

Review

Structure and function of a paramyxovirus fusion protein

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Abstract

Paramyxoviruses initiate infection by attaching to cell surface receptors and fusing viral and cell membranes. Viral attachment proteins, hemagglutinin-neuraminidase (HN), hemagglutinin (HA), or glycoprotein (G), bind receptors while fusion (F) proteins direct membrane fusion. Because paramyxovirus fusion is pH independent, virus entry occurs at host cell plasma membranes. Paramyxovirus fusion also usually requires co-expression of both the attachment protein and the fusion (F) protein. Newcastle disease virus (NDV) has assumed increased importance as a prototype paramyxovirus because crystal structures of both the NDV F protein and the attachment protein (HN) have been determined. Furthermore, analysis of structure and function of both viral glycoproteins by mutation, reactivity of antibody, and peptides have defined domains of the NDV F protein important for virus fusion. These domains include the fusion peptide, the cytoplasmic domain, as well as heptad repeat (HR) domains. Peptides with sequences from HR domains inhibit fusion, and characterization of the mechanism of this inhibition provides evidence for conformational changes in the F protein upon activation of fusion. Both proteolytic cleavage of the F protein and interactions with the attachment protein are required for fusion activation in most systems. Subsequent steps in membrane merger directed by F protein are poorly understood.

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1. Introduction

Membrane fusion directed by paramyxovirus glycoproteins is of particular interest, in part because of its pH independence and because of the requirement of two separately synthesized proteins for most members of this family. Paramyxoviruses are negative-stranded, nonsegmented RNA viruses. This family of viruses includes agents of common childhood diseases such as measles, mumps, and respiratory infections, as well as Hendra and Nipah viruses, more serious central nervous system pathogens [1] (Table 1). Also in this family are many animal pathogens, including Newcastle disease virus (NDV), an avian virus that is a serious agricultural problem in many regions of the world [2].

Paramyxovirus virions are pleomorphic, enveloped particles whose membrane is modified with two, and sometimes three, transmembrane proteins and an M protein that lines the

inner surface of the membrane [1]. The core of the virus includes the genome, a 15–19-kb single-stranded RNA, and associated proteins, the nucleocapsid protein (NP), phosphoprotein (P), and virion associated polymerase (L) [1].

2. Paramyxovirus entry

Paramyxoviruses initiate infection by attaching to cell surface receptors allowing fusion of the viral membrane with host cell plasma membranes. All members of this family encode two transmembrane glycoproteins that direct these steps in virus infection, the attachment protein and the fusion (F) protein. The virus attachment proteins are called variously hemagglutinin-neuraminidase (HN), hemagglutinin (HA), or glycoprotein (G) depending upon the virus [1] (Table 1). The receptor for many of these viruses is a sialic acid-containing molecule [1] while two different molecules have been identified as measles virus receptors, CD46 and CD150 (reviewed in Ref. [3]).

The fusion (F) protein directly mediates membrane fusion. Perhaps the best proof of this conclusion is that some paramyxovirus F proteins can direct this process by

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Table 1
 Prototype members of the family of paramyxoviruses

Virus	Attachment protein	Fusion protein	F cleavage site sequence
Respiroviruses	HN	F	
Sendai virus (SV)			V-P-Q-S-R
Parainfluenza virus 1 (PIV1)			N-P-Q-S-R
Parainfluenza virus 3 (PIV3)			P-R-T-K-R
Rubulaviruses	HN	F	
Simian virus 5 (SV5)			R-R-R-R-R
Newcastle disease virus (NDV)			
Virulent			R-R-Q-K/R-R
Avirulent			G-R/K-Q-G-R
Mumps virus (MuV)			R-R-H-K-R
Parainfluenza virus 2 (PIV2)			T-R-Q-K-R
Parainfluenza virus 4 (PIV4)			E-I-Q-S-R
Morbilliviruses	H or HA	F	
Measles virus (MV)			R-R-H-K-R
Canine distemper virus (CDV)			R-R-H-K-R
Rinderpeste virus (RPV)			R-R-H-K-R
Pneumoviruses	G	F	
Respiratory syncytial virus (RSV)			K-K-R-K-R-R and R-A-R-R
Henipaviruses	G	F	
Hendravirus (HeV)			V-G-D-V-K
Nipahvirus (NiV)			V-G-D-V-R

themselves [4–9]. Some paramyxoviruses also encode a small hydrophobic protein (SH) that has been implicated in membrane fusion [1], however, its role is unclear. It has been shown that the SH protein of respiratory syncytial virus (RSV) enhances cell fusion in a transient transfection of viral envelope genes although the protein has inhibitory effects on fusion in the context of virus infection [9,10].

In studies of paramyxovirus entry, NDV has recently assumed increased importance as a prototype paramyxovirus because crystal structures of both the NDV F protein [11] and the attachment protein (HN) [12] have been determined. In addition, there is a comprehensive analysis of the structure and function of both viral glycoproteins by mutation, reactivity of antibody, and peptides. For these reasons, the focus below is on the structure and function of the NDV F protein.

3. Overview of paramyxovirus fusion

Membrane fusion generally proceeds in a series of specific steps: docking of an attack membrane to a target membrane, F protein activation, close approach of membranes, membrane merger, pore formation, and pore expansion [13–15]. The paramyxovirus attachment protein serves to dock the target and attack membranes, at least initially. Current models [16] for how the F protein directs fusion have relied heavily upon models developed in other systems, notably HIV and influenza virus. However, as previously noted [11], several properties of the F protein,

including the crystal structure, suggest that the paramyxovirus system may not be entirely analogous. Further complicating the issue is that most F proteins expressed alone do not mediate membrane fusion [17] but require the co-expression of the attachment protein. It is clear that these attachment proteins provide more than just a docking function. While the subject of a great deal of speculation and hypothesis [1,17], the function of the attachment protein in fusion is one of the unsolved questions in paramyxovirus fusion.

Paramyxovirus fusion is pH independent (reviewed in Refs. [1,13,17]). That is, infection does not require the acid pH of endosomes to activate fusion and infection can occur at the host cell plasma membrane. Thus other mechanisms must be invoked for F protein activation. As a result of the acid independence of fusion, infected cells expressing viral glycoproteins can fuse with adjacent cells resulting in syncytia formation, a hallmark of paramyxovirus infection [1]. While it has been widely assumed that virus–cell fusion is comparable to cell–cell fusion, there is no direct proof.

To describe paramyxovirus fusion, several questions must be considered. First, what is the structure of the pre-fusion or fusion competent F protein and how does this presumably metastable structure form? Second, how is the F protein activated and what conformational changes are involved? Third, how does the F protein mediate the actual membrane merger? Lastly, what is the conformation of the post-fusion F protein?

4. F protein structure

4.1. Primary structure

All paramyxovirus F proteins are type 1 glycoproteins with an amino-terminal signal sequence, a hydrophobic transmembrane domain (TM) located near the carboxyl terminus, and a 25–30-amino-acid cytoplasmic domain (CT) [18]. Typical of paramyxovirus F proteins, the NDV F protein is a 553-amino-acid protein and is synthesized as a precursor, F₀ (diagramed in Fig. 1). The F₀ must be proteolytically cleaved to F₁ and F₂ for fusion activity (reviewed in Ref. [1]). Like all paramyxovirus F proteins, the NDV F protein is glycosylated. Rather surprisingly, given the overall conservation of determinants of protein structure in all the paramyxovirus F proteins [18], the location and numbers of carbohydrate addition sites are not at all conserved [18]. Thus, there are few generalizations that can be made about the roles of carbohydrate in this family of proteins. NDV F protein has five carbohydrate addition sites in the presumed ectodomain, four of which have been shown to be used by analysis of addition site mutants [19]. Some of the side chains are important in folding while others influence fusion activity of the protein.

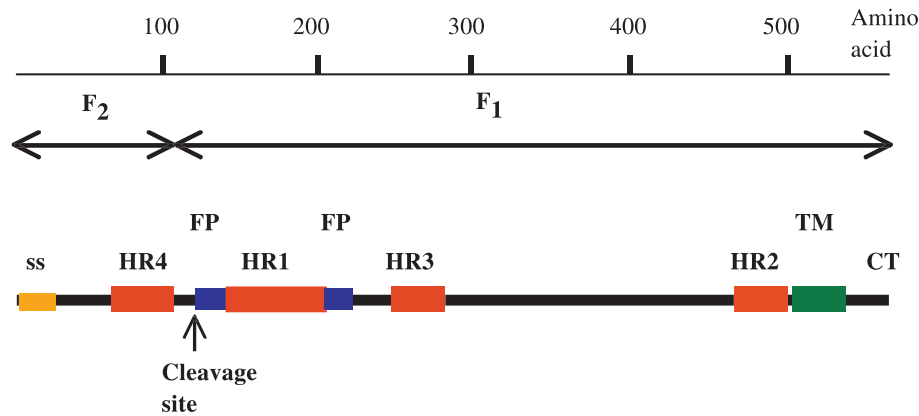


Fig. 1. Diagram of the primary sequence of the NDV F protein. Important domains in the primary sequence of the NDV F protein are diagrammed. The top line indicates amino acid position while the arrows identify polypeptides (F_1 and F_2) generated by proteolytic cleavage. Red domains are heptad repeat (HR) sequences. Other domains are: ss, signal sequence; FP, fusion peptide; TM, transmembrane domain; CT, cytoplasmic tail or cytoplasmic domain.

The primary sequence of the F protein also has several striking domains. The fusion peptide is a 10–15-amino-acid sequence at the amino terminus of the F_1 polypeptide (reviewed in Refs. [1,13]). Such sequences, found in many F proteins, are composed of hydrophobic and short-chain amino acids and are thought to insert into the target membranes to initiate fusion (reviewed in Ref. [13]). It has recently been proposed that paramyxovirus F proteins have a second fusion peptide located at an internal site in F_1 [20,21] (Fig. 1).

Also present in the sequence are several heptad repeat (HR) domains. One is located in the ectodomain adjacent to the transmembrane region (HR2 in Fig. 1). This sequence was first recognized due to similarities to leucine zipper motifs in transcription factors [22]. A second, remarkably long HR is located just carboxyl terminal to the fusion peptide (HR1 in Fig. 1) [23]. More recently, another leucine zipper motif (HR3) was recognized in some paramyxovirus F proteins, including the NDV F protein [24]. This domain, however, does not form a helix in the crystal structure of the F protein. In addition, a fourth HR (HR4) in the F_2 region of F proteins was noted and is also present in the NDV F protein [25,26].

4.2. Crystal structure

The recently solved crystal structure of the NDV F protein [11] should provide a structural basis for many biological observations about the activity of this protein. The structure, which was determined with an uncleaved F protein from an avirulent strain of NDV, was a homotrimer as had been predicted by cross-linking and sucrose gradients [27,28]. The three monomers are remarkably intertwined (Fig. 2A and B). The oligomer, which is wedge-shaped viewed from the side (Fig. 2A), is divided into a head, neck, and stalk domain. The head and neck regions are composed of sequences from both the F_2 and F_1 polypeptides (Fig. 2C and D). The stalk region is a long,

coiled-coil trimer extending from amino acid 171 to 221 and includes the carboxyl terminal region of the HR1 domain. Viewed end-on, the trimer is triangular in shape (Fig. 2B). Two remarkable and intriguing features are a central axial channel that extends through the head and neck regions as well as three radial channels that intersect the axial channel.

The structure is missing several crucial domains, which complicate considerations of the mechanisms of fusion. First, as is typical of crystal structures of glycoproteins, the transmembrane and cytoplasmic domains are missing. The crystallized protein was derived from a mutant F protein truncated at amino acid 499 eliminating the TM and CT domains in order to allow secretion from cells [29]. This source of material for crystal derivation may be important since expression of a mutant NDV F protein missing its CT domain resulted in decreased efficiency of cleavage and surface expression [30], suggesting that the cytoplasmic domain may influence the conformation of the ectodomain. Glycosylation of this truncated form of the protein was also different than the intact protein, with a glycosylation site used that is not used in the intact protein [19]. Another consideration is that the protein was expressed in the absence of the HN protein, which may affect the conformation of the molecule. The conformation of the NDV F protein on cell surfaces, as measured by binding of a peptide antibody, changed when co-expressed with HN protein [31].

Two other important domains are missing from the structure. One missing region, from amino acid 106 to 170, includes the cleavage site, the more amino terminal fusion peptide, and the amino terminal half of the HR1 domain. Also missing is the region from amino acid 455 to 499, which includes the HR2 domain. Fig. 2E shows missing domains inserted as dashed lines. Absence of these domains may be the result of degradation of the purified protein as well as conformational disorder [11]. These missing domains complicate consideration of the structure of the pre- and post-fusion conformations of the protein as

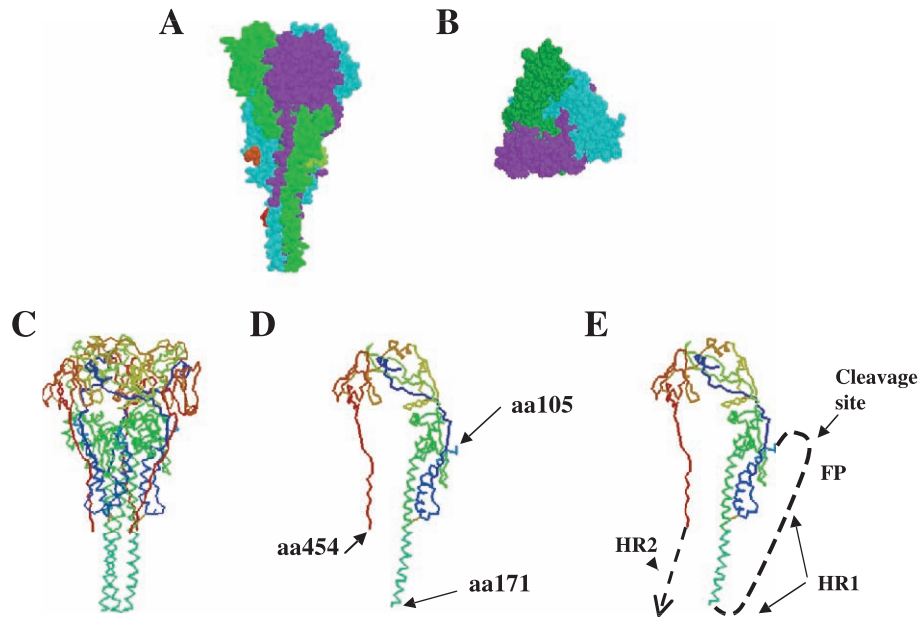


Fig. 2. Crystal structure of the NDV F protein. The structures shown are generated using coordinates deposited in protein data base [11] and presented using Protein Explorer (University of Massachusetts). A. Side view of a space filling model of the F protein trimer with each monomer colored differently; red corresponds to carbohydrate B. End view of a space filling model of the F protein trimer with each monomer colored differently; C. Backbone presentation of the F trimer with each monomer colored to key location within the primary sequence. Blue corresponds to amino terminal sequences (F_2 domain). Colors progress from green to yellow to red, which colors the most carboxyl terminal regions. D. Backbone presentation of a monomer as it rests in the trimer. Colors are the same as in C. E. Backbone presentation of a monomer with missing domains as dashed lines in black. Location of missing domains, FP, cleavage site, HR1, and HR2 are indicated.

well as conformational changes required for activation of fusion. Importantly, the disposition of the HR1 and HR2 domains before and after cleavage is also unknown. Furthermore, the orientation of the trimer with respect to membranes is not clear although Chen et al. [11] suggested that the head or wide end of the oligomer is facing away from viral membranes based on the location of antibody neutralization escape mutations.

4.3. Alternate forms of the F protein

Analyses of the primary sequence and the crystal structure of the F protein are entirely consistent with the classification of this protein as a type 1 glycoprotein, a protein anchored in membranes with the amino terminus in the ectodomain. However, analysis of synthesis of the NDV F protein in a cell-free protein synthesizing system containing membranes led to the surprising observation that the products of the reaction contained, in nearly equimolar amounts, two different topological forms of the protein with respect to membranes [32]. One form was associated with membranes typical of a type 1 glycoprotein. A second form was partially translocated. In addition, the carboxyl terminus appeared to be translocated. We have also reported evidence that this second form of the F protein exists in infected cells. While the functional significance of this second form of the NDV F protein is unclear, these observations raise the

possibility that the structural correlates of NDV F protein function are incompletely understood. There are examples of other viral glycoproteins that assume alternate topologies with respect to membranes. The hepatitis B virus L protein [33], the hepatitis C virus E2 protein [34], and the transmissible gastroenteritis M protein [35] are all reported in at least two different topological forms with different functions.

5. Functional analysis of F protein domains

Most of the domains missing in the crystal structure have been shown to be very important in the fusion activity of the protein by mutational analysis and the analysis of the structure and function of peptides with sequences from those domains. Most significant of these domains are the cleavage site, the fusion peptide, the cytoplasmic domain, and two of the HR domains.

5.1. Cleavage site

A classic property of many viral F proteins, including paramyxovirus F proteins, is the requirement of proteolytic cleavage for fusion activity [36,37]. Cleavage of paramyxovirus F proteins results in disulfide-linked F_2 and F_1 polypeptides derived from the amino-terminal and carboxyl-

terminal domains, respectively. The cellular site of this cleavage depends upon the sequence at the cleavage site (reviewed in Ref. [1]). Some F proteins have a furin recognition site (R-X-K/R-R) and are, therefore, cleaved in the trans-Golgi membranes. In this case, the majority of F proteins delivered to the plasma membrane are potentially active F proteins. Other F proteins have single basic residues at the cleavage site and are delivered to the plasma membrane in an inactive, uncleaved form. In order to direct membrane fusion, these F proteins must be cleaved by an extracellular host cell enzyme, usually found exclusively in the respiratory tract. Thus, infections by paramyxoviruses encoding F proteins without a furin sequence are usually limited to the respiratory tract. The cleavage site sequences of F proteins of different paramyxoviruses are shown in Table 1.

In contrast to other paramyxovirus systems, there exist numerous different strains of NDV, some of which encode F proteins with a furin recognition site at the cleavage site and some of which encode F proteins without this site [2]. The presence or absence of the furin recognition site in the F proteins is correlated with the virulence of the strain of virus [38]. Virulent strains, which can result in systemic infections, encode F proteins with a furin site, while avirulent strains, which result largely in respiratory tract infections, do not have a furin recognition site (reviewed in Refs. [1,2]). That both types of NDV exist in nature indicates that the cleavage of the F protein is not related to successful delivery of the protein to plasma membranes. Indeed, mutations of the furin recognition cleavage site in an F protein from a virulent strain can result in a transport-competent but uncleaved F protein and the fusion activity can be activated by addition of exogenous trypsin [39].

Cleavage of the F protein is also affected in undefined ways by other regions of the molecule. For example, mutation at amino acid 154 in the HR1 domain inhibited cleavage of the molecule [40,41]. Similarly, as noted above, deletion of the CT domain of the protein inhibited cleavage [30]. These changes must affect the conformation of the protein such that the furin site is inaccessible.

Recently it has been shown that the RSV F protein must be cleaved in two places for fusion activity, one at the F₁–F₂ junction and the other within F₂. This surprising finding suggests that the RSV F protein may have structural differences from other paramyxovirus F proteins [42,43]. Indeed the RSV F₂ has an unusually long sequence between the two cleavage sites, a sequence not present in other F proteins [44]. Perhaps this region of the RSV F₂ protein must be excised for fusion activity.

5.2. Fusion peptides

Extensive studies of fusion peptides in many viral systems have suggested that this domain inserts into membranes disordering the bilayer in preparation for membrane merger (reviewed in Refs. [13,15]). The amino terminal

sequence of the F₁ polypeptides of all paramyxovirus F protein is remarkably similar and led to its identification as a fusion peptide. Analysis of conservative and nonconservative point mutations in the SV5 F [45] and the NDV F proteins [40,41] has shown that this sequence is important for fusion activity. All mutations in the NDV F protein fusion peptide inhibited fusion as assayed by syncytia formation, content mixing, and hemifusion. Surprisingly, some of the same mutations in the SV5 F protein fusion peptide actually enhanced fusion. The reasons for these different results are unclear and point to the differences in these two F proteins.

Recently, Shai and coworkers have shown that another sequence, present in both Sendai and measles virus F proteins, has properties of a fusion peptide based on the analysis of short peptides with sequences from this domain [20,21]. This sequence is at the carboxyl terminus of the HR1 domain and is located at an internal site in the head domain of the crystal structure of F protein. This location suggests that it would become accessible to membranes only upon opening of the trimer head domain. It will be important to determine if mutations in this sequence in the intact protein can negatively affect fusion in ways similar to mutations in the more amino-terminal fusion peptide.

5.3. HR domains

The central role of HR1 and HR2 domains in paramyxovirus fusion is indicated from analysis of mutant F proteins and, most importantly, by the structure and function of peptides with sequences of these two domains. There has been much less analysis of the two other HR domains, but available evidence does suggest that they also have a role in the folding of the pre-fusion F protein and the fusion activity of the protein.

5.3.1. HR1 and HR2 domains

The NDV HR2 domain has leucine or isoleucine residues at “a” positions in four HRs. Mutation of these “a” position residues inhibited fusion, providing evidence for the importance of the domain in fusion. Two or more of these “a” residues must be changed to affect fusion if the changes are leucine to alanine. However, single, more nonconservative changes in the middle of the HR inhibited fusion [28,46]. Similar results were reported for other paramyxovirus F proteins [47]. Alterations at other positions within the heptads had no effect on fusion [28,46]. In addition, no mutation significantly affected intracellular transport and surface expression, indicating that this domain does not play a direct role in formation of the pre-fusion F protein.

Results of mutational analysis of the HR1 domain of NDV [40,41] are less straightforward but, in sum, indicate that the region is important in fusion as well as initial folding of the protein. First, the HR1 domain seems to be divided into at least two regions. While analysis is limited, all mutations in the region carboxyl terminal to amino acid

175 inhibited folding and surface expression of the protein. In contrast, some mutations amino terminal to this position did not inhibit surface expression. The crystal structure also suggested the presence of two domains [11]. Residues carboxyl terminal to position 171 form a coiled-coil trimer while the more amino terminal residues were not visible in the structure (Fig. 2E). Likely the coiled-coil trimer visible in the crystal structure from amino acid 171 to 220 must form during the folding of the molecule. The amino-terminal end of the HR1 domain may be conformationally flexible and more directly related to fusion and the conformational changes in the molecule upon activation of fusion.

Many mutations in the amino terminal region of the HR1 domain (approximately amino acids 130–170) also inhibited initial protein folding. Indeed, all mutations in the “a” position of the HRs inhibited surface expression of the protein, indicating that this side of the helix plays some role in initial folding. However, most mutations in this region in the “d” position in the HRs did not affect initial folding but did block fusion, indicating that the region is involved in fusion activity of the protein. Mutations nearer the amino terminus of the domain inhibited fusion more completely than mutations further along the domain [41].

Motivated by reports that peptides with sequences from HR domains of the HIV gp41 inhibited fusion [48–50], several laboratories characterized the effects of paramyxovirus HR2 peptides on fusion and found that these peptides also inhibited fusion [26,51–54]. In addition, peptides with sequences from the HR1 domain inhibited fusion in several systems [26,54,55]. Key to understanding the role of the HR domains in fusion was the finding that peptides from the HR1 and HR2 domains can form a complex. This complex has been demonstrated functionally in the NDV system. Two different laboratories have shown that mixtures of HR1 and HR2 peptides no longer inhibited fusion, suggesting the formation of a complex between the peptides which eliminated the fusion activities of each of the peptides [55,56]. In contrast, mixtures of HR1 and HR2 peptides with sequences from the SV5 F protein still inhibited fusion [54]. Complexes between these two SV5 peptides as well as complexes of HR1 and HR2 peptides with sequences from the RSV HR domains were demonstrated on polyacrylamide gels, and the structures of the peptide complexes were solved by X-ray crystallography [16,54,57]. These complexes form a six-stranded structure with an interior core trimer of HR1 peptides and associated HR2 peptides bound in the grooves of the trimer in an anti-parallel fashion (Fig. 3).

These findings, coupled with analogous studies of HIV gp41 and influenza virus HA, led to the hypothesis [16,57] that paramyxovirus F proteins are folded such that the HR domains are not complexed. Upon activation of fusion, the protein undergoes a cascade of conformational changes that result in insertion of the fusion peptide into target membranes followed by complexing of the HR1 and HR2 domains. Because of the location of the HR domains in

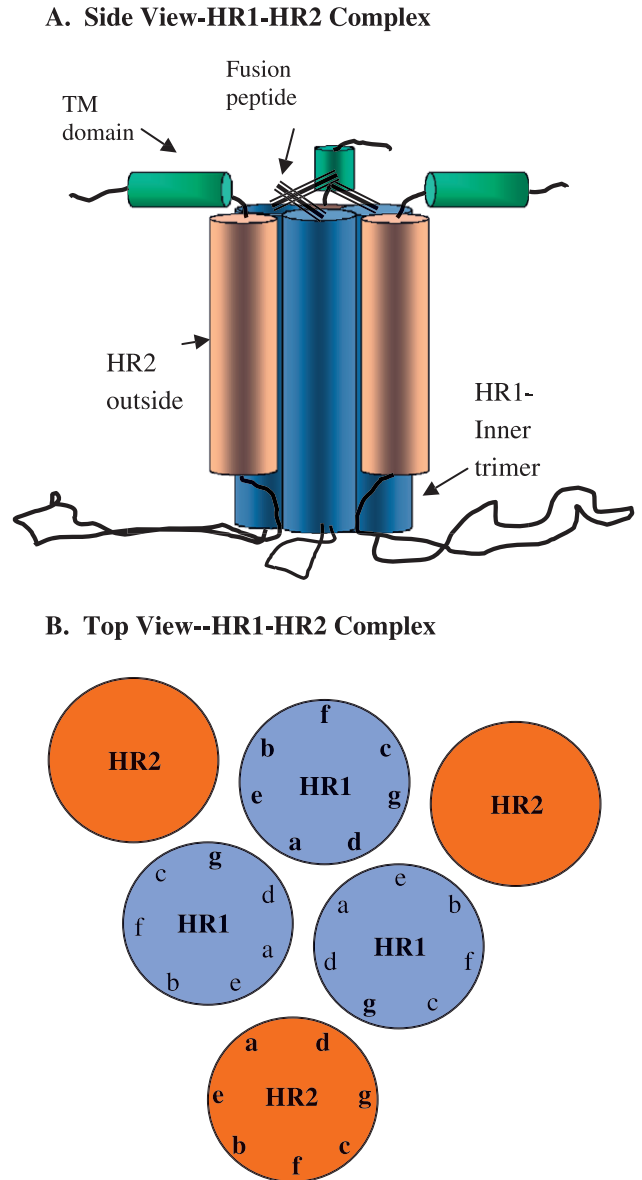


Fig. 3. Cartoon representation of HR1–HR2 complex. Panel A shows representation of the side view of the peptide complex. Three HR1 peptides (blue) form a central trimer and three HR2 (red) polypeptides bind in the grooves of the trimer. The position of the fusion peptides (black triple line) and the TM domains (green) with respect to the peptide complex as it would exist in the intact protein are diagramed. The sequences in between the HR1 and HR2 domains are represented by black lines. Panel B represents an end view of the HR1–HR2 peptide complex with the orientation of the hydrophobic side (“a” and “d” residues) of each HR helix shown.

the F protein sequence and the anti-parallel nature of the HR1–HR2 interactions, formation of this complex would bring the TM domain and the fusion peptide in close proximity. This close proximity would result in the close approach of the target membrane and the attack membrane (Fig. 3A). It is logical, therefore, that peptides with sequences from either of these domains inhibit fusion by binding to the other HR domain within the intact protein, interfering

with the formation of the complex in the intact protein. Given the structure of the peptide complex as well as implications of the F protein crystal structure, it seems possible that HR1 peptides could also inhibit by binding to HR1 domains prior to the formation of the amino-terminal region of the HR1 core trimer.

The structure of the complex also provides a rational explanation of the phenotype of some of the HR mutants. The hydrophobic “a” position residues in the predicted HR2 helix form the surface that interacts with the HR1 core trimer (Fig. 3B). Introduction of charged residues along this hydrophobic face should interfere with the interaction of HR1 and HR2 and block initial stages in membrane fusion. More conservative changes should have less effect on complex formation and, therefore, fusion. Indeed, this was the result as described above [28,46]. It is also possible that these mutations interfere with HN protein interactions. Mutations in the “d” position of HR1 may affect fusion activity of the protein by destabilizing the core HR1 trimer or by interfering with HR2 interactions (Fig. 3B).

5.3.2. HR3 domain

A second leucine zipper-like domain present in F₁ of many paramyxovirus F protein sequences (HR3) was identified by Ghosh and Shai [58] (Fig. 1). Peptides with sequences from this region of the Sendai F protein inhibited fusion although similar studies in SV5 system reported no effects of peptides on fusion mediated by the SV5 F protein [59]. Mutational analysis of this domain in the NDV F protein indicated that the domain was important in the folding of the molecule [7]. The NDV HR3 domain extends to 28 amino acids with four HRs of leucine residues. Mutation of two of the four leucine residues resulted in misfolded proteins that were not transported to the cell surface. One mutation resulted in a surface expressed protein that was conformationally abnormal and defective in fusion. Surprisingly, mutation of the fourth leucine in the HR has no effect on the folding or surface expression of the molecule but rather enhanced fusion in the presence of HN protein co-expression. This mutation also eliminated the requirement for HN protein co-expression for syncytia formation. This residue is located at an internal position in the globular head of the crystal structure. Chen et al. [11] proposed that this alteration facilitates structural transitions in the molecule important for fusion by reducing hydrophobic interactions with an adjacent domain.

5.3.3. HR4 domain

In the sequence of the NDV F₂ protein, as well as other paramyxovirus F proteins, is a striking HR from amino acid 81 to 102 (HR4) and, indeed, the crystal structure shows an alpha helix from amino acid 76 to 105 [11]. Limited mutational analysis of this region showed it to be critical to the folding of the molecule since all mutations made have resulted in no surface expression (unpublished observations). Recent mutational analyses of the comparable region

in the measles virus F protein have shown that mutations in this domain affect syncytia formation [60].

5.4. Cytoplasmic domain

Mutational analyses of the NDV, SV5, PIV3, MV, and PIV2 F protein cytoplasmic domains have been reported and the role of this domain in fusion varies with the F protein [30,61,62]. Deletion of the entire domain from the PIV2 F protein and the MV F protein had no effect on surface expression or fusion, while deletion from the PIV3 F protein and NDV F protein interfered with their proper folding and surface expression. Clearly, in some cases, the CT domain influences the folding of the ectodomain of the protein. Elimination of the carboxyl-terminal half of the NDV F protein cytoplasmic domain resulted in a surface-expressed protein that was defective in syncytia formation. It has been reported that cytoplasmic domain mutations in the SV5 F protein allow content mixing, a result that suggests that the domain is involved in later stages of fusion related to pore expansion [63,64].

6. Conformational changes in the F protein

The model outlined above and based on the structure and function of HR1 and HR2 inhibitory peptides predicts that the F protein undergoes a series of conformational changes upon activation of fusion. Indeed, by addition of these inhibitory peptides at different stages during the onset of fusion, indirect evidence for such conformational shifts has been obtained. However, results obtained in two different paramyxovirus systems, NDV and SV5, are not consistent and may reflect differences in paramyxovirus F proteins as well as differences in assays.

In both systems, onset of fusion was controlled by addition of exogenous trypsin to cells expressing an uncleaved F protein. Upon addition of trypsin and cleavage of F₀, fusion rapidly ensued. Using such a system, the NDV HR2 peptide inhibited fusion, as assayed by syncytia formation, if added prior to cleavage activation, but did not inhibit if added only after a 10-min incubation with trypsin at 25 °C [55]. Furthermore, the peptide inhibited fusion directed by cells expressing a cleaved F protein, suggesting that cleaved F protein delivered to the cell surface is, at least transiently, accessible to HR2 peptide [52]. These combined results are consistent with the idea that both uncleaved and cleaved F proteins, in a pre-fusion conformation, are accessible to binding of HR2 peptide. In contrast, HR1 peptide inhibited fusion only if added prior to F protein cleavage and not after [55].

In contrast, similar studies in the SV5 system indicated that the SV5 F protein was accessible to peptide inhibition at steps only after F protein cleavage [65]. These more recent results dissected steps in F protein conformational changes using fusion of fluorescence dye-labeled red blood cells in

order to control docking as well as subsequent steps in fusion. Using this assay, Russell et al. [65] concluded that both HR1 and HR2 peptides bound to their targets only after F cleavage and after attachment of red blood cells. Furthermore, their results indicated that HR1 peptide bound to its target at a step earlier than the HR2 peptide. They concluded that the F protein undergoes at least three conformational changes, one upon cleavage, one upon docking to red blood cells opening up the site for HR1 peptide binding, and one upon a shift to 37°, which opens the site for HR2 peptide binding.

The reasons for these contradictory results may be due to differences in assay systems used, the peptides used, and, most importantly, F proteins. Not only are the proteins from different viruses but they also differ in their requirements for an attachment protein. NDV F protein is absolutely dependent upon HN protein co-expression for fusion while the SV5 F protein used is not [6]. Furthermore, it has been recently reported that the conformation of this SV5 F protein, derived from strain W3A, is different from that of SV5 F protein derived from strain WR, an F protein that reassembles the NDV F protein in its requirement for HN protein co-expression [66]. Perhaps steps leading up to membrane merger vary somewhat with different F proteins, or perhaps initiation of fusion begins at different points along a sequence of conformational changes. In any event, it is clear that paramyxovirus F proteins undergo conformational changes related to the onset of fusion, changes that are initiated upon F protein activation. Further clarification of these changes will be important to a full understanding of the mechanism of F protein-directed membrane fusion.

7. Activation of F protein fusion activity

In all fusion reactions, a key step is the activation of fusion activity in the appropriate time and place. The acid pH of the endosome activates most viral F proteins by triggering conformational changes in the protein required to initiate fusion. In acid-independent fusion, mechanisms of activation are less well defined although in some systems it is clear that attachment of the protein to its receptor activates the F protein [67]. Implicit in studies of peptide inhibition of paramyxovirus fusion is the idea that upon activation, the F protein conformation changes. So, then, how are these conformational changes activated?

Proteolytic cleavage of paramyxovirus F proteins is necessary and results in conformational changes in the protein that were initially detected as an increase in hydrophobicity [68]. Cleavage also changes the shape of the trimer, as visualized in electron micrographs of purified protein, from a cone shape to a lollipop shape [29,69]. It was proposed that the lollipop shape corresponds to the stable, post-fusion form of the protein. Importantly, it is not clear how, upon cleavage in the Golgi membranes, the premature

formation of the most stable, post-fusion form of the protein is prevented.

While necessary, proteolytic cleavage of F protein is not sufficient for fusion, at least in most paramyxovirus systems, since expression of a cleaved F protein alone usually does not result in membrane fusion [1,17]. Rather fusion also usually requires the co-expression of the attachment protein, a finding that suggests that attachment is somehow involved in F protein activation. The role of HN protein in fusion promotion is, however, a subject of some controversy.

Initiation of membrane fusion requires some form of docking to target membranes. Indeed, a fundamental tenant of paramyxovirus fusion has been that binding of attachment protein to receptors is necessary for fusion activation [1,17]. This idea was based largely on the finding that treatment of cells with neuraminidase to remove surface sialic acid receptors blocked fusion [1,17,70–72]. However, this conclusion is now considerably complicated by several more recent observations. First, there is a recent report of mutants of NDV HN protein that have little or no demonstrable attachment activity but still efficiently promote fusion of a co-expressed wild type F protein [73]. These mutations, in the sialic acid binding site, also depress neuraminidase activity, which could complicate assays for attachment activity [74]. However, we have also made a mutation in the HN protein, at a position not in the sialic acid binding site, that also eliminates HN protein attachment activity without significantly affecting neuraminidase or fusion promotion activities (in preparation). Second, some F proteins can mediate fusion without attachment protein co-expression. The RSV F protein [8,9] and the SV5, strain W3A, F protein [6] can direct fusion without an attachment protein. As noted above, a point mutation in the NDV F protein HR3 domain eliminates the absolute requirement for HN protein in syncytia formation [7]. Thus, the relationship between the attachment function of HN protein and fusion promotion is not straightforward. In cases where an attachment protein is not required, how target and attack membranes are docked is unclear. Perhaps, there is a second receptor that interacts with F proteins. Indeed, it is reported that the RSV F protein interacts with glycosaminoglycans [75,76] and possibly other molecules [77]. Alternatively, the fusion peptide, by inserting into target membranes, may serve to dock the two membranes. How such a docking would be controlled is not clear.

The attachment function of HN protein is clearly not sufficient for fusion activation in most systems. Two lines of evidence support this idea. First, there are mutations in the NDV HN protein that eliminate fusion promotion but not attachment [78–80]. Second, the requirement for attachment proteins is virus-specific, that is, fusion requires that the attachment protein and the F protein be from the same virus [81]. This observation has been interpreted to indicate a virus-specific interaction between the attachment

and F proteins necessary for F protein activation. Indeed, several laboratories have demonstrated such a virus-specific HN–F protein interaction by co-immunoprecipitation or by co-capping of the proteins on cell surfaces [82–85]. Furthermore, the presumed region of the HN protein that interacts with F protein was identified as the membrane proximal domain, amino acids 50 to 141 in the NDV HN protein sequence, by mapping the region of the sequence that confers virus specificity to fusion promotion [86]. Similar results have been reported in two other paramyxovirus systems [87,88]. Importantly, the fusion promotion negative mutants of the NDV HN protein also map to this region of the protein (amino acids 74 to 142) (Ref. [80] and in preparation) suggesting that these mutations affect the HN–F protein interactions involved in fusion.

A plausible model for the role of HN protein in NDV fusion has been suggested by Taylor and Portner and their colleagues based on the crystal structure of the HN protein as well as HN protein mutational analysis [12,73]. These investigators crystallized two forms of the HN protein, one which they proposed was a binding form and the other a catalytic form. They proposed that there is a single sialic acid recognition site that can switch between binding and catalysis. The conformational switch that occurs upon binding to sialic acid is linked to conformational changes in the dimer interface and/or the membrane proximal regions of the molecule that, in turn, trigger conformational changes in the F protein to activate fusion. Furthermore, they propose that HN mutant proteins that still promote fusion but are attachment-negative are in the fusion-promoting conformation in the absence of binding. This hypothesis would predict that F proteins capable of fusing

without HN protein expression are already in an activated form or readily switch to that form. Indeed, using monoclonal antibody reactivity, Tsurudome et al. [66] have demonstrated that the conformation of HN protein-dependent and HN protein-independent F proteins of two different strains of SV5 are different. One epitope, accessible in the HN protein-independent F protein but not in the HN protein-dependent F protein, was masked in the comparable region of the crystal structure of the HN-dependent NDV F protein.

How might a conformational switch in the HN protein activate the F protein? Key to this question are identification of domains of F protein that interact with HN protein, the timing of the interactions relative to attachment, and definition of conformational changes that F protein undergoes upon activation, questions that remain unresolved. Two models have been invoked (diagramed in Fig. 4). Initially it was proposed that HN and F proteins interact only after HN protein receptor binding and this interaction initiates F protein conformational changes required for fusion (model 1) [17,79]. An alternative model is that HN and F proteins form a metastable complex prior to HN protein attachment [83,89,90]. HN protein attachment and switch to a catalytic form releases the F protein stimulating the cascade of conformational changes required for fusion (model 2). This second model describes a mechanism that is more analogous to current understanding of mechanisms involved in activation of HIV- and influenza virus-mediated membrane fusion [91]. In this second model, it is logical to propose that interaction of the HN protein membrane proximal domain with the F protein blocks the release of the fusion peptide and the formation

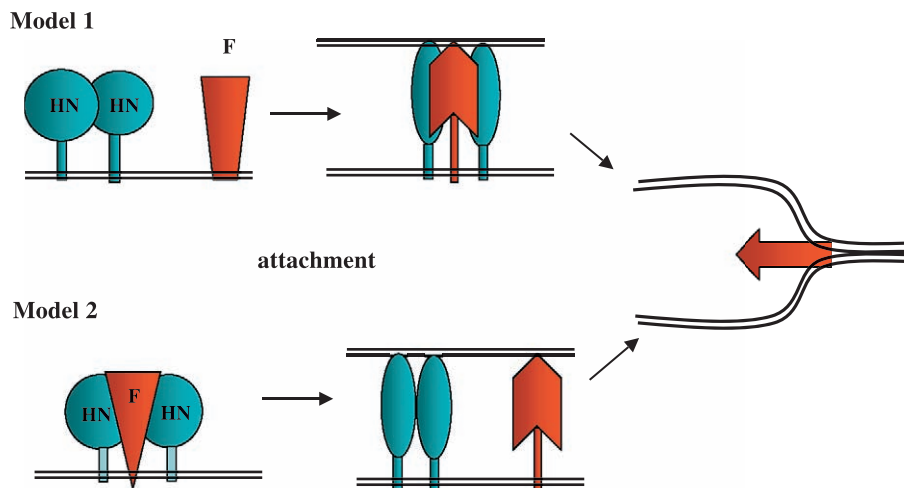


Fig. 4. Models for the role of attachment protein in F protein activation. Model 1: The HN and F proteins are transported to cell surfaces independently. Upon HN protein attachment to its receptor, the HN protein interacts transiently with the F protein altering its conformation. The F protein then undergoes a series of conformational changes that result in the insertion of the fusion peptide into the target membrane, the formation of the HR1–HR2 complex, and the close approach of the target and attack membranes. Model 2: The HN and F proteins form a metastable complex on cell surfaces prior to attachment. Upon attachment of the HN protein to its receptor, the HN protein switches conformation, releasing the F protein. The release activates conformational changes in the F protein that result in insertion of fusion peptides into target membranes, formation of the HR1–HR2 complex, and the close approach of target and attack membranes. Both models are intended to describe a general sequence of events. There would likely be additional intermediate steps not depicted.

of the HR1–HR2 complex until the target membranes are in appropriate proximity [31,92]. Indeed, we have evidence for a direct interaction between the HN protein membrane proximal domain and the F protein HR2 domain (in preparation). The HR2 domain was earlier suggested to be involved in HN protein interactions by mapping F protein domains that confer virus specificity to HN protein-promoted fusion [93].

Experimental evidence relevant to these models is conflicting. A point mutation in HN protein is reported to eliminate attachment as well as co-immunoprecipitation of HN and F proteins, a result that might support model 1 [82]. However, there are other possible interpretations of this result. There are reports of intracellular interactions of HN and F proteins, findings that would support model 2 [83,90,94]. However, there is no evidence that these interactions are directly related to fusion. In support of model 2, we have reported that co-expression of HN and F proteins alters the conformation of cell surface F protein and this alteration can be detected prior to attachment [31].

8. Models for initiation of paramyxovirus fusion

In sum, therefore, how paramyxovirus F proteins draw two membranes together to initiate membrane merger remains unresolved due to lack of information about the structure of the pre-fusion and post-fusion F proteins,

uncertainties about F protein conformational changes involved, and the mechanism of attachment protein activation of those changes. In addition, the orientation of the pre-fusion form of the protein with respect to membranes is uncertain. Two possible orientations are shown in Fig. 5, one in which the wide end of the trimer is adjacent to viral membranes (A) and the other with the wide end facing away from virion membranes (B). The orientation shown in B is favored because of the location of antigenic sites on the molecule [11]. Several different models for conformational changes upon activation have been proposed and are well described by Peisajovich and Shai [95]. Most models require that the fusion peptide of the cleaved F protein be sequestered in some way in the post-cleavage, pre-fusion form of the protein. Indeed, Chen et al. [11] have proposed that the fusion peptide is inserted into radial channels seen in the structure. Upon activation, the fusion peptide inserts into its target membrane. It has been suggested that the globular head domain of the F protein opens up upon activation, releasing the amino terminal fusion peptide and exposing the more carboxyl terminal fusion peptide for membrane insertion [20,95]. Most models propose that the molecule then refolds such that the HR1 and HR2 domains complex, drawing the attack and target membranes in close proximity. How this refolding occurs is unclear and the structure of the post-fusion protein is unknown. Peisajovich and Shai have proposed that, upon activation, the F protein trimer opens like an umbrella. Then the HR1 and HR2 domains zip together, rather like closing of an umbrella,

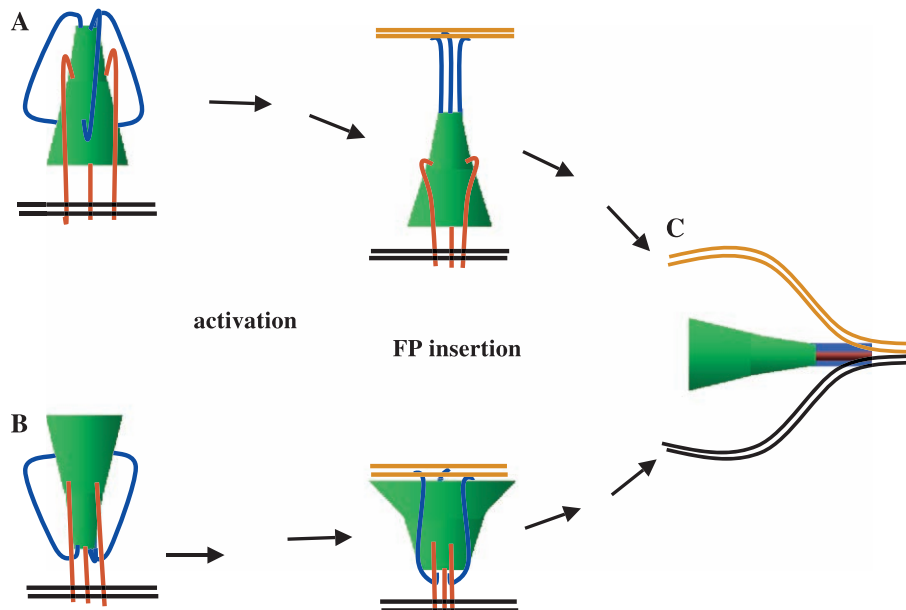


Fig. 5. Conformational changes in the F protein upon activation. Two possible conformations of uncleaved F protein trimer with respect to virion membranes are shown on the left. The top (A) shows the F protein oriented with the wide end of the trimer adjacent to virion membranes. The bottom (B) shows the F protein oriented with the wide end facing away from virion membranes. The FP and HR1 domains are shown in blue and the HR2, TM, and CT domains are in red. Upon cleavage activation and HN protein attachment the protein undergoes a series of undefined conformational changes (represented as arrows). Two possible intermediate conformations are shown in the middle of the figure although there are other possibilities. Finally the HR1 and HR2 domains complex into a six-stranded, coiled coil pulling the virion (black) and target (yellow) membranes into close proximity.

drawing the target membrane towards the attack membrane [20,95].

9. Membrane merger and pore expansion

Subsequent to close approach, membrane fusion is proposed to proceed by hemi-fusion, pore formation, and pore expansion [13]. In paramyxovirus systems, the steps following close approach are not well characterized. It has been proposed that close approach and subsequent steps are closely coupled [65] and, indeed, there are few instances where these subsequent steps have been separated from close approach. It has been reported that an F protein with a GPI anchor in place of the TM domain and CT domain can mediate hemi-fusion but not pore formation [96]. This report suggests a role for either the TM or CT domains in pore formation. In addition, mutant NDV F proteins with nonconservative changes of amino acids at the HR2–TM interface also direct hemi-fusion but not pore formation [46]. As yet, there are no reported studies of F protein TM domains in fusion and it will be interesting to characterize mutations in this domain. As described above, deletions in the SV5 F protein CT domains block syncytia formation but not pore formation [63,64], indicating a role of this domain in the final stages of fusion, pore expansion. The interactions of this domain with underlying cell structures and the role of such interactions in membrane fusion will be interesting avenues for future investigations.

Most models of fusion include the idea that multiple oligomers of F protein must be involved for successful membrane fusion as reported for influenza virus [97,98]. However, by measuring fusion at different ratios of expression of wild type and a cleavage mutant of the NDV F protein, the minimal functional unit was calculated to be a trimer [39]. By determining the UV target size of Sendai virus for hemolysis, the size of the functional unit was also determined to be a trimer [99].

10. Conclusions

While the general outlines of mechanisms involved in paramyxovirus fusion are emerging, there are numerous issues that remain to be clarified. Future studies of paramyxovirus fusion will need to focus on several questions. Prior to fusion, what is the conformation of the HR1 and HR2 domains in the uncleaved and cleaved F protein and how does co-expression of the attachment protein influence this conformation? What domains of F protein interact with activating attachment proteins? When does the F protein interact with attachment protein with respect to F protein cleavage and HN protein attachment to receptors? Is there a second virus receptor protein? What is the structure of the post-fusion form of F protein? What F protein domains are involved in actual membrane merger? What are the roles of

host proteins including underlying cellular structures in final stages of fusion, pore expansion, and syncytia formation? Are the mechanisms of cell–cell fusion and cell–virus fusion similar?

References

- [1] R.A. Lamb, D. Kolakofsky, in: D.M. Knipe, P.M. Howley (Eds.), *Fields Virology*, 3rd ed., vol. 1, Lippincott-Williams & Wilkins, Philadelphia, 2001, pp. 1305–1340.
- [2] Y. Nagai, M. Hamaguchi, T. Toyoda, *Prog. Vet. Microbiol. Immunol.* 5 (1989) 16–64.
- [3] C. Erlenhofer, W.P. Duprex, B.K. Rima, V.T. Meulen, J. Schneider-Schaulies, *J. Gen. Virol.* 83 (2002) 1431–1436.
- [4] M. Ito, M. Nishio, M. Kawano, S. Kusagawa, H. Komada, Y. Ito, M. Tsurudome, *J. Virol.* 71 (1997) 9855–9858.
- [5] M. Ito, M. Nishio, H. Komada, Y. Ito, M. Tsurudome, *J. Gen. Virol.* 81 (2000) 719–727.
- [6] S. Bagai, R.A. Lamb, *J. Virol.* 69 (1995) 6712–6719.
- [7] T. Sergel, L. McGinnes, T. Morrison, *J. Virol.* 74 (2000) 5101–5107.
- [8] R.A. Karron, D.A. Buonagurio, A.F. Georgiu, S.S. Whiteheat, J.E. Adamus, M.L. Clements-Mann, D.O. Harris, V.B. Randolph, S.A. Udem, B.R. Burphy, M.S. Sidhu, *Proc. Natl. Acad. Sci. U. S. A.* 94 (1997) 13961–13966.
- [9] S. Techaarpomkul, N. Barretto, M.E. Peeples, *J. Virol.* 75 (2001) 6825–6834.
- [10] B. Heminway, Y. Yu, Y. Tanaka, K. Perrine, E. Gustafson, J. Bernstein, M. Galinski, *Virology* 200 (1994) 801–805.
- [11] L. Chen, J.J. Gorman, J. McKimm-Breschkin, L.J. Lawrence, P.A. Tulloch, B.J. Smith, P.M. Colman, M.C. Lawrence, *Structure* 9 (2001) 255–266.
- [12] S. Crennell, T. Takimoto, A. Portner, G. Taylor, *Nat. Struct. Biol.* 7 (2000) 1068–1074.
- [13] L.D. Hernandez, L.R. Hoffman, T.G. Wolfsberg, J.M. White, *Annu. Rev. Cell Biol.* 12 (1996) 627–661.
- [14] J.R. Monck, J.M. Fernandez, *J. Cell Biol.* 119 (1992) 1395–1404.
- [15] J.M. White, *Science* 258 (1992) 917–924.
- [16] K.A. Baker, R.E. Dutch, R.A. Lamb, T.S. Jardetzky, *Mol. Cell* 3 (1999) 309–319.
- [17] R.A. Lamb, *Virology* 197 (1993) 1–11.
- [18] T. Morrison, A. Portner, in: D. Kingsbury (Ed.), *The Paramyxoviruses*, Plenum, New York, 1991, pp. 347–375.
- [19] L. McGinnes, T. Sergel, J. Reitter, T. Morrison, *Virology* 283 (2001) 332–342.
- [20] S.G. Peisajovich, O. Samuel, Y. Shai, *J. Mol. Biol.* 296 (2000) 1353–1365.
- [21] O. Samuel, Y. Shai, *Biochemistry* 40 (2000) 1340–1349.
- [22] R. Buckland, F. Wild, *Nature (Lond.)* 338 (1989) 547.
- [23] P. Chambers, C.R. Pringle, J.J. Easton, *J. Gen. Virol.* 71 (1990) 3075–3080.
- [24] J.K. Ghosh, M. Ovadia, Y. Shai, *Biochemistry* 36 (1997) 15451–15462.
- [25] J.M. Matthews, T.F. Yound, S.P. Tucker, J.P. Mackay, *J. Virol.* 74 (2000) 5911–5920.
- [26] D.M. Lambert, S. Barney, A.L. Lambert, K. Guthrie, R. Medinas, D.E. Davis, T. Bucy, J. Erickson, G. Merutka, S.R. Petteway, *Proc. Natl. Acad. Sci. U. S. A.* 93 (1996) 2186–2191.
- [27] R. Russell, R.G. Paterson, R.A. Lamb, *Virology* 199 (1994) 160–168.
- [28] J. Reitter, T. Sergel, T. Morrison, *J. Virol.* 69 (1995) 5995–6004.
- [29] L. Chen, P.M. Colman, L.J. Cosgrove, M.C. Lawrence, L.J. Lawrence, P.A. Tulloch, J.J. Gorman, *Virology* 290 (2001) 290–299.
- [30] T. Sergel, T. Morrison, *Virology* 210 (1995) 264–272.
- [31] L.W. McGinnes, K. Gravel, T.G. Morrison, *J. Virol.* 73 (2002).
- [32] L.W. McGinnes, J.N. Reitter, K. Gravel, T.G. Morrison, *J. Virol.* 77 (2003) 1951–1963.

- [33] C. Lambert, R. Prange, *J. Biol. Chem.* 276 (2001) 22265–22272.
- [34] N. Pavio, D.R. Taylor, M.M.C. Lai, *J. Virol.* 76 (2002) 1265–1272.
- [35] D. Escors, E. Camafeita, J. Ortego, H. Laude, L. Enjuanes, *J. Virol.* 75 (2001) 12228–12240.
- [36] A. Scheid, P.W. Choppin, *Virology* 80 (1978) 51–66.
- [37] A. Scheid, P.W. Choppin, *Virology* 57 (1974) 470–490.
- [38] R.L. Glickman, R.J. Syddall, R.M. Sheehan, M.A. Bratt, *J. Virol.* 62 (1988) 354–356.
- [39] Z. Li, T. Sergel, E. Razvi, T. Morrison, *J. Virol.* 72 (1998) 3789–3795.
- [40] T. Sergel-Germano, C. McQuain, T. Morrison, *J. Virol.* 68 (1994) 7654–7658.
- [41] T. Sergel, L. McGinnes, T. Morrison, *J. Virol.* 75 (2001) 7934–7943.
- [42] L. Gonzalez-Reyes, M.B. Ruiz-Arguello, B. Garcia-Barreno, L. Calder, J.A. Lopez, J.P. Albar, J.J. Skehel, D.C. Wiley, J.A. Melero, *Proc. Natl. Acad. Sci. U. S. A.* 98 (2001) 9859–9864.
- [43] G. Zimmer, L. Budz, G. Herrler, *J. Biol. Chem.* 276 (2001) 31642–31650.
- [44] B.J. Smith, M.C. Lawrence, P.M. Colmen, *Protein Eng.* 15 (2002) 365–371.
- [45] C.M. Horvath, R.A. Lamb, *J. Virol.* 66 (1992) 2443–2455.
- [46] L.W. McGinnes, T. Sergel, H. Chen, L. Hamo, S. Schwartz, T.G. Morrison, *Virology* 289 (2001) 343–352.
- [47] R. Buckland, E. Malvoisin, P. Beauverger, F. Wild, *J. Gen. Virol.* 73 (1992) 1703–1707.
- [48] C. Wild, J.W. Dubay, T. Greenwell, J.T. Baird, T.G. Oas, C. McDanal, E. Hunter, T. Mathews, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 12676–12680.
- [49] C. Wild, T. Oas, C. McDanal, D. Bolognesi, T. Mathews, *Proc. Natl. Acad. Sci. U. S. A.* 89 (1992) 10537–10541.
- [50] C.T. Wild, D.C. Shugars, T.K. Greenwell, C.B. McDanal, T.J. Mathews, *Proc. Natl. Acad. Sci. U. S. A.* 91 (1994) 9770–9774.
- [51] T.F. Wild, R. Buckland, *J. Gen. Virol.* 78 (1997) 107–111.
- [52] J.K. Young, R.P. Hicks, G.E. Wright, T.G. Morrison, *Virology* 238 (1997) 291–304.
- [53] D. Rapaport, M. Ovadia, Y. Shai, *EMBO* 14 (1995) 5524–5531.
- [54] S.B. Joshi, R.E. Dutch, R.A. Lamb, *Virology* 248 (1998) 20–34.
- [55] J.K. Young, D. Li, M.C. Abramowitz, T.G. Morrison, *J. Virol.* 73 (1999) 5945–5956.
- [56] K.S. Roman, E. Villar, I. Munoz-Barroso, *Int. J. Biochem. Cell Biol.* 34 (2002) 1207–1220.
- [57] X. Zhao, M. Singh, V.N. Malashkevich, P.S. Kim, *Proc. Natl. Acad. Sci. U. S. A.* 97 (2000) 14172–14177.
- [58] J.K. Ghosh, Y. Shai, *J. Biol. Chem.* 273 (1998) 7252–7259.
- [59] R.E. Dutch, G.P. Leser, R.A. Lamb, *Virology* 254 (1999) 147–159.
- [60] R.K. Plemper, R.W. Compans, *J. Virol.* 77 (2003) 4181–4190.
- [61] M. Moll, H.-D. Klenk, A. Maisner, *J. Virol.* 76 (2001) 7174–7186.
- [62] Q. Yao, R.W. Compans, *J. Virol.* 69 (1995) 7045–7053.
- [63] S. Bagai, R.A. Lamb, *J. Cell Biol.* 135 (1996) 73–84.
- [64] R.E. Dutch, R.A. Lamb, *J. Virol.* 75 (2001) 5363–5369.
- [65] C.J. Russell, T.S. Jardetzky, R.A. Lamb, *EMBO* 20 (2001) 4024–4034.
- [66] M. Tsurudome, M. Ito, M. Nishio, M. Kawano, H. Komada, Y. Ito, *J. Virol.* 75 (2001) 8999–9009.
- [67] L.D. Hernandez, R.J. Peters, S.E. Delos, J.A. Young, D.A. Agard, J.M. White, *J. Cell Biol.* 139 (1997) 1455–1464.
- [68] M. Hsu, A. Scheid, P. Choppin, *J. Biol. Chem.* 256 (1981) 3557–3563.
- [69] L.J. Calder, L. Gonzalez-Reyes, B. Garcia-Barreno, S.A. Wharton, J.J. Skehel, D.C. Wiley, J.A. Melero, *Virology* 271 (2000) 122–131.
- [70] A. Moscona, R. Peluso, *J. Virol.* 66 (1992) 6280–6287.
- [71] A. Moscona, R.W. Peluso, *J. Virol.* 65 (1991) 2773–2777.
- [72] A. Moscona, R.W. Peluso, *J. Virol.* 67 (1993) 6463–6468.
- [73] H. Connaris, T. Takimoto, R. Russell, S. Crennell, I. Moustafa, A. Portner, G. Taylor, *J. Virol.* 76 (2002) 1816–1824.
- [74] R.M. Iorio, G.M. Field, J.M. Sauvron, A.M. Mirza, R. Deng, P.J. Mahon, J.P. Langedijk, *J. Virol.* 75 (2000) 1918–1927.
- [75] J.A. Feldman, S. Audet, J.A. Beeler, *J. Virol.* 74 (2000) 6442–6447.
- [76] L.K. Hallak, D. Spillmann, M.E. Peeples, *J. Virol.* 74 (2000) 10508–10513.
- [77] S. Techaarpornkul, P.L. Collins, M.E. Peeples, *Virology* 294 (2002) 296–304.
- [78] T. Sergel, L.W. McGinnes, M.E. Peeples, T.G. Morrison, *Virology* 193 (1993) 717–726.
- [79] T. Sergel, L.W. McGinnes, T.G. Morrison, *Virology* 196 (1993) 831–834.
- [80] J. Stone-Hulslander, T. Morrison, *J. Virol.* 73 (1999) 3630–3637.
- [81] X. Hu, R. Ray, R.W. Compans, *J. Virol.* 66 (1992) 1528–1534.
- [82] R. Deng, Z. Wang, P. Mahan, M. Marinello, A. Mirza, R.M. Iorio, *Virology* 253 (1999) 43–54.
- [83] J. Stone-Hulslander, T.G. Morrison, *J. Virol.* 71 (1997) 6287–6295.
- [84] Q. Yao, X. Hu, R. Compans, *J. Virol.* 71 (1997) 650–656.
- [85] E. Malvoisin, T.F. Wild, *J. Gen. Virol.* 74 (1993) 2365–2372.
- [86] R. Deng, Z. Wang, A. Mirza, R. Iorio, *Virology* 209 (1995) 457–469.
- [87] M. Tsurudome, M. Kawano, T. Yuasa, N. Tabata, M. Nishio, H. Komada, Y. Ito, *Virology* 213 (1995) 190–203.
- [88] K. Tanabayashi, R.W. Compans, *J. Virol.* 70 (1996) 6112–6118.
- [89] Y. Tanaka, B.R. Heminway, M.S. Galinski, *J. Virol.* 70 (1996) 5005–5015.
- [90] S. Tong, R. Compans, *J. Gen. Virol.* 80 (1999) 107–115.
- [91] D.M. Eckert, P.S. Kim, *Ann. Rev. Biochem.* 70 (2001) 777–810.
- [92] T. Takimoto, G.L. Taylor, H.C. Connaris, S.J. Crennell, A. Portner, *J. Virol.* 76 (2002) 13028–13033.
- [93] M. Tsurudome, M. Ito, M. Nishio, M. Kawano, K. Okamoto, S. Kusagawa, H. Komada, Y. Ito, *J. Gen. Virol.* 79 (1998) 279–289.
- [94] R.K. Plemper, A.L. Hammond, R. Cattaneo, *J. Biol. Chem.* 47 (2001) 44239–44246.
- [95] S.G. Peisajovich, Y. Shai, *TIBS* 27 (2002) 183–190.
- [96] S. Tong, R.W. Compans, *Virology* 270 (2000) 368–376.
- [97] R.E. Dutch, S.B. Joshi, R.A. Lamb, *J. Virol.* 72 (1998) 7745–7753.
- [98] T. Danieli, S.L. Pelletier, Y.I. Henis, J.M. White, *J. Cell Biol.* 133 (1996) 559–569.
- [99] K. Bundo-Morita, S. Gibson, J. Lenard, *Biochemistry* 26 (1987) 6223–6227.