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Paramyxoviruses:

Different receptors - different mechanisms of fusion

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Abstract

Paramyxovirus-mediated membrane fusion usually requires an interaction between the viral-attachment and -fusion proteins. The mechanism by which this interaction regulates fusion differs between paramyxoviruses that bind to sialic acid-containing receptors and those that recognize specific proteins. The recently solved structure of the globular head of the measles virus hemagglutinin suggests that this difference might be related to the location of the receptor-binding sites on the attachment proteins of the two classes of paramyxoviruses.

Paramyxoviruses use different receptors

The Paramyxoviruses are enveloped, negative-stranded RNA viruses, each of which contains two surface glycoproteins, an attachment protein and a fusion (F) protein [1]. The most common type of paramyxovirus attachment protein, called the hemagglutinin-neuraminidase (HN), found on viruses, such as Newcastle disease virus (NDV), human parainfluenza virus 3 (hPIV3) and parainfluenza virus 5 (PIV5) (known formerly as simian virus 5), recognizes the ubiquitous sugar, sialic acid, on cell-surface moieties and has the ability to cleave the same moiety by virtue of its neuraminidase (NA) activity [1]. The structures of the globular heads of all of the above HN proteins [2–4] reveal a conserved β -sheet propeller motif, as identified originally in the influenza virus NA protein [5], but with a sialic acid-binding site on each monomer of the homotetramer that can mediate both receptor binding and NA (hereafter referred to as the NA site). A second binding site was identified subsequently in NDV HN [6] and has been postulated in hPIV3 HN [7]. Unlike HN, the hemagglutinin (H) of measles virus (MV) lacks NA and recognizes specific proteins. Although both wild-type and vaccine strains recognize signal lymphocyte-activating molecule (SLAM), vaccine strains also use CD46 as a receptor [8–11]. The recent publication by two groups of the structure of the globular domain of the MV H protein [12,13] provides our first look at a paramyxovirus attachment protein that binds to a specific protein receptor, revealing differences from the HN structures that might be related to the differences in the mechanisms by which these viruses mediate fusion.

Location of receptor-binding sites on paramyxovirus attachment proteins

The NA site in the various HN proteins is highly conserved and located in a pocket a short distance from the dimer interface (Figure 1a). The second sialic acid-binding site in NDV HN spans the membrane-distal end of the dimer interface and is composed of residues from both

monomers [6]. Both binding sites are on the top of the globular domain, oriented toward the target membrane. In MV H, both receptor-binding sites have a more lateral orientation and have been inactivated [13]. The NA site is inactivated by mutations, whereas the second site is shielded by an *N*-linked glycan. Two new distinct sites that bind CD46 or SLAM [14-16] are situated much farther from the dimer interface, although still at the top of the molecule [13] (Figure 1b). The retention of the β -sheet propeller motif characteristic of NA proteins (despite the absence of NA activity), the inactivation of the sialic acid-binding sites, the reorientation of the monomers in the dimer and the introduction of new receptor-binding sites raise the possibility that MV H might have evolved from a HN-like molecule. This is consistent with the morbilliviruses being found only in higher animals, whereas sialic acid-binding viruses are more widespread. Although the β -sheet propeller motif is retained in MV H, it is apparently no longer involved directly in receptor binding or fusion-helper function, although an indirect role in either of these functions cannot be ruled out. This is consistent with the use of four or five intramonomeric disulfide bonds to stabilize the β -sheet motif in the NA site of the HN monomers [2-4,6] but only two being present in MV H [12,13].

Glycoprotein interactions in paramyxovirus fusion

The promotion of fusion by most paramyxoviruses is dependent on an interaction between the two surface glycoproteins. In retrospect, this is not surprising because the attachment and fusion functions reside on different protein-spike structures in these viruses. If, as commonly thought, receptor binding is the trigger for fusion, there must be a mechanism to link the two events physically.

Through the analysis of the F protein-specificity of chimeras composed of regions from the HN proteins of different viruses, we and others have shown that F specificity is determined by the stalk region of HN [17-19]. More recently, we have shown that the decrease in fusion-promoting activity exhibited by NDV HN carrying mutations in a short region in the stalk is directly proportional to the extent of the HN-F interaction at the cell surface as measured by co-immunoprecipitation [20]. In addition to suggesting strongly that the F-interactive region on NDV HN is located in the stalk, this is consistent with a mechanism of fusion by sialic acid-binding viruses in which the extent of fusion is determined by the formation of the HN-F complex, which might be triggered at the cell surface by HN binding to a receptor. In other words, the extent of fusion is directly proportional to the strength of the HN-F interaction, suggesting that it is the association of HN and F that regulates fusion by viruses that bind sialic acid-containing receptors.

The altered locations of the receptor-binding sites of MV H relative to those on the HN proteins correlate with recently observed differences in the mechanisms by which the glycoprotein interactions in these two classes of paramyxoviruses regulate fusion. Plemper *et al.* [21] were the first to show that the extent of MV-induced fusion correlates inversely with the strength of the H-F interaction. Recently, we have shown that mutations in the stalk of MV H, corresponding to those cited earlier in NDV HN [20], also decrease fusion but have the opposite effect on the amount of the H-F complex detectable at the cell surface [22]. These fusion-deficient H proteins interact significantly more efficiently with the F protein than the wild-type H protein interacts with F. Thus, the extent of MV-induced fusion is inversely related to the strength of the H-F interaction. These results are consistent with MV fusion being regulated by the dissociation of a H-F complex at the cell surface that was formed inside the cell. Moreover, it seems likely that the binding of MV H to a receptor is the trigger that dissociates the complex, inducing fusion.

This proposed difference in the mechanism by which HN and MV H regulate fusion is consistent with additional evidence garnered using an endoplasmic reticulum co-retention

approach. These studies detect an intracellular complex between MV H and F [23], whereas the same approach does not detect complexes between hPIV3 HN and F or PIV5 HN and F [24].

The change in orientation of the monomers in the dimeric structure of MV H (relative to NDV HN), along with the formation of two novel protein receptor-binding sites elsewhere on the molecule, is consistent with the H molecule transmitting a somewhat different message to F when it binds receptors. The integrity of the dimer interface in HN is important to the promotion of fusion in NDV [25,26]. This might be related to the proximity of the two sialic acid-binding sites to the interface; indeed, the second site spans the dimer interface [6]. It seems plausible that the signal from the sialic acid-binding sites in the globular domain of HN to the F-interactive site in the stalk passes through the dimer interface. Because the MV H lost the NA site and developed the CD46- and SLAM-binding sites farther away from the interface, it might also have evolved a different way to interact with F to regulate its fusion-promoting activity that might not involve the dimer interface. This might be related to the relative abundance of sialic acid compared with CD46 and SLAM, as suggested originally by Plemper *et al.* [23].

An inverse relationship between the strength of the attachment-protein-fusion-protein interaction and the extent of fusion has also been demonstrated for two recently emerged paramyxoviruses, Nipah virus and Hendra virus. Like MV H, the attachment proteins (G) of these viruses also lack NA and recognize specific proteins as receptors, ephrinB2 and ephrinB3 [27-29]. Similar to our findings with MV H [22], mutations in Nipah and Hendra G, which decrease fusion, increase the avidity of the G-F interaction [30,31]. However, although the reciprocal relationship has been demonstrated for Nipah virus [30], no mutation has been identified in MV H that increases fusion and decreases the H-F interaction. As the structures of Nipah and Hendra G become available, it will be interesting to compare the location of the receptor-binding site(s) in these proteins to those of MV H and the HN proteins. Indeed, a model for the structure of Nipah virus G [32] proposes that the receptor-binding site co-localizes with the SLAM-binding site on MV H.

Concluding remarks

It is apparent that paramyxovirus attachment proteins that have evolved binding sites for specific protein receptors have also evolved a different mechanism to regulate fusion. No mutation in MV H has been identified that decreases the interaction with the homologous F protein. Instead, all H mutations that decrease fusion act by stabilizing the H-F complex. We have no evidence as to the location of the F-interactive site on MV H. Future work will focus on understanding how the MV H and F proteins interact and how the modulation of the interaction by receptor binding triggers fusion. Given the demonstrated differences between viruses that bind to sialic acid and those that bind to specific protein receptors, it seems highly likely that the signal might trigger fusion by a different mechanism. It will also be interesting to learn whether differences exist in the mechanism of fusion promotion, depending on which protein receptor, CD46 or SLAM, is used by MV H.

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References

1. Lamb, RA.; Kolakofsky, D. *Paramyxoviridae*: the viruses and their replication. In: Knipe, DM.; Howley, PM., editors. *Fundamental Virology*. 4th edn. Lippincott Williams & Wilkins; 2001. p. 689-724.

2. Crennell S, et al. Crystal structure of the multifunctional paramyxovirus hemagglutinin-neuraminidase. *Nat. Struct. Biol* 2000;7:1068–1074. [PubMed: 11062565]
3. Lawrence MC, et al. Structure of the haemagglutinin-neuraminidase from human parainfluenza virus type III. *J. Mol. Biol* 2004;335:1343–1357. [PubMed: 14729348]
4. Yuan P, et al. Structural studies of the parainfluenza virus 5 hemagglutinin-neuraminidase tetramer in complex with its receptor, sialyllactose. *Structure* 2005;13:803–815. [PubMed: 15893670]
5. Varghese JN, et al. Structure of the influenza virus glycoprotein antigen neuraminidase at 2 Å resolution. *Nature* 1983;303:35–40. [PubMed: 6843658]
6. Zaitsev V, et al. Second sialic acid binding site in Newcastle disease virus hemagglutinin-neuraminidase: implications for fusion. *J. Virol* 2004;78:3733–3741. [PubMed: 15016893]
7. Porotto M, et al. A second receptor binding site on human parainfluenza type 3 hemagglutinin-neuraminidase contributes to activation of the fusion mechanism. *J. Virol* 2007;81:3216–3228. [PubMed: 17229690]
8. Dorig RE, et al. The human CD46 molecule is a receptor for MV (Edmonston strain). *Cell* 1993;75:295–305. [PubMed: 8402913]
9. Naniche D, et al. Human membrane cofactor protein (CD46) acts as a cellular receptor for measles virus. *J. Virol* 1993;67:6025–6032. [PubMed: 8371352]
10. Hsu EC, et al. CDw150 (SLAM) is a receptor for a lymphotropic strain of MV and may account for the immunosuppressive properties of this virus. *Virology* 2001;279:9–21. [PubMed: 11145884]
11. Tatsuo H, et al. SLAM(CD150) is a cellular receptor for measles virus. *Nature* 2000;406:893–897. [PubMed: 10972291]
12. Colf LA, et al. Structure of the measles virus hemagglutinin. *Nat. Struct. Mol. Biol* 2007;14:1227–1228. [PubMed: 18026116]
13. Hashiguchi T, et al. Crystal structure of measles virus hemagglutinin provides insight into effective vaccines. *Proc. Natl. Acad. Sci. U. S. A* 2007;104:19535–19540. [PubMed: 18003910]
14. Masse N, et al. Measles virus (MV) hemagglutinin: evidence that attachment sites for MV receptors SLAM and CD46 overlap on the globular head. *J. Virol* 2004;78:9051–9063. [PubMed: 15308701]
15. Vongpunsawad S, et al. Selectively receptor-blind measles viruses: identification of residues necessary for SLAM- or CD46-induced fusion and their localization on a new hemagglutinin structural model. *J. Virol* 2004;78:302–313. [PubMed: 14671112]
16. Tahara M, et al. Multiple amino acid substitutions in hemagglutinin are necessary for wild-type measles virus to acquire ability to use receptor CD46 efficiently. *J. Virol* 2007;81:2564–2572. [PubMed: 17182683]
17. Deng R, et al. Localization of a domain on the paramyxovirus attachment protein required for the promotion of cellular fusion by its homologous fusion protein spike. *Virology* 1995;209:457–469. [PubMed: 7778280]
18. Tanabayashi K, Compans RW. Functional interaction of paramyxovirus glycoproteins: identification of a domain in Sendai virus HN which promotes cell fusion. *J. Virol* 1996;70:6112–6118. [PubMed: 8709235]
19. Tsurudome M, et al. Identification of regions on the hemagglutinin-neuraminidase protein of human parainfluenza virus type 2 important for promoting cell fusion. *Virology* 1995;213:190–203. [PubMed: 7483263]
20. 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;78:13053–13061. [PubMed: 15542657]
21. Plemper RK, et al. Strength of envelope protein interaction modulates cytopathogenicity of measles virus. *J. Virol* 2002;76:5051–5061. [PubMed: 11967321]
22. 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;81:9900–9910. [PubMed: 17626104]
23. Plemper RK, et al. Measles virus envelope glycoproteins hetero-oligomerize in the endoplasmic reticulum. *J. Biol. Chem* 2001;276:44239–44246. [PubMed: 11535597]

24. Paterson RG, et al. Paramyxovirus fusion (F) protein and hemagglutinin-neuraminidase (HN) protein interactions: Intracellular retention of F and HN does not affect transport of the homotypic HN or F protein. *Virology* 1997;237:1–9. [PubMed: 9344902]
25. Takimoto T, et al. Role of the hemagglutinin-neuraminidase protein in the mechanism of paramyxovirus-cell membrane fusion. *J. Virol* 2002;76:13028–13033. [PubMed: 12438628]
26. Corey EA, et al. Fusion deficiency induced by mutations at the dimer interface in the Newcastle disease virus hemagglutinin-neuraminidase is due to a temperature-dependent defect in receptor binding. *J. Virol* 2003;77:6913–6922. [PubMed: 12768010]
27. Bonaparte MI, et al. Ephrin-B2 ligand is a functional receptor for hendra virus and Nipah virus. *Proc. Natl. Acad. Sci. U. S. A* 2005;102:10652–10657. [PubMed: 15998730]
28. Negrete OA, et al. EphrinB2 is the entry receptor for Nipah virus, an emergent deadly paramyxovirus. *Nature* 2005;436:401–405. [PubMed: 16007075]
29. Negrete OA, et al. Two key residues in ephrinB3 are critical for its use as an alternative receptor for Nipah virus. *PLoS Pathog* 2006;2:e7. [PubMed: 16477309]
30. Aguilar HC, et al. N-glycans on Nipah virus fusion protein protect against neutralization but reduce membrane fusion and viral entry. *J. Virol* 2006;80:4878–4889. [PubMed: 16641279]
31. Bishop KA, et al. Identification of Hendra virus G glycoprotein residues that are critical for receptor binding. *J. Virol* 2007;81:5893–5901. [PubMed: 17376907]
32. Guillaume V, et al. Evidence of a potential receptor-binding site on the Nipah virus G protein (NiV-G): Identification of globular head residues with a role in fusion promotion and their localization on an NiV-G structural model. *J. Virol* 2006;80:7546–7554. [PubMed: 16840334]

Figure 1.

Receptor-binding sites on the globular domains of NDV HN and MV H. The outlines of the top views of the dimeric forms of (a) NDV HN and (b) MV H (looking down at the molecule from the target membrane) are shown. (a) Made in RASMOL from coordinates published by Zaitsev *et al.* [6]. (b) Made from the dimer published by Hashiguchi *et al.* [13]. Labeled ovals represent the locations of the two sialic acid-binding sites in (a) NDV HN and the CD46- and SLAM-binding sites in (b) MV H.