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Unity in diversity: Shared mechanism of entry among paramyxoviruses

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Abstract

The *Paramyxoviridae* family includes many viruses that are pathogenic in humans, including parainfluenza viruses, measles virus, respiratory syncytial virus and the emerging zoonotic Henipaviruses. No effective treatments are currently available for these viruses, and there is a need for efficient antiviral therapies. Paramyxoviruses enter the target cell by binding to a cell surface receptor and then fusing the viral envelope with the target cell membrane, allowing the release of the viral genome into the cytoplasm. Blockage of these crucial steps prevents infection and disease. Binding and fusion are driven by two virus encoded glycoproteins, the receptor-binding protein and the fusion protein, that together form the viral "fusion machinery". The development of efficient antiviral drugs requires a deeper understanding of the mechanism of action of the *Paramyxoviridae* fusion machinery, which is still controversial. Here we review recent structural and functional data on these proteins and the current understanding of the mechanism of the paramyxovirus cell entry process.

Keywords

Paramyxoviridae; fusion machinery; viral entry; receptor-binding protein; fusion protein

1. Introduction to paramyxoviruses

1.1 Classification and medical significance

The *Paramyxoviridae* family, among the *Mononegavirales* order, is composed of enveloped viruses containing non-segmented negative strand RNA (reviewed in Refs. 1–3). Its members are found worldwide (Fig.1) and infect a broad range of host species including humans, pigs, horses and birds. Several paramyxoviruses such as measles virus (MeV), mumps virus (MuV), human parainfluenza viruses (HPIV) and respiratory syncytial virus (RSV) continue to have a major impact on global health. These viruses cause severe infections mainly affecting the respiratory tract of children and immunocompromised patients (Table 1).

The *Paramyxoviridae* family is divided into two sub-families: *Paramyxovirinae* and *Pneumovirinae*. The *Paramyxovirinae* sub-family consists of seven genera, *Respirovirus* (which includes human parainfluenza virus type 3; HPIV3), *Rubulavirus* (which includes MuV); *Morbillivirus* (which includes MeV), *Avulavirus* (which includes Newcastle disease virus; NDV), *Aquaparamyxovirus* (which only includes atlantic salmon paramyxovirus; ASPV[4]), *Ferlavirus* (which only includes Fer-de-Lance virus; FDLV[5]), and *Henipavirus* (which includes Nipah virus [NiV] and Hendra virus [HeV] as well as Cedar virus [CedPV] recently discovered in bats in Australia[6]); Table 1. Other paramyxoviruses such as J-virus (JPV) and Beilong virus (BeiPV), as well as some recently discovered bat paramyxoviruses, are closely related, but remain unassigned to any sub-family[7,8]. The *Pneumovirinae* sub-family consists of two genera: *Pneumovirus* (which includes RSV) and *Metapneumovirus* (which includes human metapneumovirus; HMPV); Table 1.

Epidemiological studies have shown that HPIV is responsible for around 7% of hospitalizations for fever and/or respiratory diseases in children under five[9]. RSV alone is responsible for at least 3–9% (66,000 – 199,000) of deaths caused by acute lower respiratory tract infection worldwide, mainly in children under the age of five[10]. Human metapneumovirus (HMPV) also causes acute respiratory infections. Studies led on hospitalized patients in Virginia revealed that HMPV is involved in 90% of wheezing cases requiring hospitalization[11]. In terms of the pathogens that do not infect humans but cause problems to society, NDV infects poultry and is associated with a high mortality rate due to respiratory tract infections, generally occurring in developing countries where this disease has a negative economic impact (reviewed in Ref. 12).

The emerging henipaviruses NiV and HeV is associated with high mortality and/or lethal outbreaks. In the first outbreak of NiV in Malaysia in 1999, 265 people were infected, and 105 patients died of fatal encephalitis. HeV first emerged in Australia in 1994 primarily affecting horses, however, seven people have been infected resulting in four deaths. All Henipaviruses are classified as Biosafety level 4 agents, due to the high lethality of infection and the lack of established treatment. The main reservoir for Henipaviruses is fruit bats, notably the *Pteropus* genus. These bats are mainly present in Africa, South-East Asia and Oceania (reviewed in Refs.13,14). Recent studies have identified new paramyxoviruses in European insectivorous bats[8] which produce symptoms resembling HeV infection. While these viruses remain unassigned to any genus, they are more closely related to *Paramyxovirinae* than to Pneumovirinae[8].

The only preventive vaccines currently available for members of the *Paramyxoviridae* family are those against MeV, MuV, and NDV (for poultry). Even these viruses are still a major health concern. According to the World Health Organization (WHO), each year, around 200,000 deaths are associated with MeV infection mainly in developing countries. The Centers for Disease Control and Prevention (CDC) declares that more people have been infected with measles in the United States during the first four months of 2014 than have been infected in the first four months of the past 18 years.

In 2012, 687,000 cases of MuV infection were reported across the world. (WHO). Usually, MuV infection is not lethal but it can lead to complications such as meningitis, encephalitis,

and orchitis, with possible permanent sequelae (WHO). Furthermore, there are currently no therapies to treat patients infected by any paramyxovirus (reviewed in Ref. 2), making these viruses a significant public health issue.

1.2 Structure

Paramyxoviruses are 150 to 300nm in diameter with envelopes composed of host cell lipids and viral glycoproteins (reviewed in Refs. 1,2). The genome is a non-segmented RNA strand of negative polarity, between 15,210 (RSV type 2, GU591759.1, Kumaria *et al.*[15]) and 15,894 nucleotides (MeV, NC_001498.1, Takeuchi *et al.*[16]) with the exception of Henipaviruses which contain a longer genome (NC_001906, Wang *et al.*[17], Yu *et al.*[18], NC_007454.1, Jack *et al.*[19], NC_007803.1, Li *et al.*[20]). The length of the genome of each paramyxovirus is always a multiple of six nucleotides, an organization required for efficient replication by the viral polymerase[15,16]. The genomic RNA strand is encapsidated by the helical nucleocapsid protein, N or NP (Fig. 2). The Large protein (L) and the phospho protein (P) constitute the viral RNA-dependent transcriptase / replicase complex. In the virion, L and P are associated with the RNA-nucleocapsid complex (Fig. 2). The N or NP protein also interacts with the matrix protein (M), a non-structural protein that lines the envelope of the viral particle (Fig. 2). The lipid bilayer envelope of the virus is derived from the host cell membrane, formed when the virus buds from a region of membrane expressing the viral receptor-binding protein (HN/H/G) and the fusion protein (F). As for many other enveloped viruses, the virions are labile, and can be easily inactivated *ex vivo* by heat, organic solvents such as ethanol, or detergents.

The six proteins N/NP, L, P, M, HN/H/G and F are conserved among the *Paramyxoviridae* family. In addition, some structural proteins are restricted to specific viruses and their roles may be less clear, for example the small hydrophobic proteins (SH) and the transmembrane (TM) proteins[7,17–20]. *Paramyxoviridae* also encode for non-structural proteins that are involved in the inhibition of the interferon response[21]. In addition, the alternative splicing of the P-gene leads to the expression of C, V and W proteins, whose role is to counteract host innate immunity (reviewed in Ref. 22).

1.3 Viral entry and life cycle

Paramyxovirus fusion is mediated by two different viral proteins that in most cases must work in concert to accomplish viral entry. The receptor-binding protein first engages the cellular receptor, then in most cases activates the fusion protein, and the fusion protein inserts itself into the target cellular membrane, allowing the viral envelope and target cellular membrane to merge. Upon fusion of the viral envelope with the target cell membrane, the genetic material is released into the cytoplasm. The negative sense RNA, which is present in the form of a nucleocapsid or RNA/protein complex, is converted into positive sense message-length RNAs by the RNA-dependent RNA polymerase that is provided by the virus. This step allows for translation of virally encoded proteins. Replication of the viral genome occurs via transcription of a full length positive sense strand which is then copied into a full-length negative sense new genome, and encapsidated by the viral nucleocapsid protein. The matrix protein binds to the nucleocapsid and interacts with the cytosolic tails of the membrane bound HN/H/G and F proteins, facilitating the process of

budding of progeny virions. Release of new viral particles from the cell surface, in some paramyxoviruses requiring a receptor-cleaving enzymatic function carried out by the receptor binding protein[23], permits infection of new target cells and spread of infection.

Most *paramyxovirus* fusion events occur in a pH-independent manner, at the cell surface, however some viruses enter the cell via endocytosis (reviewed in Refs. 1,3). How the receptor-binding protein and the fusion protein (together called “fusion machinery’) work together to promote fusion has been an area of active investigation since it was first shown that the paramyxovirus receptor binding protein plays an active role in the fusion process during entry[24–26]. Several models have been proposed, and the molecular details of the fusion process mediated by the paramyxovirus fusion machinery remain controversial. Previous models postulate a duality amongst *Paramyxoviridae* (reviewed in Refs. 1,3). It has been proposed that for paramyxoviruses that bind a proteinaceous receptor, the role of the receptor binding protein is mainly a repressive one[27–32] (reviewed in Refs. 33–35) and that upon receptor binding, the fusion protein is released and proceeds to fusion ; on the other hand, the receptor binding proteins of sialic-acid binding viruses have been thought to interact with F only upon receptor engagement[27,28,36,37] (reviewed in Refs. 33,34,38,39). Our data suggest that a common mechanism applies to all paramyxoviruses that use a receptor-binding protein to activate a fusion protein, including those that bind a proteinaceous receptor[40,41].The debate will be detailed in the sections below. In this chapter we review recent advances in the field of paramyxovirus entry. We first summarize structural data about *Paramyxoviridae* virions and specifically HN/H/G and F. The focus then turns to receptor engagement and its effects on HN/H/G. We detail the interaction between these two surface glycoproteins before, during, and after receptor engagement, as well as the membrane fusion process mediated by F, and propose a potential unifying model for *Paramyxoviridae* fusion.

2. Structure and function of the paramyxovirus glycoproteins

2.1 The receptor binding protein

The paramyxovirus receptor binding proteins present on different members of the virus family are known as HN, H, or G. These proteins are distinguished by the type of receptor they engage, their ability to cleave sialic acid (neuraminidase activity), and their ability to agglutinate red blood cells (reviewed in Ref. 1). The HN protein carried by the *Respirovirus*, *Rubulavirus* and *Avulavirus* genera (Table 1) possesses both sialic acid binding (hemagglutinating) and sialic acid cleaving (neuraminidase) activities. Sialic acid binding is active during viral entry while neuraminidase activity is involved in viral budding and prevents the virus from self-aggregating. The H protein carried by the *Morbillivirus* genus (Table 1) does not bind to sialic acid during viral entry. Both HN and H proteins have the ability to agglutinate red blood cells, but the H binds proteinaceous receptors during MV entry. The H protein lacks neuraminidase activity, suggesting that following viral release, self-aggregation mediated by sialic acid binding does not occur. The G protein carried by the *Pneumovirinae* and *Henipaviruses* genera (Table 1) does not bind sialic acid and does not possess neuraminidase activity. Like H, G proteins bind proteinaceous receptors.

The three types of receptor-binding proteins differ in the type of receptor they bind, but share the same general architecture. HN, H, and G are type II transmembrane proteins, with N- termini inside the viral particle (Fig. 3). Each is present on the viral membrane as a tetramer composed of two dimers, an arrangement known as a dimer of dimers. A dimer consists of an association of two monomers (Fig. 3), each of which monomer contains a cytoplasmic tail domain, a transmembrane domain, a stalk domain, and a globular head domain (reviewed in Refs. 1,3).

Dimers of the receptor binding proteins are formed by disulfide bridges between the stalk domains of two monomers[42–53] and are also linked via the stalk domain of the proteins, as described for PIV5-HN[42], although the transmembrane domain may stabilize the tetramer, as described for NDV-HN[53]. These inter-dimer links mainly involve non-covalent bonds which are weaker than the intra-dimer disulfide linkages. Tetramers of HN/H/G are more suitable for crystallization than dimers, as described for HeV-G[52]. Alteration of disulfide bridges via *in vitro* mutagenesis alters dimer and tetramer stability[50,51]. Interestingly, a recent study from Navaratnarajah *et al*.[51] reported that the complete stalk domain of MeV-H is not directly involved in the tetrameric structure, and the extent of involvement of the stalk in formation of the tetramer for other members of the *Paramyxoviridae* family is unclear.

Crystallographic studies of the tetrameric globular heads show each monomer carriying an N-terminal six-blade β-propeller, characteristic of neuraminidase enzymes[43–55]. Interestingly, HN, H and G share this structure, although only HN possesses neuraminidase activity. H and G carry a structural vestigial neuraminidase site[43–47], consistent with the hypothesis of a common evolutionary origin for these three receptor-binding proteins.

In the case of HN, the sialic acid binding site of each monomer, known as sialic acid binding site I, is located at the top of the globular head domain, in the center of the β-propeller. In addition, a second sialic acid binding site (known as site II) was identified crystallographically at the dimer interface of NDV-HN.[56] This site II is involved in receptor binding[57,58]. Functional analysis have suggested a second sialic acid site on HPIV1[59,60] and have identified a second sialic acid binding site on HPIV3 that is also important for activating F[61], although these sites have not been demonstrated crystallographically. The receptor binding site of G shares the same location as site I[46,47] whereas the H binding sites are located on the side of the β-propeller[45,55, 138]. The structure of the tetrameric receptor-binding protein ectodomain, comprised of the head and stalk domains, have been solved for PIV5[48] and NDV[53]. The stalk domain adopts a four helix bundle conformation (Fig. 3) with a hydrophobic core located at the upper part of the stalk domain[48,53].

2.2 The fusion protein

The paramyxovirus fusion protein, F, is a type I transmembrane protein, with its N-terminus outside the viral particle. It is synthesized as an inactive F_0 precursor (reviewed in Refs.1,3). F_0 is then cleaved into its active form, F, which is composed of two sub-units, F_1 and F_2. The two sub-units are linked by a disulfide bridge between the HRN of F_1 and F_2 (Fig. 4). The cleavage creates the hydrophobic fusion peptide, which is inserted into the target membrane during the fusion process, once an activation step exposes the peptide at the surface of the molecule.

F acts as a homotrimer in which each monomer is linked to each other via a transmembrane domain[62,63] and contains a cytoplasmic tail, a transmembrane domain, a heptad repeat C-terminal domain (HRC), a heptad repeat N-terminal domain (HRN), and the fusion peptide (Fig. 4). The heptad repeat domains are regions of 7-mer repeats in which every seventh residue is either a Leucine, Isoleucine or Valine, and the whole structure is an amphipatic α-helix.

The F cleavage step is crucial for the viruses, as uncleaved F proteins are unable to promote fusion. For most *Paramyxoviridae* (with the exception of Henipaviruses) furin proteases within the trans-Golgi network cleave F0 at an R-X-K/R-R consensus motif (reviewed in Ref. 1). Unlike most *Paramyxoviridae*, RSV F possesses two cleavage sites which are required for efficient fusion[64]. For Henipaviruses, F_0 is cleaved in the endosomal compartment by cathepsins L and B at a VGDVR/K consensus motif[65–68]. Henipavirus F_0 is expressed at the plasma membrane, re-internalized and then cleaved before associating with the rest of the viral particle. The cleavage also seems highly dependent on the valine content of the fusion peptide, as reported for HeV[69].

The structure of cleaved PIV5-F in its pre-fusion state has been solved[70]. The fusion peptide is initially buried in a hydrophobic pocket, preventing premature exposure[70]. This pocket is composed of the HRN (Fig. 4). In this pre-fusion state, HRC forms an α-helix close to the viral membrane[71]. The crystallographic data suggested that few conformational changes occurred after cleavage, when compared to uncleaved forms of PIV5 F and HPIV3 F[72,73]. However, uncleaved F is fusion incompetent. Established models describe the state of pre-fusion F as being metastable, and destabilized following activation by the receptor-binding protein.

In the post-fusion state, the fusion peptide is exposed in an open α-helical domain, and the heptad repeat domains associate, forming a highly stable 6-helix bundle. The formation of this stable structure is a significant driver of the process of membrane fusion[74,75] (reviewed in Ref. 76) (Fig. 4). Like the receptor binding proteins, F is highly glycosylated. For NiV-F, it seems that some of these glycosylation sites decrease the fusogenicity of the virus[77]. *In vivo* these additional carbohydrates may protect the virus from recognition by the host immune system[77].

3. Proposed mechanisms of receptor binding protein and fusion protein interactions

3.1 The globular heads of the receptor-binding protein selectively engage specific cellular receptors

The fusion process begins when the receptor-binding protein engages its receptor. HN recognizes sialic acid bearing membrane proteins, whereas H and G bind proteinaceous receptors. H binds different proteinaceous receptors for each virus. For example, MeV H engages CD46, CD150/SLAM (signaling lymphocyte-activation molecule), and Nectin 4[45,55,78–82]. CD46 binding seems to be unique to laboratory-adapted strains. CD150 is expressed on the cell surface of macrophages and dendritic cells, and MeV engages this receptor to infect the host immune system[83]. Nectin-4 is expressed on the basal surface of

the epithelium cells, allowing MeV to be spread from macrophages to epithelium and then into the lung lumen.[81,82] The neurotropic Henipavirus G engages Ephrin B2 and B3 on cell surfaces[6,46,47,84]; these molecules are expressed in neurons in the brain. Ephrin B2 and B3 are also found in other cell types, and are conserved among many species, allowing Henipaviruses to infect a range of species including humans, pigs, horses and bats. Henipaviruses can spread within the host by binding lymphocytes and using them as transporters[85]. The G protein of *Pneumovirinae* binds heparan sulfate proteoglycans[86–89]. RSV-G has been shown to interact with the chemokine receptor CXC3CR1, through a CX3C motif[90]. While it is unlikely that this interaction would promote fusion, this interaction strongly inhibits the host immune response[91]. The diversity in receptor usage confers paramyxoviruses the ability to adapt, gain access and infect new tissues and new hosts.

3.2 The stalk domain the receptor-binding protein interacts with and activates F

HN/H/G is the driving force for fusion initiation and then for sustaining F's role in mediating viral entry[40] (reviewed in Refs.[1,92]). Under a variety of *in vitro* experimental situations, F can fuse alone[40,58,93,94], or a "headless" HN/H/G may be sufficient to mediate F activation[41,94,99] ; see specific examples below. However, as discussed in section 3.6 the function of specific residues in the globular head of HN is essential for infection in the host, and any subtle change at the dimer interface of the globular domain can affect HN dimer association, impact the HN/F fusion machinery, and markedly alter host infection.

The globular heads of the HN/H/G proteins bind the cellular receptor. The stalk domains of HN/H/G proteins are responsible for specific interaction with the homologous F proteins and are critical for F activation once they receive the signal from the receptor bound globular head[27,37,42,49–51,53,95–98]. After initial identification of the importance of the stalk of the receptor binding protein for activating F, this stalk function has been assessed using a variety of approaches including the use of the "headless" receptor binding proteins mentioned above. A construct consisting of the PIV5 HN stalk domain (residues 1–117) lacking the globular binding domain was sufficient to activate F. This activation seemed to be specific; the PIV5 HN stalk could not activate heterotypic Fs, and required direct interaction with F[94]. This set of experiments was used to postulate that for PIV5 HN, activation of F requires that the stalk domain be "freed". Receptor engagement would drive the movement of the heads that would free the stalk. Similar experiments have been performed using different "headless" stalks proteins with varied results. However, only very specific MeV H, NiV G, and PIV5 stalk lengths can activate the F protein[94,96,99] and, for MeV H, the stalk must be partially stabilized in order to be functional[96]. Only one out of several different headless stalk constructs of mumps HN[100], NDV HN[100], and NiV G[99] can activate F suggesting that the specific sequence of the receptor binding protein stalk and the F protein are crucial for this activity. For HPIV3, a headless HN does not seem to be capable of activating HPIV3 F. Thus how the stalk domain of paramyxovirus HN/H/G activates F remains to be further characterized, and as described below, we contend that the interaction of the globular head of the receptor binding protein with its receptor provides a critical signal to the stalk in the process of F activation.

Chimeric proteins bearing the globular domain from NDV and the stalk domain of either HPIV3-HN, NiV-G, or MeV-H revealed that receptor engagement by the NDV-HN globular head is sufficient for transmitting the activating signal through the stalk domain of these other paramyxoviruses and trigger the homologous fusion protein[58,101]. These chimeric receptor-binding proteins are only capable of triggering an F protein that is homologous to the stalk domain of the chimeric protein. Thus a chimeric protein with an NDV-HN globular head and an HPIV3-HN stalk can only activate HPIV3-F[58]. The only exceptions are Henipaviruses NiV-G and HeV-G whose stalks demonstrate enough sequence similarity to activate both F proteins[102]. Closely related Henipaviruses, such as the recently discovered Cedar virus[6], may share the same property. The chimeric receptor-binding proteins reveal one of the ways in which HN, H, and G protein function is conserved at least amongst the *Paramyxovirinae* sub-family, and support the hypothesis of a unified model for the paramyxovirus fusion machinery in which the globular head domain of the receptor-binding protein acts as a receiving unit that is independent of the rest of the protein. The receptor-binding protein engages its receptor and transmits a signal to the stalk domain. The stalk domain likely undergoes conformational changes allowing it to activate its homologous fusion protein[50,103] (reviewed in Ref.[39]).

3.3 The role of the receptor-binding protein before receptor engagement

Several distinct models describing the interaction between the HN/H/G protein and the F protein have been proposed (reviewed in Refs. 1,3). One model, the dissociation or clamp model, postulates that the HN/H/G and F proteins interact prior to receptor engagement and that receptor engagement abrogates this interaction. Another model, the association or provocateur model, suggests that HN/H/G only interact with the homologous F protein following receptor engagement. Recent studies from our group have uncovered elements in support of a unified model among paramyxoviruses[41,104]. We used a Bimolecular Fluorescence Complementation (BiFC) strategy where HPIV3 HN and HPIV3 F were respectively fused with the N-terminus of YFP and C- terminus of CFP. Only if HN and F proteins interact, the fluorescent protein is reconstitiuted and fluorescence is emitted upon excitation. We observed that HPIV3 HN interacts with F in the absence of receptor engagement. Upon receptor engagement, HN and F continue to interact and cluster at the point where the fusion pore will form[41]. Whether clustering occurs before or after activation of the F protein has not been firmly established, but recent data are more consistent with clustering occurring first, and F activation occurring in the cluster (unpublished). HN and F continue to interact throughout the fusion process[40] and dissociate only once fusion is complete (unpublished).

For HPIV3, it appears that non-receptor engaged HN protein stabilizes F, maintaining it in the pre-fusion state. When HPIV3 F alone is exposed to high temperatures, it enters the post fusion state (as assessed by acquisition of sensitivity to proteinase K digestion); however, in the presence of non-receptor engaged HN, F remains in its pre-fusion state, resistant to proteinase digestion[104]. These data support the idea that HPIV3 HN serves a “protective” role for the fusion protein[104]. Prior to receptor engagement the receptor-binding protein stabilizes HPIV3 F and prevents it from premature activation. Ader et *al*.[103] recently showed that the F protein of some morbilliviruses is highly stable and suggested that it is unlikely

that the H stabilizes F, however it cannot be excluded that *in vivo*, stabilization may be required since many parameters could prematurely trigger fusion.

An intriguing role of pH in the NDV cell entry process has recently emerged[105]. NDV entry is reduced when caveolin-associated traffic is inhibited. Cholesterol seems to be important in the process since the drug methyl-β-cyclodextrin, which inhibits cholesterol trafficking, also diminishes NDV-HN binding. Moreover, NDV particles were shown to colocalize with EEA1, a marker of early endosome formation suggesting that NDV could enter the cell through caveolin-mediated endocytosis. Past work showed that HMPV, NiV, and RSV can use the endosomal pathway to enter cells[106–108]. Low pH exposure increases NDV fusion and subsequent syncytia formation while, reciprocally, fusion decreases in the presence of pH-acidification inhibitors[109]. Consistent with these results, the stability of NDV F (assessed through fusion assays) decreases after exposure to low pH; F is more easily activated under these conditions[109]. There may be an accessory pH-dependent pathway through the caveolin-mediated endocytosis for NDV.

For some paramyxoviruses, the F protein can mediate fusion in the absence of the receptor-binding protein, in some cases permitting viral infection[110–116]. However very few viruses are infectious when lacking their receptor-binding protein. Infectious virions that lack a receptor-binding protein have been studied for RSV, HMPV, and laboratory adapted strains of NDV, however in these cases, infectivity is enhanced when the receptor-binding protein is present[89,111,117,118]. In the case of RSV, the F protein itself can bind nucleolin[119], the HMPV F can bind integrins[120], and both can engage heparan sulfate[121–123], potentially permitting G-independent entry. It will be important to determine whether in these cases the receptor binding protein serves a role in vivo in stabilization of F prior to receptor engagement; an F-stabilizing role for HN/H/G protein may be a conserved feature at least among the *Paramyxovirinae* sub-family.

3.4 The receptor-binding protein transmits a triggering signal to the fusion protein upon receptor engagement

Upon receptor engagement, the HN/H/G protein activates the F protein to undergo its final fusion-readiness structural changes. The mechanism whereby this activation occurs – where the signal for activation originates, and how it is transmitted from the receptor binding protein to the fusion protein – is a topic of significance. The stalk domain of HN/H/G protein is critical to this activation process[51,95–97,100,124,125]. Recent studies from our group indicate that for NiV the domain that connects the globular head to the stalk domain is required for transmission of the triggering signal to F[124]. Chimeric receptor-binding proteins containing the globular heads of NDV and the stalk domain of NiV can efficiently activate NiV-F only if specific residues are present in the head-stalk junction[124].

A proposed model deriving from PIV5 postulated that the heads of HN/H/G could change their position upon receptor engagement, shifting from a “heads-down” conformation, in which the stalk domain is masked, to a “heads-up” conformation exposing the stalk domain. A masked stalk domain would prevent HN/H/G and F interaction, and upon receptor engagement the heads would move aside, allowing the stalk to activate the F protein[42,48,53,94]. However, recent analyses of the MV fusion process do not support this

model, suggesting that at least this mechanism may not apply to other paramyxoviruses (reviewed in Ref. 39). MeV H and MeV F interact prior to receptor engagement[126], indicating that an H head must be up to allow H/F interaction during transit to the cell membrane. However F is not triggered prematurely (i.e., before receptor engagement during infection), suggesting that for MeV the exposure of the stalk is not the crucial requirement for F activation. It has been suggested that MeV receptor engagement with a "pulling" of the H molecule could induce a conformational change in the stalk that triggers F[27]. Brindley *et al*. noted that MeV H proteins with truncated globular heads promoted fusion *in vitro*[96], implying that the globular heads may mask a portion of the stalk domain responsible for activating the F protein, consistent with a "heads-down, heads-up" model. However, the truncated H proteins that were studied are highly specific, with truncation at precise sites on H being required for fusion complementation. Headless Hs, to promote F-mediated fusion, require stabilization at the C-terminus, e.g. by a yeast-derived GCN4 motif[96]. Thus it seems likely that the specific truncated H proteins do not represent a general mechanism, except when they are stabilized or otherwise modified to adopt a structure similar to the receptor-engaged conformation that activates F. Finally, key residues on the MeV H that are involved in F protein activation are located at the membrane-proximal part of the stalk domain, where the globular heads of MeV H do not reach even when the protein is in a "heads-down" conformation[51,95,100,124].

How does receptor engagement affect the receptor binding protein and modulate the F activation step? The first studies to analyze how receptor engagement modifies the receptor binding protein structure showed that receptor engagement does not appear to affect the monomer-monomer interface[127]. Stabilization of NDV HN monomers by disulfide bridges restricted monomer movement but enhanced HN's fusion promotion activity, suggesting that large movements between monomers are not needed for successful fusion promotion. Crystallographic data of MeV H bound to SLAM receptors revealed H present in two tetrameric conformations. In the pre-F triggering conformation H is in a planar form and upon receptor engagement, a "sliding" movement between the dimers occurs that gives rise to the post F-triggering form[45]. One recently proposed model suggests that upon receptor engagement, after exposure of the stalk of the receptor binding protein, a specific conformational change occurs in the complex formed by HN/H/G and F, to reach an "induced-fit" state that leads to F activation[100]. In this case, the nature of the signal activating F is a modulation of its interaction with HN/H/G rather than a loss or a gain of interaction (except perhaps for PIV5).

Even after F activation and during the fusion process itself, the receptor-binding protein continues to regulate the fusion process. For HPIV3, the presence of HN is crucial until merger of the viral and cellular membranes[40]. Using a mutant HN that lacks neuraminidase activity and thus constitutively engages its sialic acid receptor, we specifically disrupted receptor engagement at precise times using the small molecule zanamivir. Only when HN continuously engages its receptor can F proceed through the fusion process. Even after insertion of the fusion peptide into the target membrane, F still requires the activating signal from HN/H/G to complete fusion[40].

3.5 The fusion protein inserts its hydrophobic fusion peptide into the target membrane leading to the formation of the fusion pore

After activation, F undergoes several conformational changes (Fig. 4). Fusion can be prevented by introducing disulfide bridges to stabilize F[128]. F acts as an indivisible unit. The cytoplasmic tail of F protein must bear conserved Ser/Thr residues for fusion to properly occur, and the cytoplasmic tail is likely to be virus-specific; it cannot be interchanged with that of another virus[129]. The current model for F action is based on several available F protein structures (Fig. 4), and predicts that F inserts its fusion peptide into the target membrane by extending its α-helical domains. The amphipathic HRC and HRN domain then interact with each other, driving fusion. Supporting this hypothesis, Donald et *al* found that the α-helical domains on PIV5-F interact avidly with each other[130]. The model postulates that once the fusion peptide is inserted, the F protein is in a pre-hairpin intermediate state (Fig. 5). This transient intermediate was recently observed for PIV5[71]. Kim et *al*. used nanobeads bearing a lipid bilayer as targets for viral particles, and HRC-derived peptides that interact with the HRN domain to prevent F from refolding after fusion peptide insertion. They then measured the distance between the viral bilayer and the nanobead bilayer using EM, and compared it to *in silico* predictions based on the hypothesis of the pre-hairpin fusion intermediate. The experimental results fit the computational model, supporting the existence of the intermediate state[69]. F proteins blocked in the transient state were identified by electronic microscopy by gold coupling of HRC-derived peptides, and were observed to be present in clusters. This observation is consistent with our model for HPIV3, where receptor-binding and fusion protein clustering is required in order for fusion to occur[41]. Attaining this unstable fusion intermediate is likely to be the event that is prevented by the non-receptor-engaged HN/H/G protein[104].

After the unstable intermediate state, F adopts a hairpin intermediate state (Fig. 5). In this form, the two heptad repeat regions (HRN and HRC) interact with each other, leading to a 6 Helix-Bundle conformation, which mechanically forces the two membranes together (Fig. 5). In this process, the length of the HRC-linking region separating the HRC domain from the membrane (Fig. 4) is crucial. Linkers that are too short or too long were associated with fusion-incompetent F proteins, as described for NDV[75]. This is consistent with the hypothesis that the HRC-linker contributes to the stabilization of the HRC domain and thus can modulate its function, as previously described for HeV-F[63].

A critical element of this refolding is that the HRN and HRC domains form a close interaction. Inhibitors of this interaction an attractive strategy for blocking fusion and viral entry, and peptides derived from the HRC domain of paramyxovirus F molecules have recently been shown to inhibit the fusion process *in vivo*[131,132]. The peptide inhibitors are far more efficient when conjugated with lipids. This conjugation allows for peptide insertion into target cell membranes, placing the anti-fusion peptides in close proximity to the viral glycoproteins. These conjugated peptides can reach the brain and may be efficient against neurotropic viruses such as NiV, or neurotropic variants of MeV[131–133].

At the end of the F refolding process, F is in its stable post-fusion state. Experimentally expressed, soluble F protein naturally adopts this post-fusion state[73,134]. The stability of this

final state implies that the fusion process is irreversible, and supports the importance of the "protective role"[104] of the receptor binding protein in preventing premature initiation of the activation process. Indeed, virions whose F proteins have been prematurely activated cannot fuse and are non-infectious[135,136].

3.6 The interaction between HN/H/G and F modulates infection in the natural host

A series of recent experiments showed that the communication between the HPIV3 HN and F directly impacts infection in the natural host[101,135]. An HPIV3 virus bearing HN molecules that trigger F rapidly or interact with F avidly are effective at fusing in cell culture, but fare poorly in natural tissues or *in vivo*[26,41,61,135]. Enhanced F-triggering by HN, while advantageous in vitro, results in non-infectious viral particles in airway epithelium[137], in which the F protein may have been prematurely activated before contacting target cells[135,136]. Recent structural analysis of the HN molecules with specific biological effects of revealed properties that are critical for infection *in vivo*. The second sialic acid binding site that was identified by functional assays as a key operative site on HPIV3 HN (site II)[61] has a clear structural correlate and modulates viral growth *in vivo*[101]. Specific structural changes at the HN dimer interface, where site II exists, modulate the interaction between HN and F, impact fusion triggering, and directly impact viral infection. For example, the HN from a virus that is well adapted to growth in lung tissue has a wider separation across the dimer interface near the regions of site II than a variant that is restricted in growth in the lung. These conformational changes are propagated to nearby loops at the dimer interface and reduce dimer association in the lung-adapted virus's HN, as measured by buried interface areas, indicating that the dimer interface (and its modulation of HN/F interaction) is critical to infection in the host[101]. The viruses that grow well in lung tissue and in vivo bear a less active HN/F fusion machinery, with lower receptor avidity and less efficient fusion triggering[137]. One may speculate that during natural infection in the host, receptors may be widely available, and an overly fusogenic virus may prematurely trigger its F before reaching the target membrane, a notion consistent with the evolution of laboratory-adapted HPIV3 variants *in vivo* to become less fusogenic than reference strains[137]. In support of this concept, several highly fusogenic glycosylation mutants from NiV have not been identified i*n vivo*[77]. It seems apparent that most paramyxoviruses require a specific balance between the various properties of the fusion machinery for viability *in vivo*.

Conclusions

A variety of models that reflect the diversity of the paramyxoviruses have been proposed for the steps in entry. However, recent data may be taken together to support a common fusion mechanism. Reviewing recent advances in the field of *Paramyxoviridae* entry, we propose a unified model for the fusion process (Fig. 5). Before receptor engagement, the receptor-binding protein interacts with the fusion protein and, if necessary, prevents its untimely activation. After the globular heads of the receptor binding protein engage the target receptor, an activating signal is transmitted via the stalk domain to the fusion protein. This signal appears likely to be in the form of a conformational change in the stalk domain and -- in an "induced-fit" model -- induces a structural change in the fusion protein. The fusion protein is destabilized and inserts its hydrophobic fusion peptide into the target membrane.

The two heptad repeat domains of the fusion protein interact with each other as the molecule progresses to its stable post-fusion state. This process drives the formation of the fusion pore (Fig. 5).

Many open questions remain about the process of fusion activation. While the importance of the HN/H/G stalk domain is well-established[42,95,96] the mechanism by which the signal is transmitted to the homologous F protein is just beginning to be understood. Structural analysis of the complete HN/H/G molecule both engaged to its receptor and free, has been challenging in the face of the important hydrophobic domains[42,48,52,53]. Measuring the precise structural changes that trigger F protein activation will require novel approaches including cryo-electron microscopic analysis of specific stages in the fusion activation process.

Understanding the fusion and entry processs of paramyxoviruses is key for the design of new therapies. The unifying model we suggest for the *Paramyxoviridae* fusion machinery can support the development of new anti-viral strategies that may have broad-spectrum potential for inhibiting entry. Potentially promising therapies that target the entry step include small molecules that prematurely trigger the fusion protein[136], or lipid conjugated HRC-derived peptides[131,132]. Together with other antiviral approaches that target common mechanisms, these strategies hold promise for wide applicability for preventing and treating this important group of pathogens.

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References

1. Chang A, Dutch RE. Paramyxovirus fusion and entry: multiple paths to a common end. Viruses. 2012; 4(4):613–636. [PubMed: 22590688]
2. Enders, G. Paramyxoviruses. In: Baron, S., editor. Medical Microbiology. 4th ed.. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Available at: http://www.ncbi.nlm.nih.gov/books/NBK8461/ [Accessed April 11, 2014]
3. Bossart KN, Fusco DL, Broder CC. Paramyxovirus entry. Adv Exp Med Biol. 2013; 790:95–127. [PubMed: 23884588]
4. Falk K, Batts WN, Kvellestad A, Kurath G, Wiik-Nielsen J, Winton JR. Molecular characterisation of Atlantic salmon paramyxovirus (ASPV): a novel paramyxovirus associated with proliferative gill inflammation. Virus Res. 2008; 133(2):218–227. [PubMed: 18304670]
5. Kurath G, Batts WN, Ahne W, Winton JR. Complete genome sequence of Fer-de-Lance virus reveals a novel gene in reptilian paramyxoviruses. J Virol. 2004; 78(4):2045–2056. [PubMed: 14747569]
6. Marsh GA, de Jong C, Barr JA, et al. Cedar virus: a novel Henipavirus isolated from Australian bats. Plos Pathog. 2012; 8(8):e1002836. [PubMed: 22879820]
7. Magoffin DE, Mackenzie JS, Wang L-F. Genetic analysis of J-virus and Beilong virus using minireplicons. Virology. 2007; 364(1):103–111. [PubMed: 17397895]

8. Kurth A, Kohl C, Brinkmann A, et al. Novel paramyxoviruses in free-ranging European bats. Plos One. 2012; 7(6):e38688. [PubMed: 22737217]
9. Weinberg GA, Hall CB, Iwane MK, et al. Parainfluenza virus infection of young children: estimates of the population-based burden of hospitalization. J Pediatr. 2009; 154(5):694–699. [PubMed: 19159905]
10. Nair H, Nokes DJ, Gessner BD, et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. Lancet. 2010; 375(9725):1545–1555. [PubMed: 20399493]
11. Williams JV, Tollefson SJ, Heymann PW, Carper HT, Patrie J, Crowe JE. Human metapneumovirus infection in children hospitalized for wheezing. J Allergy Clin Immunol. 2005; 115(6):1311–1312. [PubMed: 15940152]
12. Ganar K, Das M, Sinha S, Kumar S. Newcastle disease virus: Current status and our understanding. Virus Res. 2014; 184C:71–81. [PubMed: 24589707]
13. Croser EL, Marsh GA. The changing face of the henipaviruses. Vet Microbiol. 2013
14. Clayton BA, Wang LF, Marsh GA. Henipaviruses: an updated review focusing on the pteropid reservoir and features of transmission. Zoonoses Public Heal. 2013; 60(1):69–83.
15. Hausmann S, Jacques JP, Kolakofsky D. Paramyxovirus RNA editing and the requirement for hexamer genome length. Rna New York N. 1996; 2(10):1033–1045.
16. Halpin K, Bankamp B, Harcourt BH, Bellini WJ, Rota PA. Nipah virus conforms to the rule of six in a minigenome replication assay. J Gen Virol. 2004; 85(Pt 3):701–707. [PubMed: 14993656]
17. Jin H, Zhou H, Cheng X, Tang R, Munoz M, Nguyen N. Recombinant respiratory syncytial viruses with deletions in the NS1, NS2, SH, and M2-2 genes are attenuated in vitro and in vivo. Virology. 2000; 273(1):210–218. [PubMed: 10891423]
18. Takeuchi K, Tanabayashi K, Hishiyama M, Yamada A. The mumps virus SH protein is a membrane protein and not essential for virus growth. Virology. 1996; 225(1):156–162. [PubMed: 8918542]
19. He B, Leser GP, Paterson RG, Lamb RA. The paramyxovirus SV5 small hydrophobic (SH) protein is not essential for virus growth in tissue culture cells. Virology. 1998; 250(1):30–40. [PubMed: 9770417]
20. Biacchesi S, Pham QN, Skiadopoulos MH, Murphy BR, Collins PL, Buchholz UJ. Infection of nonhuman primates with recombinant human metapneumovirus lacking the SH, G, or M2-2 protein categorizes each as a nonessential accessory protein and identifies vaccine candidates. J Virol. 2005; 79(19):12608–12613. [PubMed: 16160190]
21. Atreya PL, Peeples ME, Collins PL. The NS1 protein of human respiratory syncytial virus is a potent inhibitor of minigenome transcription and RNA replication. J Virol. 1998; 72(2):1452–1461. [PubMed: 9445048]
22. Audsley MD, Moseley GW. Paramyxovirus evasion of innate immunity: Diverse strategies for common targets. World J Virol. 2013; 2(2):57–70. [PubMed: 24175230]
23. Huberman K, Peluso RW, Moscona A. Hemagglutinin-neuraminidase of human parainfluenza 3: role of the neuraminidase in the viral life cycle. Virology. 1995; 214(1):294–300. [PubMed: 8525632]
24. Moscona A, Peluso RW. Fusion properties of cells persistently infected with human parainfluenza virus type 3: participation of hemagglutinin-neuraminidase in membrane fusion. J Virol. 1991; 65(6):2773–2777. [PubMed: 1851852]
25. Morrison T, McQuain C, McGinnes L. Complementation between avirulent Newcastle disease virus and a fusion protein gene expressed from a retrovirus vector: requirements for membrane fusion. J Virol. 1991; 65(2):813–822. [PubMed: 1987376]
26. Moscona A, Peluso RW. Relative affinity of the human parainfluenza virus type 3 hemagglutinin-neuraminidase for sialic acid correlates with virus-induced fusion activity. J Virol. 1993; 67(11): 6463–6468. [PubMed: 8411349]
27. Navaratnarajah CK, Oezguen N, Rupp L, et al. The heads of the measles virus attachment protein move to transmit the fusion-triggering signal. Nat Struct Mol Biol. 2011; 18(2):128–134. [PubMed: 21217701]

28. Mirza AM, Aguilar HC, Zhu Q, et al. Triggering of the newcastle disease virus fusion protein by a chimeric attachment protein that binds to Nipah virus receptors. J Biol Chem. 2011; 286(20): 17851–17860. [PubMed: 21460213]
29. Corey EA, Iorio RM. Mutations in the stalk of the measles virus hemagglutinin protein decrease fusion but do not interfere with virus-specific interaction with the homologous fusion protein. J Virol. 2007; 81(18):9900–9910. [PubMed: 17626104]
30. Aguilar HC, Matreyek KA, Filone CM, et al. N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. J Virol. 2006; 80(10):4878–4889. [PubMed: 16641279]
31. Bishop KA, Hickey AC, Khetawat D, et al. Residues in the stalk domain of the hendra virus g glycoprotein modulate conformational changes associated with receptor binding. J Virol. 2008; 82(22):11398–11409. [PubMed: 18799571]
32. Plemper RK, Hammond AL, Gerlier D, Fielding AK, Cattaneo R. Strength of envelope protein interaction modulates cytopathicity of measles virus. J Virol. 2002; 76(10):5051–5061. [PubMed: 11967321]
33. Iorio RM, Melanson VR, Mahon PJ. Glycoprotein interactions in paramyxovirus fusion. Future Virol. 2009; 4(4):335–351. [PubMed: 20161127]
34. Lee B, Ataman ZA. Modes of paramyxovirus fusion: a Henipavirus perspective. Trends Microbiol. 2011; 19(8):389–399. [PubMed: 21511478]
35. Iorio RM, Mahon PJ. Paramyxoviruses: different receptors - different mechanisms of fusion. Trends Microbiol. 2008; 16(4):135–137. [PubMed: 18346895]
36. Porotto M, Murrell M, Greengard O, Doctor L, Moscona A. Influence of the human parainfluenza virus 3 attachment protein's neuraminidase activity on its capacity to activate the fusion protein. J Virol. 2005; 79(4):2383–2392. [PubMed: 15681439]
37. Porotto M, Murrell M, Greengard O, Moscona A. Triggering of human parainfluenza virus 3 fusion protein (F) by the hemagglutinin-neuraminidase (HN) protein: an HN mutation diminishes the rate of F activation and fusion. J Virol. 2003; 77(6):3647–3654. [PubMed: 12610140]
38. Dutch RE. Entry and fusion of emerging paramyxoviruses. Plos Pathog. 2010; 6(6):e1000881. [PubMed: 20585631]
39. Plattet P, Plemper RK. Envelope protein dynamics in paramyxovirus entry. MBio. 2013; 4(4)
40. Porotto M, Devito I, Palmer SG, et al. Spring-loaded model revisited: paramyxovirus fusion requires engagement of a receptor binding protein beyond initial triggering of the fusion protein. J Virol. 2011; 85(24):12867–12880. [PubMed: 21976650]
41. Porotto M, Palmer SG, Palermo LM, Moscona A. Mechanism of fusion triggering by human parainfluenza virus type III: communication between viral glycoproteins during entry. J Biol Chem. 2012; 287(1):778–793. [PubMed: 22110138]
42. Bose S, Welch BD, Kors CA, Yuan P, Jardetzky TS, Lamb RA. Structure and mutagenesis of the parainfluenza virus 5 hemagglutinin-neuraminidase stalk domain reveals a four-helix bundle and the role of the stalk in fusion promotion. J Virol. 2011; 85(24):12855–12866. [PubMed: 21994464]
43. Colf LA, Juo ZS, Garcia KC. Structure of the measles virus hemagglutinin. Nat Struct Mol Biol. 2007; 14(12):1227–1228. [PubMed: 18026116]
44. Hashiguchi T, Kajikawa M, Maita N, et al. Crystal structure of measles virus hemagglutinin provides insight into effective vaccines. Proc Natl Acad Sci U S A. 2007; 104(49):19535–19540. [PubMed: 18003910]
45. Hashiguchi T, Ose T, Kubota M, et al. Structure of the measles virus hemagglutinin bound to its cellular receptor SLAM. Nat Struct Mol Biol. 2011; 18(2):135–141. [PubMed: 21217702]
46. Bowden TA, Aricescu AR, Gilbert RJC, Grimes JM, Jones EY, Stuart DI. Structural basis of Nipah and Hendra virus attachment to their cell-surface receptor ephrin-B2. Nat Struct Mol Biol. 2008; 15(6):567–572. [PubMed: 18488039]
47. Xu K, Rajashankar KR, Chan Y-P, Himanen JP, Broder CC, Nikolov DB. Host cell recognition by the henipaviruses: crystal structures of the Nipah G attachment glycoprotein and its complex with ephrin-B3. Proc Natl Acad Sci U S A. 2008; 105(29):9953–9958. [PubMed: 18632560]

48. Welch BD, Yuan P, Bose S, Kors CA, Lamb RA, Jardetzky TS. Structure of the parainfluenza virus 5 (PIV5) hemagglutinin-neuraminidase (HN) ectodomain. Plos Pathog. 2013; 9(8):e1003534. [PubMed: 23950713]

49. Lawrence MC, Borg NA, Streltsov VA, et al. Structure of the haemagglutinin-neuraminidase from human parainfluenza virus type III. J Mol Biol. 2004; 335(5):1343–1357. [PubMed: 14729348]

50. Navaratnarajah CK, Negi S, Braun W, Cattaneo R. Membrane fusion triggering: three modules with different structure and function in the upper half of the measles virus attachment protein stalk. J Biol Chem. 2012; 287(46):38543–38551. [PubMed: 23007387]

51. Navaratnarajah CK, Kumar S, Generous A, Apte-Sengupta S, Mateo M, Cattaneo R. The measles virus hemagglutinin stalk: structure and function of the central fusion-activation and membrane-proximal segments. J Virol. 2014

52. Bowden TA, Crispin M, Harvey DJ, Jones EY, Stuart DI. Dimeric architecture of the Hendra virus attachment glycoprotein: evidence for a conserved mode of assembly. J Virol. 2010; 84(12):6208–6217. [PubMed: 20375167]

53. Yuan P, Swanson KA, Leser GP, Paterson RG, Lamb RA, Jardetzky TS. Structure of the Newcastle disease virus hemagglutinin-neuraminidase (HN) ectodomain reveals a four-helix bundle stalk. Proc Natl Acad Sci U S A. 2011; 108(36):14920–14925. [PubMed: 21873198]

54. Crennell S, Takimoto T, Portner A, Taylor G. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. Nat Struct Biol. 2000; 7(11):1068–1074. [PubMed: 11062565]

55. Santiago C, Celma ML, Stehle T, Casasnovas JM. Structure of the measles virus hemagglutinin bound to the CD46 receptor. Nat Struct Mol Biol. 2010; 17(1):124–129. [PubMed: 20010840]

56. Zaitsev V, von Itzstein M, Groves D, et al. Second sialic acid binding site in Newcastle disease virus hemagglutinin-neuraminidase: implications for fusion. J Virol. 2004; 78(7):3733–3741. [PubMed: 15016893]

57. Bousse TL, Taylor G, Krishnamurthy S, Portner A, Samal SK, Takimoto T. Biological significance of the second receptor binding site of Newcastle disease virus hemagglutinin-neuraminidase protein. J Virol. 2004; 78(23):13351–13355. [PubMed: 15542686]

58. Porotto M, Salah Z, DeVito I, et al. The second receptor binding site of the globular head of the Newcastle disease virus hemagglutinin-neuraminidase activates the stalk of multiple paramyxovirus receptor binding proteins to trigger fusion. J Virol. 2012; 86(10):5730–5741. [PubMed: 22438532]

59. Bousse T, Takimoto T. Mutation at residue 523 creates a second receptor binding site on human parainfluenza virus type 1 hemagglutinin-neuraminidase protein. J Virol. 2006; 80(18):9009–9016. [PubMed: 16940513]

60. Alymova IV, Portner A, Mishin VP, McCullers JA, Freiden P, Taylor GL. Receptor-binding specificity of the human parainfluenza virus type 1 hemagglutinin-neuraminidase glycoprotein. Glycobiology. 2012; 22(2):174–180. [PubMed: 21846691]

61. Porotto M, Fornabaio M, Kellogg GE, Moscona A. A second receptor binding site on human parainfluenza virus type 3 hemagglutinin-neuraminidase contributes to activation of the fusion mechanism. J Virol. 2007; 81(7):3216–3228. [PubMed: 17229690]

62. Smith EC, Smith SE, Carter JR, et al. Trimeric transmembrane domain interactions in paramyxovirus fusion proteins: roles in protein folding, stability, and function. J Biol Chem. 2013; 288(50):35726–35735. [PubMed: 24178297]

63. Smith EC, Culler MR, Hellman LM, Fried MG, Creamer TP, Dutch RE. Beyond anchoring: the expanding role of the hendra virus fusion protein transmembrane domain in protein folding, stability, and function. J Virol. 2012; 86(6):3003–3013. [PubMed: 22238302]

64. González-Reyes L, Ruiz-Argüello MB, García-Barreno B, et al. Cleavage of the human respiratory syncytial virus fusion protein at two distinct sites is required for activation of membrane fusion. Proc Natl Acad Sci U S A. 2001; 98(17):9859–9864. [PubMed: 11493675]

65. Diederich S, Moll M, Klenk H-D, Maisner A. The nipah virus fusion protein is cleaved within the endosomal compartment. J Biol Chem. 2005; 280(33):29899–29903. [PubMed: 15961384]

66. Pager CT, Dutch RE. Cathepsin L is involved in proteolytic processing of the Hendra virus fusion protein. J Virol. 2005; 79(20):12714–12720. [PubMed: 16188974]

67. Pager CT, Craft WW Jr, Patch J, Dutch RE. A mature and fusogenic form of the Nipah virus fusion protein requires proteolytic processing by cathepsin L. Virology. 2006; 346(2):251–257. [PubMed: 16460775]

68. Diederich S, Sauerhering L, Weis M, et al. Activation of the Nipah virus fusion protein in MDCK cells is mediated by cathepsin B within the endosome-recycling compartment. J Virol. 2012; 86(7):3736–3745. [PubMed: 22278224]

69. Smith EC, Gregory SM, Tamm LK, Creamer TP, Dutch RE. Role of sequence and structure of the Hendra fusion protein fusion peptide in membrane fusion. J Biol Chem. 2012; 287(35):30035–30048. [PubMed: 22761418]

70. Welch BD, Liu Y, Kors CA, Leser GP, Jardetzky TS, Lamb RA. Structure of the cleavage-activated prefusion form of the parainfluenza virus 5 fusion protein. Proc Natl Acad Sci U S A. 2012; 109(41):16672–16677. [PubMed: 23012473]

71. Kim YH, Donald JE, Grigoryan G, et al. Capture and imaging of a prehairpin fusion intermediate of the paramyxovirus PIV5. Proc Natl Acad Sci U S A. 2011; 108(52):20992–20997. [PubMed: 22178759]

72. Yin H-S, Wen X, Paterson RG, Lamb RA, Jardetzky TS. Structure of the parainfluenza virus 5 F protein in its metastable, prefusion conformation. Nature. 2006; 439(7072):38–44. [PubMed: 16397490]

73. Yin H-S, Paterson RG, Wen X, Lamb RA, Jardetzky TS. Structure of the uncleaved ectodomain of the paramyxovirus (hPIV3) fusion protein. Proc Natl Acad Sci U S A. 2005; 102(26):9288–9293. [PubMed: 15964978]

74. McLellan JS, Yang Y, Graham BS, Kwong PD. Structure of respiratory syncytial virus fusion glycoprotein in the postfusion conformation reveals preservation of neutralizing epitopes. J Virol. 2011; 85(15):7788–7796. [PubMed: 21613394]

75. Swanson K, Wen X, Leser GP, Paterson RG, Lamb RA, Jardetzky TS. Structure of the Newcastle disease virus F protein in the post-fusion conformation. Virology. 2010; 402(2):372–379. [PubMed: 20439109]

76. Harrison SC. Viral membrane fusion. Nat Struct Mol Biol. 2008; 15(7):690–698. [PubMed: 18596815]

77. Biering SB, Huang A, Vu AT, et al. N-Glycans on the Nipah virus attachment glycoprotein modulate fusion and viral entry as they protect against antibody neutralization. J Virol. 2012; 86(22):11991–12002. [PubMed: 22915812]

78. Dörig RE, Marcil A, Chopra A, Richardson CD. The human CD46 molecule is a receptor for measles virus (Edmonston strain). Cell. 1993; 75(2):295–305. [PubMed: 8402913]

79. Tatsuo H, Ono N, Tanaka K, Yanagi Y. SLAM (CDw150) is a cellular receptor for measles virus. Nature. 2000; 406(6798):893–897. [PubMed: 10972291]

80. Erlenhoefer C, Wurzer WJ, Löffler S, Schneider-Schaulies S, ter Meulen V, Schneider-Schaulies J. CD150 (SLAM) is a receptor for measles virus but is not involved in viral contact-mediated proliferation inhibition. J Virol. 2001; 75(10):4499–4505. [PubMed: 11312320]

81. Noyce RS, Bondre DG, Ha MN, et al. Tumor cell marker PVRL4 (nectin 4) is an epithelial cell receptor for measles virus. Plos Pathog. 2011; 7(8):e1002240. [PubMed: 21901103]

82. Mühlebach MD, Mateo M, Sinn PL, et al. Adherens junction protein nectin-4 is the epithelial receptor for measles virus. Nature. 2011; 480(7378):530–533. [PubMed: 22048310]

83. Kato S-I, Nagata K, Takeuchi K. Cell tropism and pathogenesis of measles virus in monkeys. Front Microbiol. 2012; 3:14. [PubMed: 22363320]

84. Bonaparte MI, Dimitrov AS, Bossart KN, et al. Ephrin-B2 ligand is a functional receptor for Hendra virus and Nipah virus. Proc Natl Acad Sci U S A. 2005; 102(30):10652–10657. [PubMed: 15998730]

85. Mathieu C, Pohl C, Szecsi J, et al. Nipah virus uses leukocytes for efficient dissemination within a host. J Virol. 2011; 85(15):7863–7871. [PubMed: 21593145]

86. Krusat T, Streckert HJ. Heparin-dependent attachment of respiratory syncytial virus (RSV) to host cells. Arch Virol. 1997; 142(6):1247–1254. [PubMed: 9229012]

87. Bourgeois C, Bour JB, Lidholt K, Gauthray C, Pothier P. Heparin-like structures on respiratory syncytial virus are involved in its infectivity in vitro. J Virol. 1998; 72(9):7221–7227. [PubMed: 9696816]

88. Feldman SA, Hendry RM, Beeler JA. Identification of a linear heparin binding domain for human respiratory syncytial virus attachment glycoprotein G. J Virol. 1999; 73(8):6610–6617. [PubMed: 10400758]

89. Thammawat S, Sadlon TA, Hallsworth PG, Gordon DL. Role of cellular glycosaminoglycans and charged regions of viral G protein in human metapneumovirus infection. J Virol. 2008; 82(23): 11767–11774. [PubMed: 18786997]

90. Harcourt JL, Karron RA, Tripp RA. Anti-G protein antibody responses to respiratory syncytial virus infection or vaccination are associated with inhibition of G protein CX3C-CX3CR1 binding and leukocyte chemotaxis. J Infect Dis. 2004; 190(11):1936–1940. [PubMed: 15529257]

91. Chirkova T, Boyoglu-Barnum S, Gaston KA, et al. Respiratory syncytial virus G protein CX3C motif impairs human airway epithelial and immune cell responses. J Virol. 2013; 87(24):13466–13479. [PubMed: 24089561]

92. Steffen DL, Xu K, Nikolov DB, Broder CC. Henipavirus mediated membrane fusion, virus entry and targeted therapeutics. Viruses. 2012; 4(2):280–308. [PubMed: 22470837]

93. Russell CJ, Jardetzky TS, Lamb RA. Membrane fusion machines of paramyxoviruses: capture of intermediates of fusion. Embo J. 2001; 20(15):4024–4034. [PubMed: 11483506]

94. Bose S, Zokarkar A, Welch BD, Leser GP, Jardetzky TS, Lamb RA. Fusion activation by a headless parainfluenza virus 5 hemagglutinin-neuraminidase stalk suggests a modular mechanism for triggering. Proc Natl Acad Sci U S A. 2012; 109(39):E2625–E2634. [PubMed: 22949640]

95. Ader N, Brindley MA, Avila M, et al. Structural rearrangements of the central region of the morbillivirus attachment protein stalk domain trigger F protein refolding for membrane fusion. J Biol Chem. 2012; 287(20):16324–16334. [PubMed: 22431728]

96. Brindley MA, Suter R, Schestak I, Kiss G, Wright ER, Plemper RK. A Stabilized Headless Measles Virus Attachment Protein Stalk Efficiently Triggers Membrane Fusion. J Virol. 2013

97. Talekar A, Moscona A, Porotto M. Measles fusion machinery activated by sialic acid binding globular domain. J Virol. 2013

98. Melanson VR, Iorio RM. Amino acid substitutions in the F-specific domain in the stalk of the newcastle disease virus HN protein modulate fusion and interfere with its interaction with the F protein. J Virol. 2004; 78(23):13053–13061. [PubMed: 15542657]

99. Liu Q, Stone JA, Bradel-Tretheway B, et al. Unraveling a three-step spatiotemporal mechanism of triggering of receptor-induced Nipah virus fusion and cell entry. Plos Pathog. 2013; 9(11):e1003770. [PubMed: 24278018]

100. Bose S, Song AS, Jardetzky TS, Lamb RA. Fusion activation through attachment protein stalk domains indicates a conserved core mechanism of paramyxovirus entry into cells. J Virol. 2014

101. Xu R, Palmer SG, Porotto M, et al. Interaction between the Hemagglutinin-Neuraminidase and Fusion Glycoproteins of Human Parainfluenza Virus Type III Regulates Viral Growth In Vivo. MBio. 2013; 4(5)

102. Bossart KN, Wang L-F, Flora MN, et al. Membrane fusion tropism and heterotypic functional activities of the Nipah virus and Hendra virus envelope glycoproteins. J Virol. 2002; 76(22): 11186–11198. [PubMed: 12388678]

103. Ader N, Brindley M, Avila M, et al. Mechanism for active membrane fusion triggering by morbillivirus attachment protein. J Virol. 2013; 87(1):314–326. [PubMed: 23077316]

104. Porotto M, Salah ZW, Gui L, et al. Regulation of paramyxovirus fusion activation: the hemagglutinin-neuraminidase protein stabilizes the fusion protein in a pretriggered state. J Virol. 2012; 86(23):12838–12848. [PubMed: 22993149]

105. Cantín C, Holguera J, Ferreira L, Villar E, Muñoz-Barroso I. Newcastle disease virus may enter cells by caveolae-mediated endocytosis. J Gen Virol. 2007; 88(Pt 2):559–569. [PubMed: 17251575]

106. Schowalter RM, Chang A, Robach JG, Buchholz UJ, Dutch RE. Low-pH triggering of human metapneumovirus fusion: essential residues and importance in entry. J Virol. 2009; 83(3):1511–1522. [PubMed: 19036821]

107. Kolokoltsov AA, Deniger D, Fleming EH, Roberts NJ Jr, Karpilow JM, Davey RA. Small interfering RNA profiling reveals key role of clathrin-mediated endocytosis and early endosome formation for infection by respiratory syncytial virus. J Virol. 2007; 81(14):7786–7800. [PubMed: 17494077]
108. Diederich S, Thiel L, Maisner A. Role of endocytosis and cathepsin-mediated activation in Nipah virus entry. Virology. 2008; 375(2):391–400. [PubMed: 18342904]
109. Sánchez-Felipe L, Villar E, Muñoz-Barroso I. Entry of Newcastle Disease Virus into the host cell: Role of acidic pH and endocytosis. Biochim Biophys Acta. 2013
110. Kahn JS, Schnell MJ, Buonocore L, Rose JK. Recombinant vesicular stomatitis virus expressing respiratory syncytial virus (RSV) glycoproteins: RSV fusion protein can mediate infection and cell fusion. Virology. 1999; 254(1):81–91. [PubMed: 9927576]
111. Techaarpornkul S, Barretto N, Peeples ME. Functional analysis of recombinant respiratory syncytial virus deletion mutants lacking the small hydrophobic and/or attachment glycoprotein gene. J Virol. 2001; 75(15):6825–6834. [PubMed: 11435561]
112. Schowalter RM, Smith SE, Dutch RE. Characterization of Human Metapneumovirus F Protein-Promoted Membrane Fusion: Critical Roles for Proteolytic Processing and Low pH. J Virol. 2006; 80(22):10931–10941. [PubMed: 16971452]
113. Sergel TA, McGinnes LW, Morrison TG. A single amino acid change in the Newcastle disease virus fusion protein alters the requirement for HN protein in fusion. J Virol. 2000; 74(11):5101–5107. [PubMed: 10799584]
114. Seth S, Vincent A, Compans RW. Mutations in the cytoplasmic domain of a paramyxovirus fusion glycoprotein rescue syncytium formation and eliminate the hemagglutinin-neuraminidase protein requirement for membrane fusion. J Virol. 2003; 77(1):167–178. [PubMed: 12477822]
115. Ito M, Nishio M, Kawano M, Komada H, Ito Y, Tsurudome M. Effects of multiple amino acids of the parainfluenza virus 5 fusion protein on its haemagglutinin-neuraminidase-independent fusion activity. J Gen Virol. 2009; 90(Pt 2):405–413. [PubMed: 19141450]
116. Karron RA, Buonagurio DA, Georgiu AF, et al. Respiratory syncytial virus (RSV) SH and G proteins are not essential for viral replication in vitro: clinical evaluation and molecular characterization of a cold-passaged, attenuated RSV subgroup B mutant. Proc Natl Acad Sci U S A. 1997; 94(25):13961–13966. [PubMed: 9391135]
117. Dutch RE, Joshi SB, Lamb RA. Membrane fusion promoted by increasing surface densities of the paramyxovirus F and HN proteins: comparison of fusion reactions mediated by simian virus 5 F, human parainfluenza virus type 3 F, influenza virus HA. J Virol. 1998; 72(10):7745–7753. [PubMed: 9733810]
118. Herfst S, Mas V, Ver LS, et al. Low-pH-induced membrane fusion mediated by human metapneumovirus F protein is a rare, strain-dependent phenomenon. J Virol. 2008; 82(17):8891–8895. [PubMed: 18596097]
119. Tayyari F, Marchant D, Moraes TJ, Duan W, Mastrangelo P, Hegele RG. Identification of nucleolin as a cellular receptor for human respiratory syncytial virus. Nat Med. 2011; 17(9): 1132–1135. [PubMed: 21841784]
120. Wei Y, Zhang Y, Cai H, et al. Roles of the putative integrin-binding motif of the human metapneumovirus fusion (f) protein in cell-cell fusion, viral infectivity, and pathogenesis. J Virol. 2014; 88(8):4338–4352. [PubMed: 24478423]
121. Feldman SA, Audet S, Beeler JA. The fusion glycoprotein of human respiratory syncytial virus facilitates virus attachment and infectivity via an interaction with cellular heparan sulfate. J Virol. 2000; 74(14):6442–6447. [PubMed: 10864656]
122. Barretto N, Hallak LK, Peeples ME. Neuraminidase treatment of respiratory syncytial virus-infected cells or virions, but not target cells, enhances cell-cell fusion and infection. Virology. 2003; 313(1):33–43. [PubMed: 12951019]
123. Chang A, Masante C, Buchholz UJ, Dutch RE. Human metapneumovirus (HMPV) binding and infection are mediated by interactions between the HMPV fusion protein and heparan sulfate. J Virol. 2012; 86(6):3230–3243. [PubMed: 22238303]

124. Talekar A, DeVito I, Salah Z, et al. Identification of a region in the stalk domain of the nipah virus receptor binding protein that is critical for fusion activation. J Virol. 2013; 87(20):10980–10996. [PubMed: 23903846]

125. Bose S, Heath CM, Shah PA, Alayyoubi M, Jardetzky TS, Lamb RA. Mutations in the parainfluenza virus 5 fusion (F) protein reveal domains important for fusion triggering and metastability. J Virol. 2013

126. Brindley MA, Takeda M, Plattet P, Plemper RK. Triggering the measles virus membrane fusion machinery. Proc Natl Acad Sci U S A. 2012; 109(44):E3018–E3027. [PubMed: 23027974]

127. Mahon PJ, Mirza AM, Musich TA, Iorio RM. Engineered intermonomeric disulfide bonds in the globular domain of Newcastle disease virus hemagglutinin-neuraminidase protein: implications for the mechanism of fusion promotion. J Virol. 2008; 82(21):10386–10396. [PubMed: 18753211]

128. Zokarkar A, Connolly SA, Jardetzky TS, Lamb RA. Reversible inhibition of fusion activity of a paramyxovirus fusion protein by an engineered disulfide bond in the membrane-proximal external region. J Virol. 2012; 86(22):12397–12401. [PubMed: 22951841]

129. Zokarkar A, Lamb RA. The paramyxovirus fusion protein C-terminal region: mutagenesis indicates an indivisible protein unit. J Virol. 2012; 86(5):2600–2609. [PubMed: 22171273]

130. Donald JE, Zhang Y, Fiorin G, et al. Transmembrane orientation and possible role of the fusogenic peptide from parainfluenza virus 5 (PIV5) in promoting fusion. Proc Natl Acad Sci U S A. 2011; 108(10):3958–3963. [PubMed: 21321234]

131. Porotto M, Rockx B, Yokoyama CC, et al. Inhibition of Nipah virus infection in vivo: targeting an early stage of paramyxovirus fusion activation during viral entry. Plos Pathog. 2010; 6(10):e1001168. [PubMed: 21060819]

132. Welsch JC, Talekar A, Mathieu C, et al. Fatal Measles Infection Prevented by Brain-Penetrant Fusion Inhibitors. J Virol. 2013

133. Pessi A, Langella A, Capitò E, et al. A general strategy to endow natural fusion-protein-derived peptides with potent antiviral activity. Plos One. 2012; 7(5):e36833. [PubMed: 22666328]

134. Chen L, Gorman JJ, McKimm-Breschkin J, et al. The structure of the fusion glycoprotein of Newcastle disease virus suggests a novel paradigm for the molecular mechanism of membrane fusion. Struct Lond Engl 1993. 2001; 9(3):255–266.

135. Palermo LM, Porotto M, Yokoyama CC, et al. Human parainfluenza virus infection of the airway epithelium: viral hemagglutinin-neuraminidase regulates fusion protein activation and modulates infectivity. J Virol. 2009; 83(13):6900–6908. [PubMed: 19386708]

136. Farzan SF, Palermo LM, Yokoyama CC, et al. Premature activation of the paramyxovirus fusion protein before target cell attachment with corruption of the viral fusion machinery. J Biol Chem. 2011; 286(44):37945–37954. [PubMed: 21799008]

137. Palmer SG, Porotto M, Palermo LM, Cunha LF, Greengard O, Moscona A. Adaptation of human parainfluenza virus to airway epithelium reveals fusion properties required for growth in host tissue. MBio. 2012; 3(3)

138. Leonard VH, et al. Measles virus blind to its epithelial cell receptor remains virulent in rhesus monkeys but cannot cross the airway epithelium and is not shed. J. Clin. Invest. 2008; 118:2448–2458. [PubMed: 18568079]

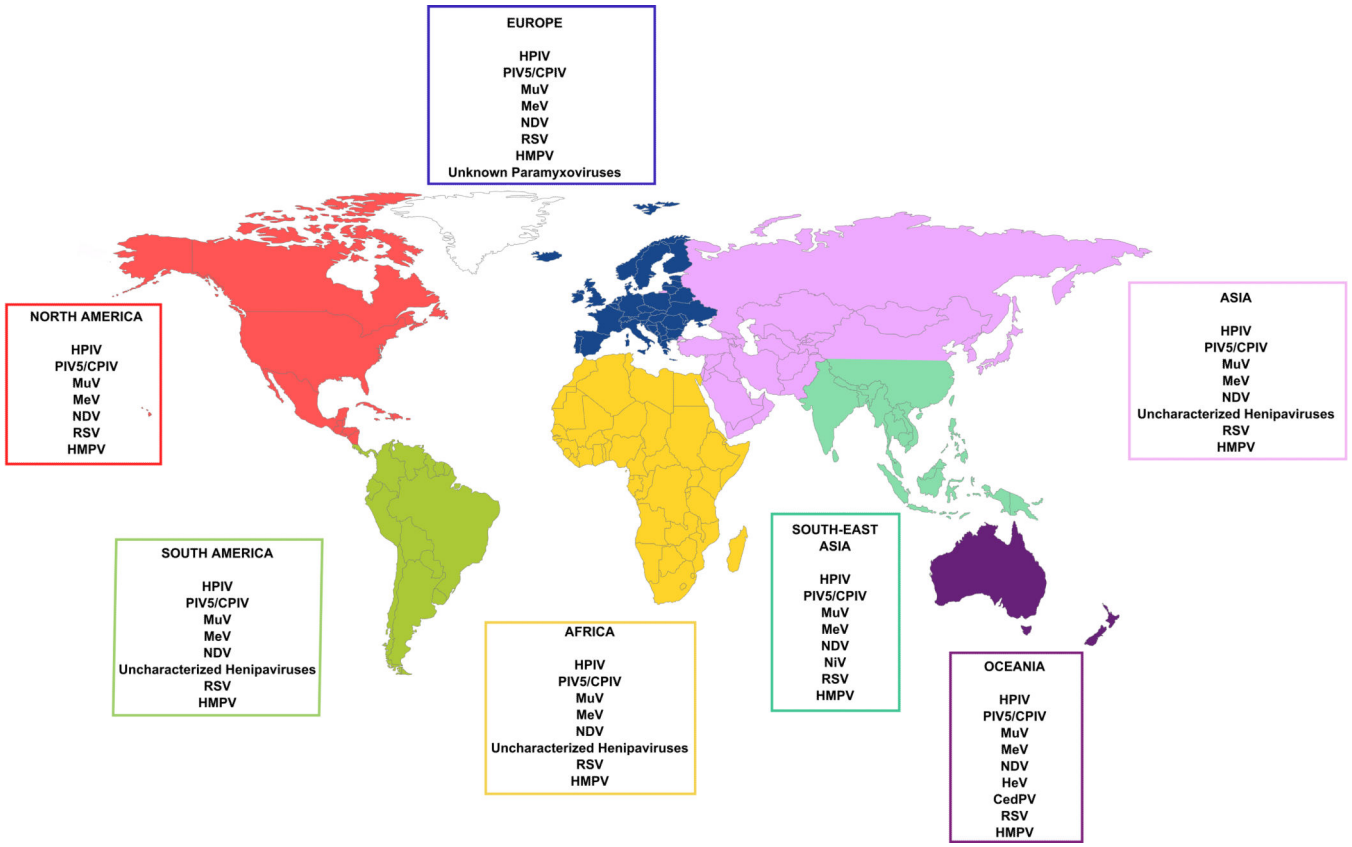


Figure 1. World distribution of major paramyxoviruses

Paramyxoviruses are found on every continent. Henipa- and Henipa-like viruses have been found in Oceania, Asia, Africa and South America, but human infections have only been reported in Oceania and South-East Asia. Data gathered from Enders *et al.*[2], Ganar *et al.*[12], Croser *et al.*[13] the World Health Organization, the World Organization for Animal Health, and recent studies[8]. Abbreviations are as in Table 1.

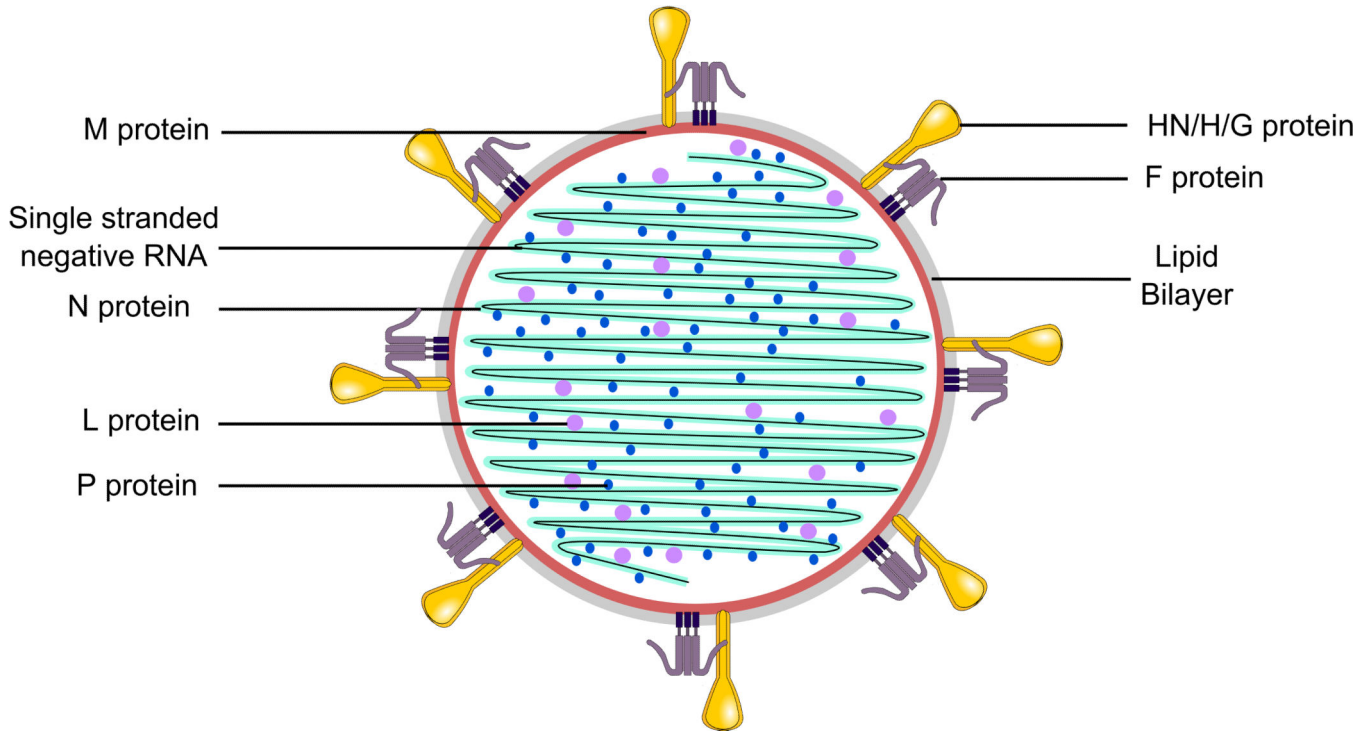


Figure 2. Schematic representation of the common structure of Paramyxoviruses

Paramyxoviridae are enveloped viruses. They contain single-stranded negative RNA coated with nucleocapsid (N) protein as well as a large (L) protein and a phosphoprotein (P) that carries out polymerase activity. The matrix (M) protein lines the viral lipid bilayer. The two viral glycoproteins -- hemagglutinin-neuraminidase (HN)/ hemagglutinin (H)/ glycoprotein (G) and fusion (F) -- protude from the viral membrane.

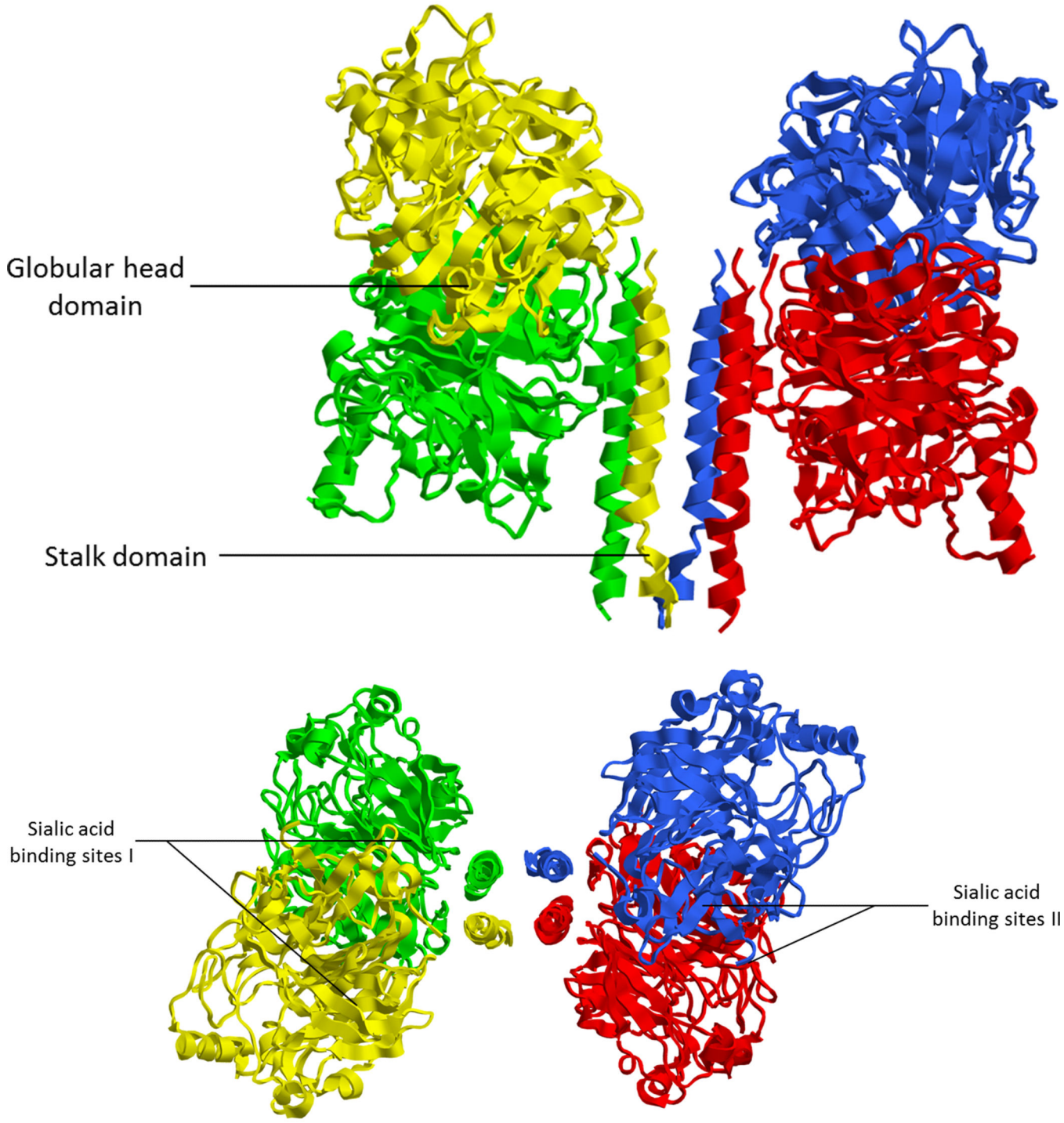
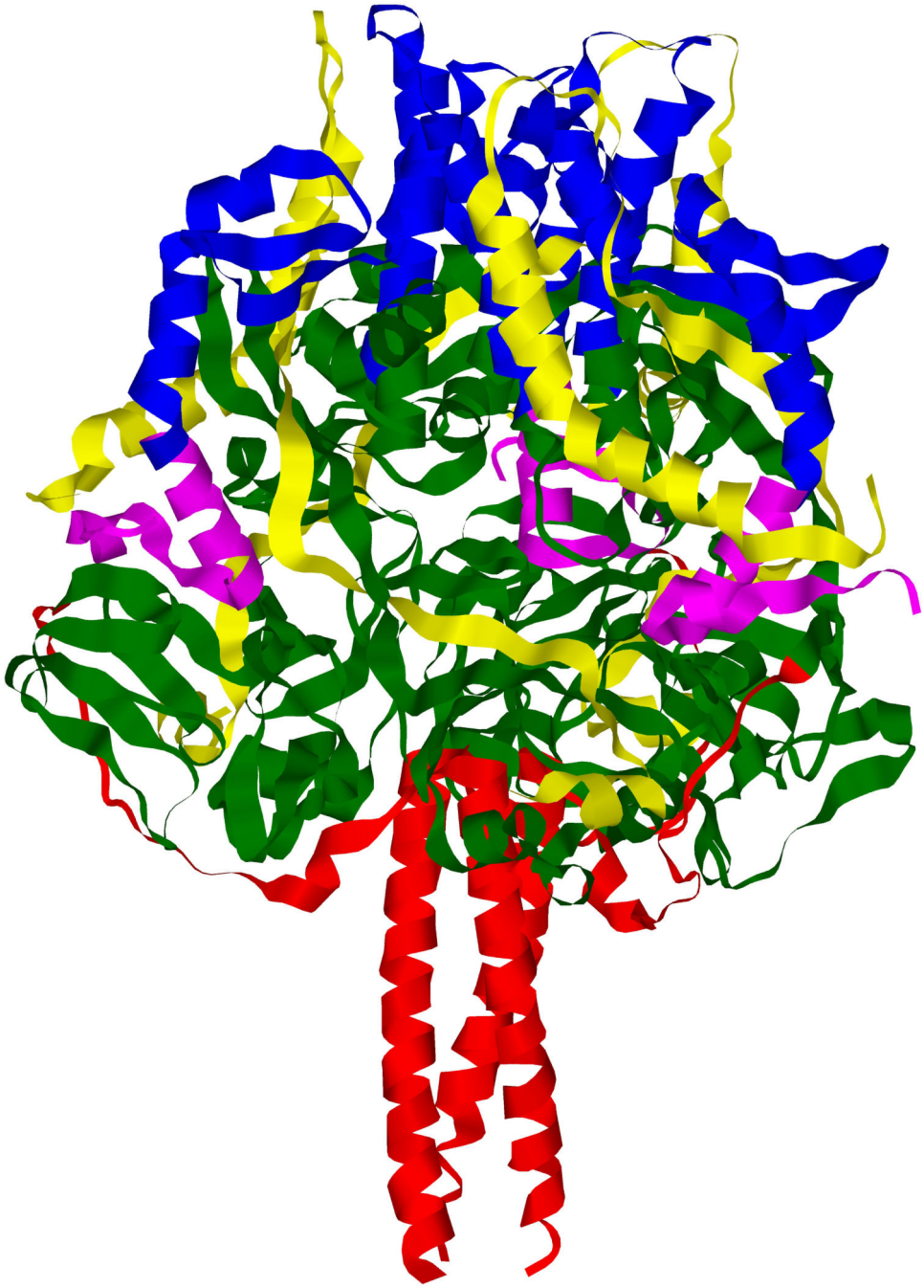
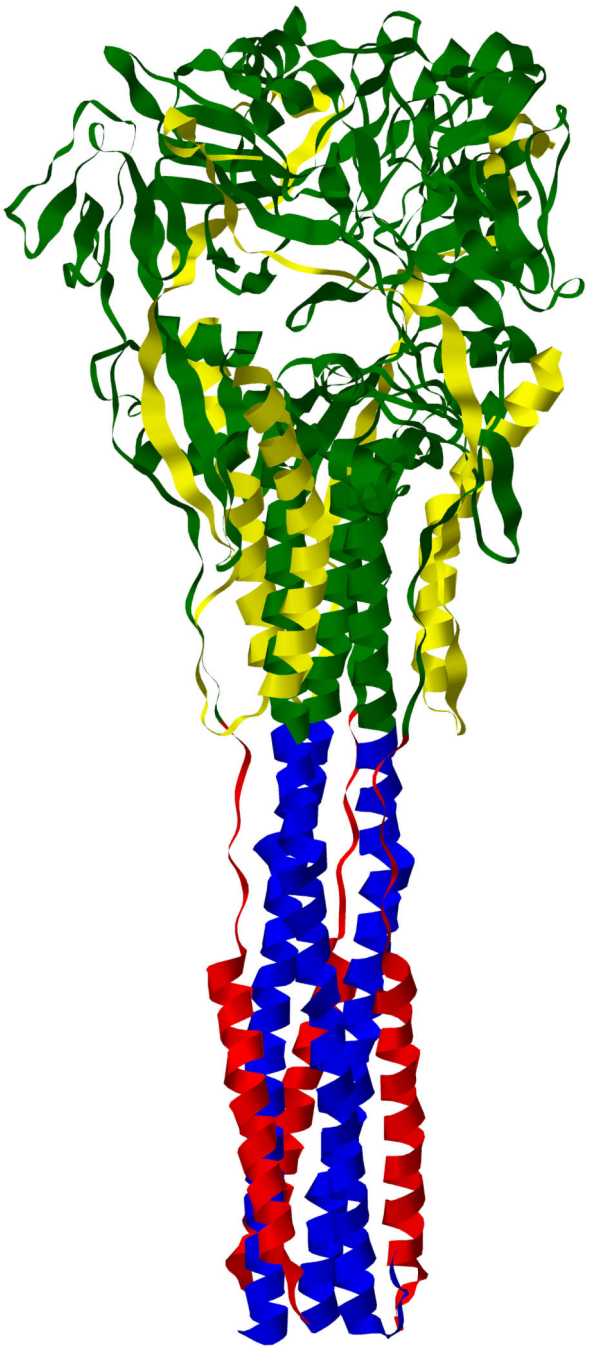


Figure 3. Structure of the Newcastle disease virus hemagglutinin-neuraminidase protein

3.1 Side view of the crystal structure of the tetramerized NDV-HN ectodomain showing the stalk and the globular domains of each monomer. Each color represents one monomer of the receptor-binding protein. One dimer is composed of green and yellow monomers, the other of red and blue monomers. (PDB ID: 3T1E; Yuan *et al.*[53]). 3.2 Top view of the crystal structure of the tetramerized NDV-HN ectodomain, showing sialic acid binding sites I and

II. Each monomer bears a site I and a site II. (PDB ID: 3T1E; Yuan *et al*.[53]) 3.3 Schematic representation of the domains of NDV HN.





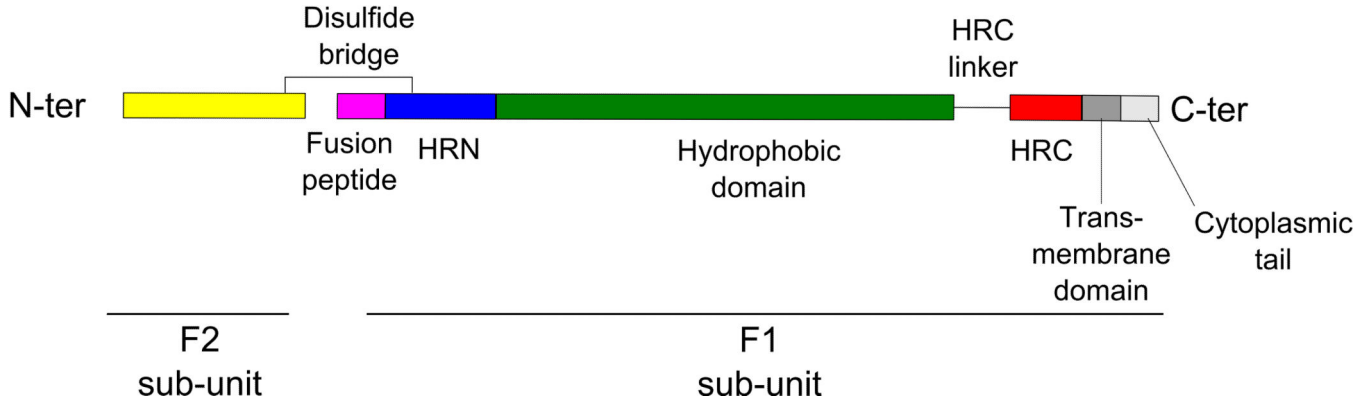


Figure 4. Structure of the paramyxovirus fusion protein

4.1 Crystal structure of the pre-fusion state of the trimeric fusion protein of PIV5 showing the fusion peptide (purple) in the hydrophobic pocket formed by ae hydrophobic domain (deep green), the HRN domain (deep blue) and the F_2 sub-unit (yellow). (PDB ID: 4GIP; Welch et *al.*[70]). 4.2 Crystal structure of the post-fusion state of the fusion protein of HPIV3 showing the 6-helix bundle structure formed by the HRN (deep blue) and HRC (red) domains interacting together. (PBDB ID: 1ZTM; Yin et *al*[73]). 4.3 Schematic representation of the main domains of a monomer of the cleaved paramyxovirus fusion protein. HRC/HRN: Heptad Repeat C-/N-terminal domain.

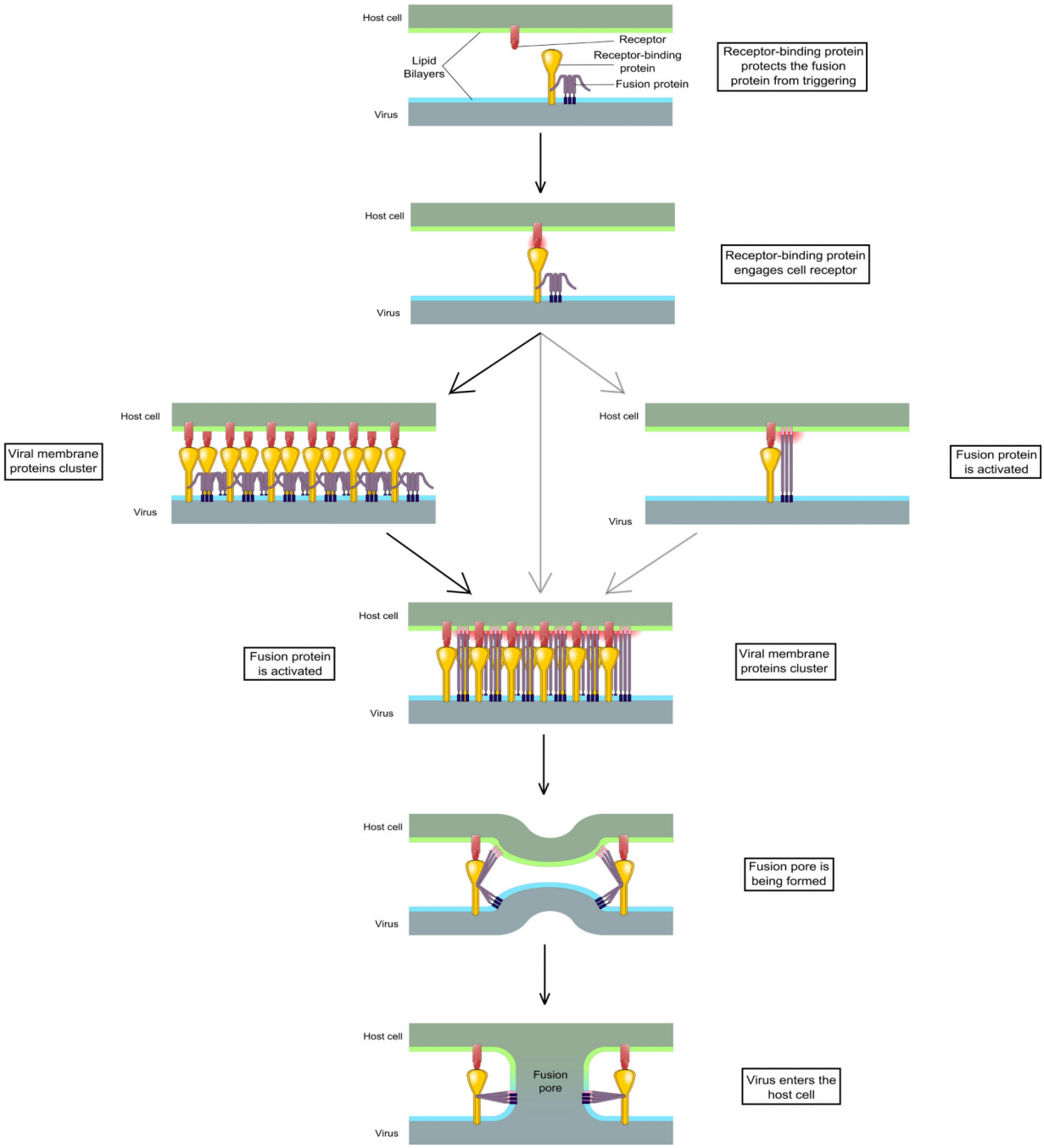


Figure 5. Unified model for *Paramyxoviridae* fusion process

See text for description. Pink: HRN domain and fusion peptide. HRC/HRN: Heptad Repeat C-/N-terminal domain.

Table 1

Paramyxoviruses classification and associated pathologies.

Order	Family	Sub-family	Genus	Species	Associated diseases
Mononegavirales	*Paramyxoviridae*	*Paramyxovirinae*	*Respirovirus*	HPIV1, HPIV3	Rhinitis, pharyngitis, pneumonia
			Rubulavirus	MuV, HPIV2, HPIV4, PIV5/CPIV	Mumps, orchitis, meningo-encephalitis, tracheobronchitis
			Morbillivirus	MeV	Measles, encephalitis
			Avulavirus	NDV	Fatal respiratory tract infection of poultry
			Aquaparamyxovirus	ASPV	Salmonid gill disease?
			Ferlavirus	FDLV	Unknown
			Henipavirus	HeV, NiV	Fatal encephalitis
		Pneumovirinae	*Pneumovirus*	RSV	Rhinitis, pneumonia
			Metapneumovirus	HMPV	Rhinitis, fever, bronchiolitis

The *Paramyxoviridae* family is divided into two sub-families and seven genera. Most paramyxoviruses cause a wide range of pathology. Data gathered from ICTV, Enders *et al*.[2] and recent articles[4,5].
PIV: parainfluenza virus. HPIV: human parainfluenza virus. CPIV: canine parainfluenza virus. MuV: mumps virus. MeV: measles virus. NDV: Newcastle disease virus. ASPV: atlantic salmon paramyxovirus. FDLV: Fer-de-Lance virus. HeV: Hendra virus. NiV: Nipah virus. RSV: respiratory syncytial virus. HMPV: human metapneumovirus.

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