

nism of F protein activation, particularly in the absence of an attachment protein. HMPV F protein has also been shown to mediate cell-cell fusion (34) and virus-cell fusion (3) in the absence of the attachment G protein. Interestingly, syncytium formation by the HMPV F protein expressed alone in transfected cells was dependent on low pH (34). It is therefore possible that members of the *Pneumovirinae* subfamily have evolved distinct mechanisms of F protein activation compared to those of the *Paramyxovirinae* subfamily of paramyxoviruses.

ACKNOWLEDGMENTS

This work was supported by grant SAF2006-07805, awarded to J.A.M., from the Ministerio de Educación y Ciencia. This laboratory participates in the "Virus-host" framework funded by the Comunidad de Madrid and in CIBER de Enfermedades Respiratorias, an initiative of ISCIII.

We thank L. Roux (Geneva, Switzerland) for the pGEM-F and pGEM-HN plasmids, K.-K. Conzelmann (Munich, Germany) for the BSR-T7/5 cells, and S. Wharton and J. J. Skehel (London, United Kingdom) for the GB5 monoclonal antibody. We are also grateful for advice and comments from Teresa Corral, Paulino Gómez-Puertas, and Concepción Palomo.

REFERENCES

1. Bagai, S., A. Puri, R. Blumenthal, and D. P. Sarkar. 1993. Hemagglutinin-neuraminidase enhances F protein-mediated membrane fusion of reconstituted Sendai virus envelope with cells. *J. Virol.* **67**:3312–3318.
2. Baker, K. A., R. E. Dutch, R. A. Lamb, and T. S. Jardetzky. 1999. Structural basis for paramyxovirus-mediated membrane fusion. *Mol. Cell* **3**:309–319.
3. Biacchesi, S., M. H. Skiadopoulos, L. Yang, E. W. Lamirande, K. C. Tran, B. R. Murphy, P. L. Collins, and U. J. Buchholz. 2004. Recombinant human metapneumovirus lacking the small hydrophobic SH and/or attachment G glycoprotein: deletion of G yields a promising vaccine candidate. *J. Virol.* **78**:12877–12887.
4. Buchholz, U. J., S. Finke, and K.-K. Conzelmann. 1999. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J. Virol.* **73**:251–259.
5. Collins, P. L., and J. E. Crowe. 2007. Respiratory syncytial virus and metapneumovirus, p. 1601–1646. *In* D. M. Knipe, P. M. Howley, D. E. Griffin, R. A. Lamb, M. A. Martin, B. Roizman, and S. E. Straus (ed.), *Fields virology*, 5th ed. Lippincott Williams and Wilkins, Philadelphia, PA.
6. Connolly, S. A., and R. A. Lamb. 2007. Paramyxovirus fusion: real-time measurement of parainfluenza virus 5 virus-cell fusion. *Virology* **355**:203–212.
7. Crim, R. L., S. A. Audet, S. A. Feldman, H. S. Mostowski, and J. A. Beeler. 2007. Identification of linear heparin-binding peptides derived from human respiratory syncytial virus fusion glycoprotein that inhibit infectivity. *J. Virol.* **81**:261–271.
8. Deng, R., Z. Wang, P. J. Mahon, M. Marinello, A. Mirza, and R. M. Iorio. 1999. Mutations in the Newcastle disease virus hemagglutinin-neuraminidase protein that interfere with its ability to interact with the homologous F protein in the promotion of fusion. *Virology* **253**:43–54.
9. Elroy-Stein, O., T. R. Fuerst, and B. Moss. 1989. Cap-independent translation of mRNA conferred by encephalomyocarditis virus 5' sequence improves the performance of the vaccinia virus/bacteriophage T7 hybrid expression system. *Proc. Natl. Acad. Sci. USA* **86**:6126–6130.
10. Feldman, S. A., S. Audet, and J. A. Beeler. 2000. The fusion glycoprotein of human respiratory syncytial virus facilitates virus attachment and infectivity via an interaction with cellular heparan sulphate. *J. Virol.* **74**:6442–6447.
11. García-Barreno, B., C. Palomo, C. Peñas, T. Delgado, P. Perez-Breña, and J. A. Melero. 1989. Marked differences in the antigenic structure of human respiratory syncytial virus F and G glycoproteins. *J. Virol.* **63**:925–932.
12. González-Reyes, L., M. B. Ruiz-Argüello, B. García-Barreno, L. Calder, J. A. López, J. P. Albar, J. J. Skehel, D. C. Wiley, and J. A. Melero. 2001. Cleavage of the human respiratory syncytial virus fusion protein at two distinct sites is required for activation of membrane fusion. *Proc. Natl. Acad. Sci. USA* **98**:9859–9864.
13. Hemingway, B. R., Y. Yang, Y. Tanaka, M. Panin, Y. T. Huang, and M. S. Galinski. 1995. Role of basic residues in the proteolytic activation of Sendai virus fusion glycoprotein. *Virus Res.* **36**:15–35.
14. Ito, M., M. Nishio, H. Komada, Y. Ito, and M. Tsurudome. 2000. An amino acid in the heptad repeat domain 1 is important for the hemagglutinin-neuraminidase-independent fusing activity of simian virus 5 fusion protein. *J. Gen. Virol.* **81**:719–727.
15. Karron, R. A., D. A. Buonagurio, A. F. Georgiu, S. S. Whitehead, J. E. Adamus, M. L. Clements-Mann, D. O. Harris, V. B. Randolph, S. A. Udem, B. R. Murphy, and M. S. Sidhu. 1997. 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. USA* **94**:13961–13966.
16. Kido, H., M. Murakami, K. Oba, Y. Chen, and T. Towatari. 1999. Cellular proteinases trigger the infectivity of the influenza A and Sendai virus. *Mol. Cell* **9**:235–244.
17. Klenk, H.-D., and W. Garten. 1994. Host cell proteases controlling virus pathogenicity. *Trends Microbiol.* **2**:39–43.
18. König, P., K. Giesow, K. Schuldt, U. J. Buchholz, and G. M. Keil. 2004. A novel protein expression strategy using recombinant bovine respiratory syncytial virus (BRSV): modifications of the peptide sequence between the two furin cleavage sites of the BRSV fusion protein yield secreted proteins, but affect processing and function of the BRSV fusion protein. *J. Gen. Virol.* **85**:1815–1824.
19. Lamb, R. A. 1993. Paramyxovirus fusion: a hypothesis for changes. *Virology* **197**:1–11.
20. Lamb, R. A., R. G. Paterson, and T. S. Jardetzky. 2006. Paramyxovirus membrane fusion: lessons from the F and HN atomic structures. *Virology* **344**:30–37.
21. Lamb, R. A., and T. S. Jardetzky. 2007. Structural basis of virus invasion: lessons from paramyxovirus F. *Curr. Opin. Struct. Biol.* **17**:427–436.
22. Lavillette, D., M. Maurice, C. Roche, S. J. Russell, M. Sitbon, and F.-L. Cosset. 1998. A proline-rich motif downstream of the receptor binding domain modulates conformation and fusogenicity of murine retroviral envelopes. *J. Virol.* **72**:9955–9965.
23. Leyrer, S., M. Bitzer, U. Lauer, J. Kramer, W. J. Neubert, and R. Sedlmeier. 1998. Sendai virus-like particles devoid of haemagglutinin-neuraminidase protein infect cells via the human asialoglycoprotein receptor. *J. Gen. Virol.* **79**:683–687.
24. McGuinness, L. W., and T. G. Morrison. 2006. Inhibition of receptor binding stabilizes Newcastle disease virus HN and F protein-containing complexes. *J. Virol.* **80**:2894–2903.
25. Melero, J. A. 2007. Molecular biology of human respiratory syncytial virus, p. 1–42. *In* P. A. Cane (ed.), *Respiratory syncytial virus. Perspectives in medical virology*, vol. 14. Elsevier, Amsterdam, The Netherlands.
26. Melikyan, G. B., R. M. Markosyan, H. Hemmati, M. K. Delmedico, D. M. Lambert, and F. S. Cohen. 2000. Evidence that the transition of HIV-1 gp41 into a six helix bundle, not the bundle configuration, induces membrane fusion. *J. Cell Biol.* **151**:413–423.
27. Paterson, R. G., C. J. Russell, and R. A. Lamb. 2000. Fusion protein of the paramyxovirus SV5: destabilizing and stabilizing mutants of fusion activation. *Virology* **270**:17–30.
28. Ruiz-Argüello, M. B., L. González-Reyes, L. J. Calder, C. Palomo, D. Martin, M. J. Saiz, B. García-Barreno, J. J. Skehel, and J. A. Melero. 2002. Effect of proteolytic processing at two distinct sites on shape and aggregation of an anchorless fusion protein of human respiratory syncytial virus and fate of the intervening segment. *Virology* **298**:317–326.
29. Ruiz-Argüello, M. B., D. Martin, S. A. Wharton, L. J. Calder, S. R. Martin, O. Cano, M. Calero, B. García-Barreno, J. J. Skehel, and J. A. Melero. 2004. Thermostability of the human respiratory syncytial virus fusion protein before and after activation: implications for the membrane-fusion mechanism. *J. Gen. Virol.* **85**:3677–3687.
30. Russell, C. J., T. S. Jardetzky, and R. A. Lamb. 2001. Membrane fusion machines of paramyxoviruses: capture of intermediates of fusion. *EMBO J.* **20**:4024–4034.
31. Russell, C. J., K. L. Kantor, T. S. Jardetzky, and R. A. Lamb. 2003. A dual-functional paramyxovirus F protein regulatory switch segment: activation and membrane fusion. *J. Cell Biol.* **163**:363–374.
32. Scheid, A., and P. W. Choppin. 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity of proteolytic cleavage of an inactive precursor protein of Sendai virus. *Virology* **57**:475–490.
33. Schmidt, U., J. Beyer, U. Polster, L. J. Gershwin, and U. J. Buchholz. 2002. Mucosal immunization with live recombinant bovine respiratory syncytial virus (BRSV) and recombinant BRSV lacking the envelope glycoprotein G protects against challenge with wild-type BRSV. *J. Virol.* **76**:12355–12359.
34. Schwalter, R. M., S. E. Smith, and R. E. Dutch. 2006. Characterization of human metapneumovirus F protein-promoted membrane fusion: critical roles for proteolytic processing and low pH. *J. Virol.* **80**:10931–10941.
35. Segawa, H., T. Yamashita, M. Kawakita, and H. Taira. 2000. Functional analysis of the individual oligosaccharide chains of Sendai virus fusion protein. *J. Biochem.* **128**:65–72.
36. Stone-Hulslander, J., and T. G. Morrison. 1997. Detection of an interaction between the HN and F proteins in Newcastle disease virus-infected cells. *J. Virol.* **71**:6287–6295.
37. Tanabayashi, K., and R. W. Compans. 1996. Functional interaction of paramyxovirus glycoproteins: identification of a domain in Sendai virus HN which promotes cell fusion. *J. Virol.* **70**:6112–6118.
38. Techaarpornkul, S., N. Barretto, and M. E. Peeples. 2001. Functional anal-