leaflet to reorient into a more stable, partially fused structure: hemifusion (Fig. 6D). With a theoretical length of 25–48 amino acids, for PIV5, it is hypothesized that the TM domain of F may help stabilize hemifusion by spanning its membrane and the connecting region between the two fused outer leaflets, although this has yet to be confirmed (159). To our knowledge there is no direct evidence to support the slow HR1-HR2 zippering process; however, kinetic evidence that the transition from PHI to 6HB takes on the order of a few minutes, not seconds or milliseconds (at least for NiV F), makes this model plausible.

Fusion pore formation is thought to be mediated by the ectodomain of F. It has been shown that truncations of the PIV5 CT can progress through hemifusion and fuse the inner leaflets, forming a small pore between the virus and its host (160, 161). In contrast, truncations of SER virus CT rescued full syncytium formation, despite the wild-type F pH requirement (151, 162). Zippering likely continues into the membrane itself by interactions between the FP and TM domains (158). The FPs form the bundle's core and TM domains lodge along grooves formed by the three clustered helices, as shown for RSV, NDV, and hPIV3 post-fusion crystal structures (119, 131–133). This final conformation of F is the 6HB (Fig 6E): a low energy, irreversible state indicative of a fully expended high-energy protein (163).

While the fusion pore has formed, viral RNA cannot pass through, as the estimated 1–2nm pore is not large enough (164). Subsequent expansion of the fusion pore is thought to be mediated by the F CT (161). Also, for PIV5, actin has been shown to inhibit fusion pore expansion (165). This may indicate that a major obstacle in pore expansion could be the cytoskeleton. While fusion pore expansion has not been well studied, intracellular proteins, such as Rho GTPases for RSV and HeV, have been shown to affect syncytium formation (166, 167). Interactions with intracellular proteins by the F CT, or by unknown functional domains of these viral proteins, somehow lead to cytoskeletal rearrangements or other processes necessary to enlarge the fusion pore (168, 169).

Conclusions

In summary, membrane fusion is accomplished by two paramyxovirus glycoproteins: the attachment and fusion glycoproteins. While there are differences in how this crucial aspect of the paramyxoviral life cycle is accomplished, there are many commonalities. All steps of the membrane fusion process, from receptor binding to fusion pore expansion, may offer future targets for therapeutic development. The more we understand each of these steps, the better we will be prepared to target them.

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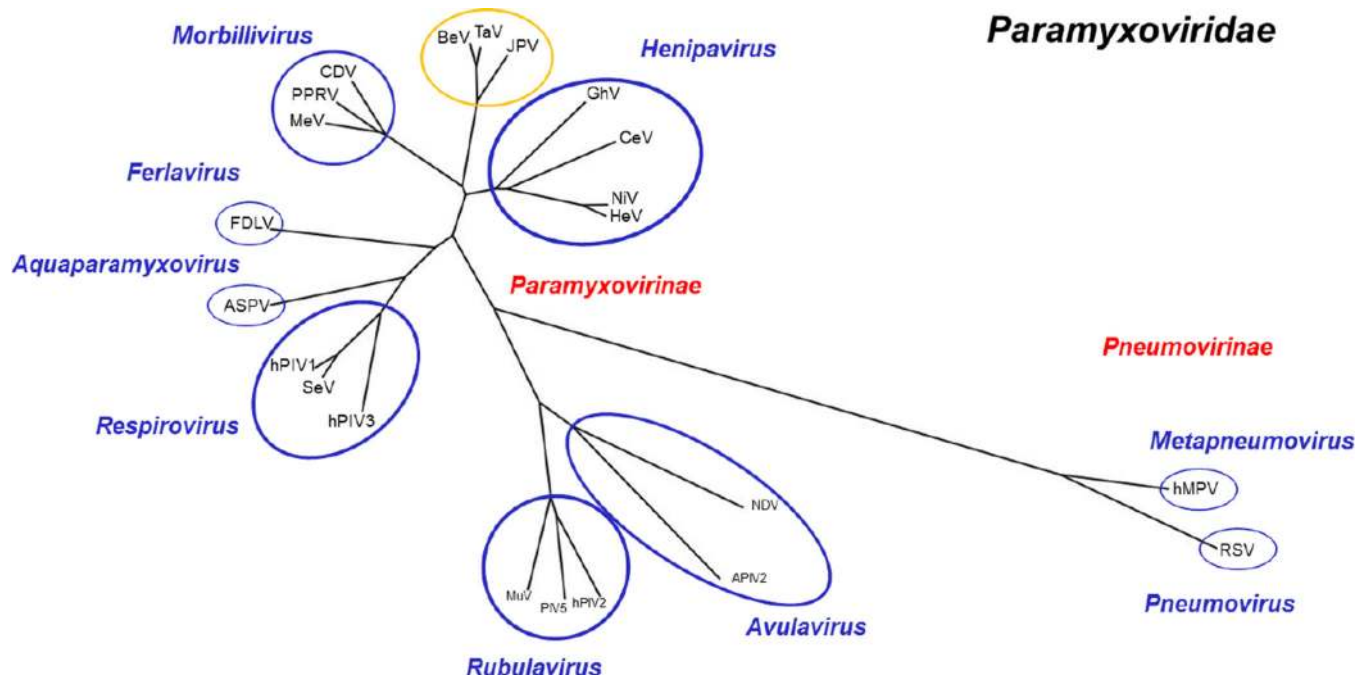
Fig. 1. Paramyxoviridae polymerase protein phylogeny tree
Polymerase/Large protein sequences of selected viruses were acquired from the NCBI Protein Database and Virus Pathogen Resource (ViPR). L protein sequences were aligned using the COBALT Multiple Alignment Tool-NCBI. Aligned sequences were then used to generate a phylogenic tree using COBALT NCBI TreeView1.8. The generated tree was visualized and modified using the FigTree Program. Abbreviations: APIV-2, avian parainfluenza virus 2; ASPV, Atlantic salmon paramyxovirus; BeV, Beilong virus; CDV, canine distemper virus; CeV, Cedar Virus; FDLV, Fer-de-Lance virus; GhV, Ghana virus; HeV, Hendra virus; hMPV, Human metapneumovirus; hPIV1, Human parainfluenza virus 1; hPIV2, Human parainfluenza virus 2; hPIV3, Human parainfluenza virus 3; JPV, J Paramyxovirus; PIV5, Parainfluenza virus 5; RSV, respiratory syncytial virus; MeV, Measles virus; MuV, Mumps virus; NDV, Newcastle disease virus; NiV, Nipah Virus; PPRV, Peste-des-petits ruminants virus; TaV, Tailam Virus. Paramyxoviridae family (black); sub-families (red); genus (blue); unclassified (yellow).
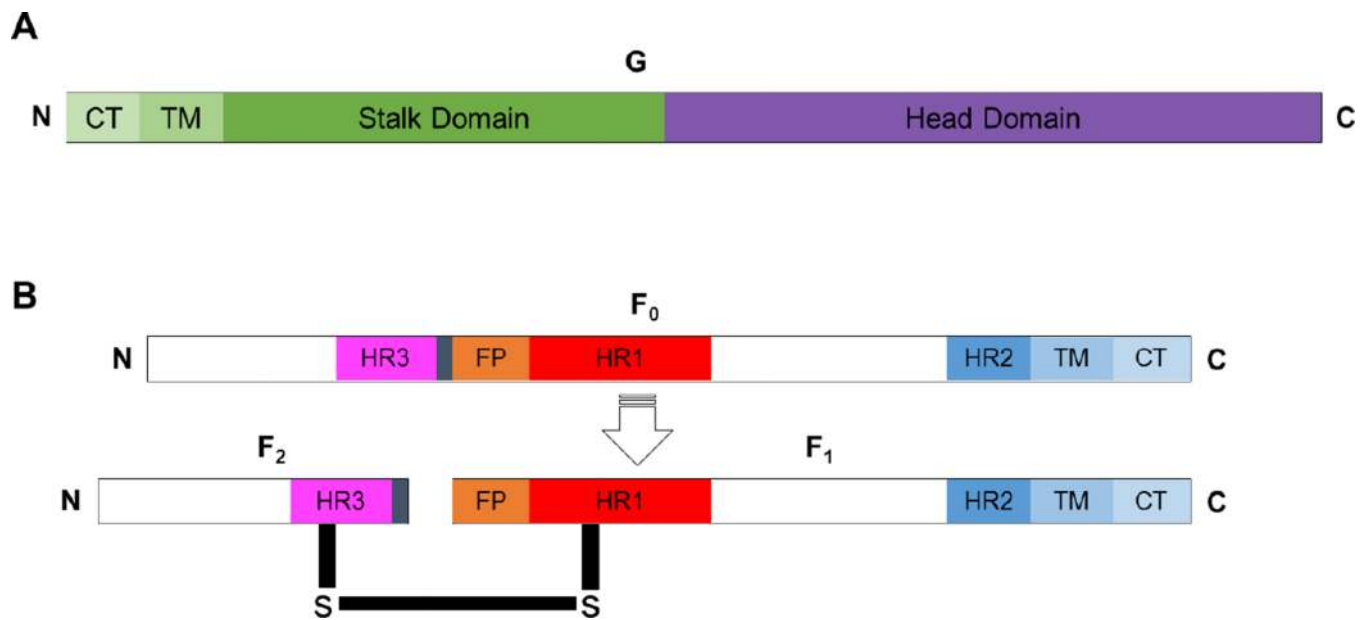
Fig. 2. Paramyxovirus attachment and fusion glycoproteins

A) Diagram depicting the attachment glycoprotein. B) Diagram depicting the precursor of the paramyxovirus fusion glycoprotein (F_0, top) and the cleaved, biologically active and disulfide linked paramyxovirus protein (F_1-F_2, bottom). The fusion peptide (FP), heptad repeats HR1, HR2, and HR3, transmembrane (TM); and cytoplasmic tail (CT) domains are shown, and the N- and C-termini are indicated.
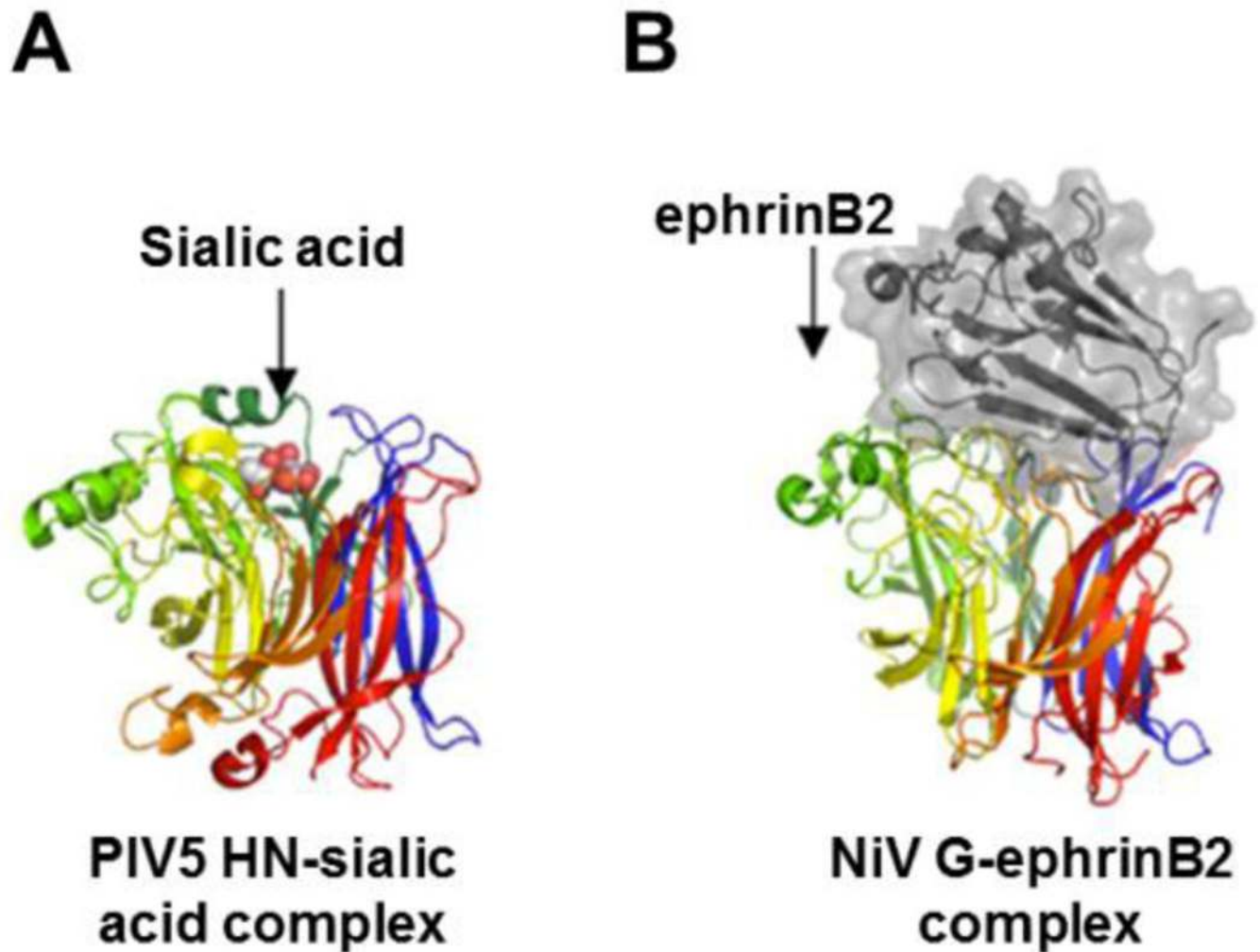
Fig. 3. Receptor binding with attachment glycoproteins occurs at the head domain
The attachment protein binds the cellular receptor within a binding pocket(s) in the globular head. As illustrated, the glycoprotein monomer heads have separate β-blades distinguished by different colors for structures of the PIV5 HN (A) or NiV G (B). In both of these ribbon models sialic acid (A; orange cluster) or ephrinB2 (B; colored grey) binds the attachment protein towards the center of the β-propeller. This figure was adapted from (123). The PDB code for the NiV G-ephrin B2 complex is 2VSM (52).

A

B
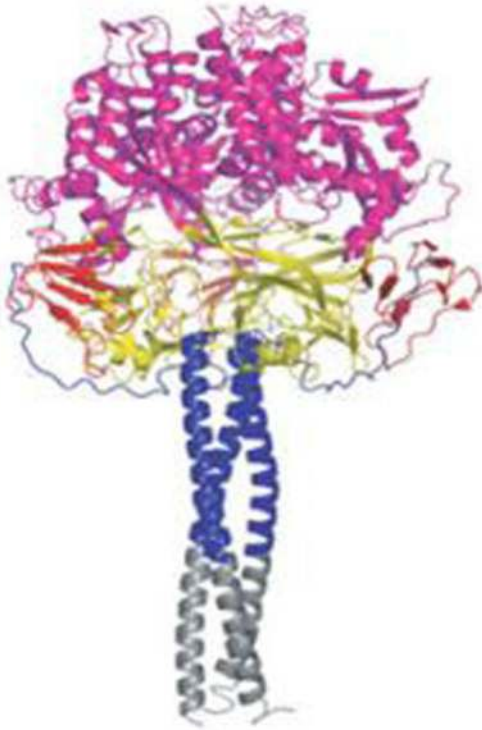
**PIV5 metastable
pre-fusion**
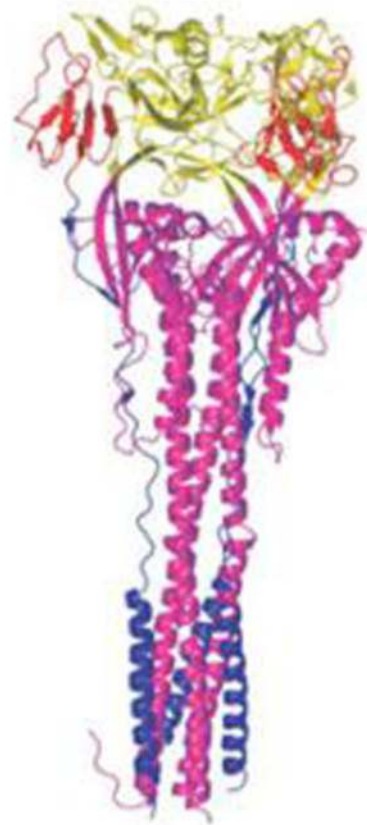
**hPIV3
post-fusion
(six-helix bundle)**

Fig. 4. The pre- and post-fusion conformations of paramyxovirus F glycoproteins
Substantial structural rearrangements occur in the progression from pre-fusion to post-fusion
F glycoprotein conformations. Several domains are distinguished by color including the
large HR1-containing region (magenta) and the HR2 domain (blue). In the pre-fusion
structure from PIV5 (A), a trimeric stabilization domain was added to support crystallization
(grey). The six-helix bundle conformation is apparent in the hPIV3 structure (B). This figure
was adapted from (111). The PDB codes for A and B are 2B9B and 1ZTM, respectively
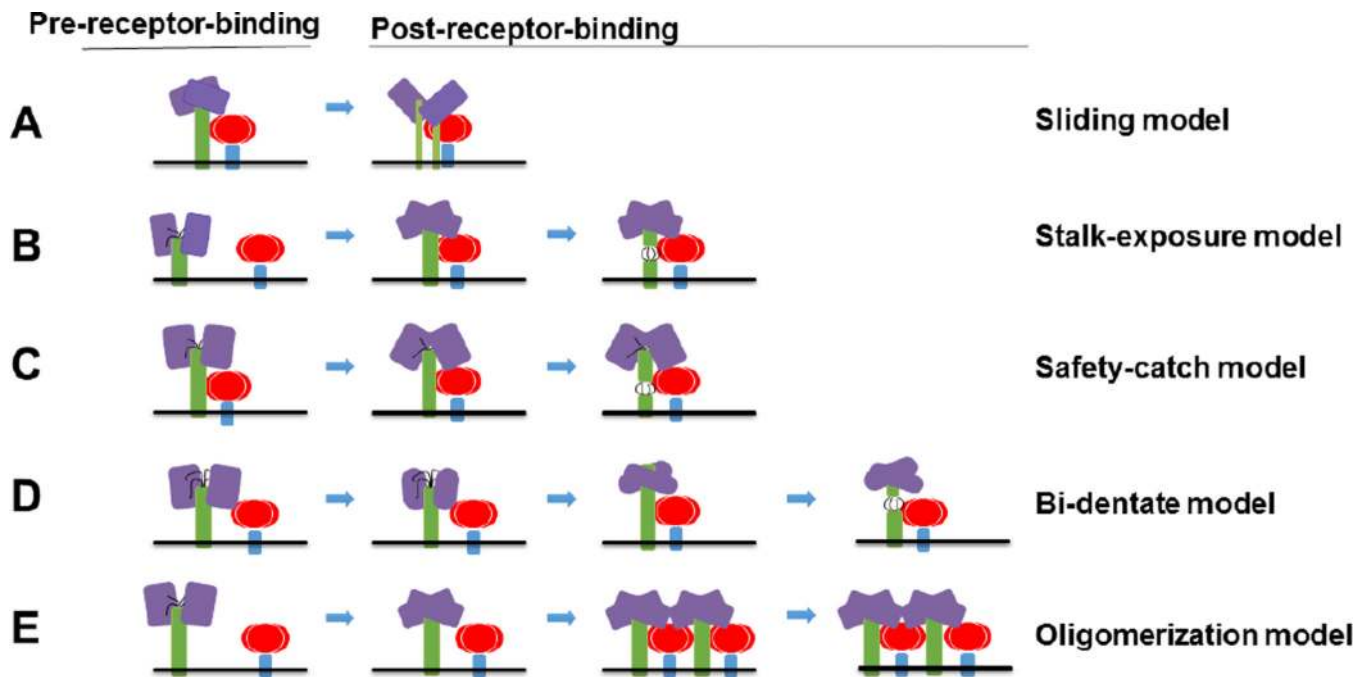(111, 119).

Fig. 5. Five models of F activation (A–E)
Attachment glycoprotein tetramers are colored in purple (heads) and green (stalks, transmembrane, and cytoplasmic tail domains). F is highlighted in red (heads) and blue (stalks, transmembrane, and cytoplasmic tail domains). In the bi-dentate model, the conformational changes of the head are shown as slightly distinct shapes. F-triggering regions in the stalk are shown as black lines (B–D).
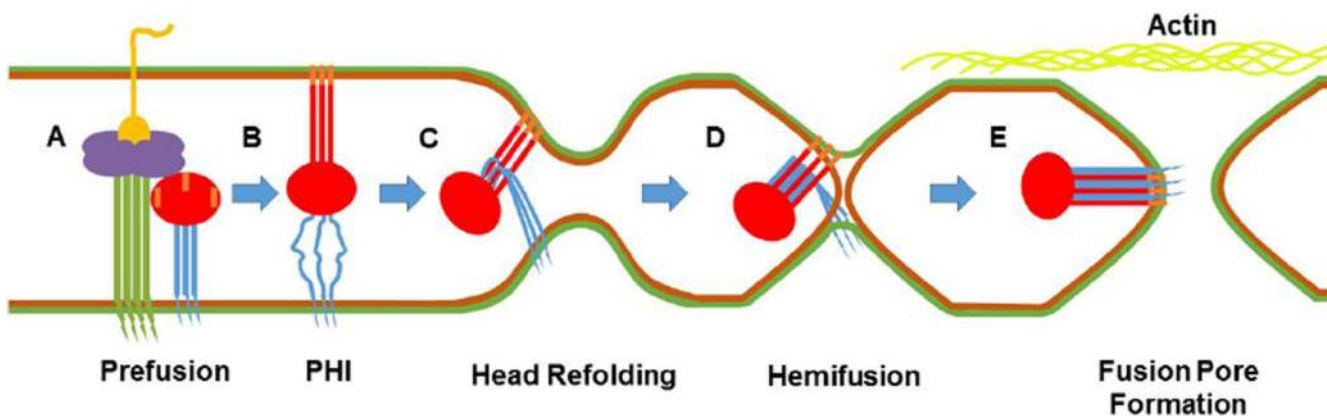
Fig. 6. Model of the late steps in paramyxovirus membrane fusion
Inner and outer membrane leaflets of viral and cellular membranes are shown as green and brown, respectively. The attachment protein is shown with a green stalk and purple head, while yellow depicts its bound receptor. For F, the cytoplasmic tail (CT), transmembrane (TM) domain, and HR2 are shown in blue. The head region through HR1 is shown in red and the fusion peptide in orange. A) The attachment glycoprotein binds its receptor and triggers prefusion F. B) To reach the prehairpin intermediate (PHI), HR2 melts and forms an open stalk. HR1 extends and projects the fusion peptide into the target membrane. (C) The head continues to refold, bringing HR1 and HR2 into close proximity, and pulls the membranes in. (D) HR1 and HR2 "zipper" together and the outer membranes fuse to form the hemifusion intermediate. (E) Inner membranes fuse as zippering continues through the transmembrane domain, forming the 6-helix bundle. F's CT is now exposed to intracellular proteins and the fusion pore expands. The actin cytoskeleton (shown in light green) is likely involved in fusion pore expansion.