The Nipah Virus Fusion Protein Is Cleaved within the Endosomal Compartment*

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Nipah virus (NiV), a highly pathogenic paramyxovirus, was isolated in 1999 after an outbreak of fatal encephalitis among pig farmers in Malaysia and Singapore (1). In 1998, the virus was transmitted from bats to pigs and then to humans, suggesting that the pig was required as an amplifying host. However, in more recent outbreaks in Bangladesh, NiV was also transmitted directly from bats to humans, and even human-to-human transmission cannot be ruled out (3, 4). Because of the broad host range and the high mortality rates associated with infection, NiV has been classified as a biosafety level 4 agent. Because of their unique genetic and biological characteristics NiV and the closely related Hendra virus form a new genus, Henipavirus, within the Paramyxoviridae family (5, 6).

NiV encodes for two surface glycoproteins, the receptor-binding G protein and the fusion (F) protein. The G protein mediates attachment to a receptor on host cells that has not yet been identified. The F protein is responsible for virus entry into host cells by initiating pH-independent fusion of the viral and cellular membranes. If coexpressed together with the G protein on the surface of infected cells or on cells expressing plasmid-encoded NiV glycoproteins, the F protein mediates fusion with adjacent cells, resulting in multinucleated syncytia (7, 8). It is known that cleavage of paramyxoviral F proteins at basic amino acid residues is a prerequisite for fusion activity and, thus, for virus infectivity (9, 10). The F proteins are synthesized as inactive precursors (F₀) and have to be activated by proteolytic cleavage into the disulfide-linked subunits F₁ and F₂, thereby releasing the hydrophobic fusion peptide at the amino terminus of F₁ (Fig. 1). So far, two mechanisms of proteolytic F activation are known. Paramyxoviruses that cause systemic infections in vivo generally possess F proteins with multiple basic amino acids at the cleavage site. These F proteins are ubiquitously activated during the transport through the secretory pathway by the Golgi protease furin. Because cleavage occurs intracellularly, F proteins with multiple basic cleavage sites reach the cell surface as fusion-active molecules. In contrast, F proteins with only one basic residue at the cleavage site usually cannot be activated intracellularly but have to be activated by extracellular trypsin-like proteases after they have reached the cell surface. As a consequence, the in vitro growth of paramyxoviruses encoding F proteins with monobasic cleavage sites depends on the addition of trypsin to the cell culture medium. In vivo, replication of these viruses is restricted to the respiratory tract where suitable trypsin-like proteases are known to be expressed (11, 12). Although the NiV F protein has a single arginine at the cleavage site (Fig. 1), NiV infection is not restricted to the respiratory tract and does not require exogenous trypsin for its growth in cell culture (5, 7, 13). This suggests that activation of the NiV F protein differs from what is known for other paramyxoviral and orthomyxoviral fusion proteins. Supporting this view, we have shown recently that the NiV F protein is cleaved in all cell lines tested. Furthermore, this ubiquitous activation does not require a basic residue at the cleavage site and is, therefore, not mediated by a member of the trypsin- or furin-like protease families known to activate other paramyxoviruses (8).

EXPERIMENTAL PROCEDURES

Cell Culture and Virus Infection—MDCK (Madin-Darby canine kidney) cells were maintained in Eagle’s minimal essential medium (Invitrogen) supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin, and 0.1 mg/ml streptomycin. Vero (African green monkey kidney) cells and HeLa cells were grown in Dulbecco’s modified minimal essential medium (DMEM) containing 10% FCS, penicillin, and streptomycin. To adapt Vero cells to serum-free conditions, the cells were cultivated in virus production serum-free medium (VP-SFM; Invitrogen). Infections with NiV were performed under biosafety level 4 conditions as described previously (8).

Plasmid Vectors—cDNA fragments spanning the F gene and the G gene of the NiV genome (GenBank accession number AF212302) were cloned into the pczCFG5 vector as described earlier (14). Generation of the endocytosis-negative mutant NiV F₄₅ (Fig. 1) has been described previously (15). The F gene of the measles virus (MV) Edmonston strain was cloned into the pCG vector (16).
Endosomal Cleavage of the Nipah Virus Fusion Protein

**Results**

Proteolytic Activation of the NiV F Protein Is Not Mediated by Soluble Serum Proteases—We have shown recently that the NiV F protein is ubiquitously cleaved in cell culture by a protease that cannot be a member of the trypsin- or furin-like protease families (8). To determine whether the ubiquitous activation is mediated by a host cell protease or by an exogenous serum protease, cleavage and fusion activity of the NiV F protein was analyzed in the absence of FCS. For this, Vero cells were cultivated for several weeks under serum-free conditions and either infected with NiV or cotransfected with the NiV F and G genes. Only if the F protein is proteolytically processed can fusogenic G/F1,2 complexes on the surface of infected or transfected cells form and mediate fusion with adjacent receptor-bearing cells (8, 13). To analyze cleavage of the NiV F protein under serum-free conditions, transfected cells were metabolically labeled with [35S]methionine and [35S]cysteine (Tfn-TR; Molecular Probes) was added during the incubation at 37°C for either 5 or 30 min. For lysosomal containing, an antibody against the lysosomal-associated membrane protein 1 (Southern Biotech) was added after fixation and permeabilization for 1 h at 4°C. Bound antibodies were detected with rhodamine-conjugated secondary antibodies. Confocal fluorescence images were recorded using a Zeiss Axiosplan 2 LSM 510.

**Metabolic Labeling**—Cultures of Vero or MDCK cells in 35-mm diameter dishes were transfected with plasmid DNA encoding NiV F or NiV Fα using the cationic lipid transfection reagent Lipofectamine 2000 (Invitrogen). At 24 h posttransfection, cells were cultured for 40 min with medium lacking cysteine and methionine followed by an incubation with medium containing [35S]methionine and [35S]cysteine (Promix; Amersham Biosciences) at a final concentration of 100 μCi/ml for 10 min. Subsequently, labeling medium was replaced with non-radioactive chase medium for 2 h at 37°C. After labeling, cells were lysed in 0.5 ml of radioimmunoprecipitation assay buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 10 mM EDTA, 20 mM Tris-HCl, pH 8.5; protease inhibitors) and centrifuged for 20 min at 100,000 × g. NiV F protein was immunoprecipitated from cell lysates with a polyclonal antiserum derived from infected guinea pigs (a kind gift from M. Czub and H. Weingartl) at a final dilution of 1:500. After addition of a suspension of protein A-Sepharose CL-4B (Sigma), immune complexes were washed three times with radioimmunoprecipitation assay buffer suspended, in reduced sample buffer for SDS-PAGE, and separated on an 12% polyacrylamide gel. Dried gels were exposed to Kodak BIOMAX films.

**Inhibitor Studies**—To block NiV F protein transport along the secretory pathway, cells were metabolically labeled in the absence or presence of monensin (10 μM; Sigma), brefeldin A (15 μg/ml; Sigma), and a mixture of 30 mM NaF and 50 μM AlCl3 (Sigma). Inhibition of clathrin-mediated endocytosis was examined in the presence of 50 μM chlorpromazine (Sigma).

**Surface Biotinylation Analysis**—Surface labeling with biotin was performed as described previously (17). Briefly, MDCK cells were transfected with plasmid DNA encoding either the wild type NiV F or mutant NiV Fα gene. At 24 h posttransfection, cells were washed and incubated twice for 20 min at 4°C with 2 mg/ml sulfo-N-hydroxysuccinimido-biotin (Calbiochem). After washing, cells were incubated for 10 min and then washed and shifted to 37°C for 5 or 30 min to allow endocytosis to occur. Surface-bound primary antibodies were blocked by incubation with a peroxidase-conjugated secondary antibody (dilution 1:5; DAKO). After fixation with 2% paraformaldehyde for 30 min and permeabilization with phosphate-buffered saline containing 0.2% Triton X-100, internalized primary antibodies were stained with a fluorescein isothiocyanate-conjugated secondary antibody. To analyze the colocalization of NiV F with transferrin, 50 μg/ml transferrin-tetramethylrhodamine (Tfn-TR; Molecular Probes) was added during the incubation at 37°C for either 5 or 30 min. For lysosomal containing, an antibody against the lysosomal-associated membrane protein 1 (Southern Biotech) was added after fixation and permeabilization for 1 h at 4°C. Bound antibodies were detected with rhodamine-conjugated secondary antibodies. Confocal fluorescence images were recorded using a Zeiss Axiosplan 2 LSM 510.
inhibitors of the secretory pathway. As a control, processing of the MV F protein was analyzed. In contrast to the NiV F protein, MV F has a multibasic cleavage site and is, therefore, activated by furin in the trans-Golgi network (TGN) (9, 10, 18). To prevent transport of the F proteins from the endoplasmic reticulum to the Golgi apparatus, F-expressing cells were pulse labeled and then chased for 2 h at 37 °C in the presence of brefeldin A (BFA) (19). To block the transport from the endoplasmic reticulum or the medial Golgi completely prevented cleavage of both MV F and NiV F, showing that NiV F activation does not occur before reaching the TGN. By using a mixture of NaF and AlCl₃, trafficking of secretory vesicles from the TGN to the cell surface was blocked (21). As expected, furin-mediated cleavage of the MV F protein in the TGN was not affected by this treatment. In contrast, cleavage of the NiV F protein could no longer be detected (Fig. 3B), showing that the NiV F protein is not activated during the transport along the secretory pathway but needs to reach the plasma membrane before it can be cleaved.

Endocytosis of the NiV F Protein Is Required for Proteolytic Cleavage—Very recently, we have shown that both NiV glycoproteins are internalized from the surface of infected and transfected cells. Whereas the G protein appeared to be internalized with the bulk flow during membrane turnover, constitutive endocytosis of the F protein was found to be signal mediated (15). To test whether F uptake is required for proteolytic activation, F cleavage was analyzed in the presence of chlorpromazine, an inhibitor of clathrin-mediated endocytosis (22). Fig. 3C shows that NiV F cleavage was completely prevented in the presence of 50 μM chlorpromazine whereas furin cleavage of the MV F protein was not affected. This clearly indicates that not only transport to the cell surface but also internalization via clathrin-coated pits is required for proteolytic activation of the NiV F protein.

To demonstrate the intracellular localization of the NiV F protein after endocytosis, an antibody uptake experiment with F-expressing HeLa cells was performed. At 24 h posttransfection, surface biotinylated and the F protein was immunoprecipitated from cell lysates and analyzed by autoradiography after separation on a 12% SDS gel under reducing conditions.

Endosomal Cleavage of the Nipah Virus Fusion Protein

![Image: Fig. 3. Proteolytic processing of NiV F and MV F in the presence of transport inhibitors. A, 24 h after transfection of MDCK cells with plasmids carrying either NiV F or measles virus (MV) F genes, cells were metabolically labeled with [35S]Promix for 10 min and incubated for 2 h in the absence (+) or presence (−) of either 15 μg/ml brefeldin A (BFA) or 10 μM monensin (A), 30 mM NaF and 50 μM AlCl₃ (B), or 50 μM chlorpromazine (C). F proteins were then immunoprecipitated from cell lysates and analyzed by autoradiography after separation on a 12% SDS gel under reducing conditions.

![Image: Fig. 4. Subcellular distribution of endocytosed NiV F protein. A and B, at 24 h posttransfection, an antibody uptake assay with NiV F-expressing HeLa cells was performed. Cells were incubated with a polyclonal anti-NiV serum for 1 h at 4 °C and then incubated at 37 °C in the presence of 50 μg/ml transferrin-tetramethylrhodamine (Tfn-TR) for either 5 (A) or 30 min (B). Following endocytosis, surface-bound primary anti-NiV F antibodies were blocked by incubation with a peroxidase-conjugated secondary antibody. After fixation and permeabilization, internalized anti-NiV F antibodies were stained with a fluorescein isothiocyanate-conjugated secondary antibody. C, after antibody uptake for 30 min and blocking of surface-bound anti-NiV F antibodies, cells were fixed and permeabilized. Endocytosed anti-NiV F antibodies were again detected by fluorescein isothiocyanate-conjugate secondary antibodies. Lysosomes were stained with an anti-lamp-1 antibody and a rhodamine-conjugated secondary antibody. Colocalization of the proteins was analyzed by confocal microscopy.

and separated by SDS-PAGE under reducing conditions. Surface-labeled F protein was detected by peroxidase-conjugated streptavidin and chemiluminescence. Fig. 5A shows that in the presence of NH₄Cl almost no cleaved F protein was expressed on the cell surface (NiV F + NH₄Cl). As a consequence, syncytia formation in NH₄Cl-treated cells coexpressing NiV G and F was barely detectable (data not shown). This result shows that not only endocytosis but also acidification of the endosome is required for NiV F activation.

A Decrease in the Extracellular pH Is Not Sufficient for Cleavage—As mentioned above, recent studies have shown that the NiV F protein possesses intrinsic internalization signals that ensure rapid endocytosis of the protein. The YSRL sequence in the F cytoplasmic domain (position 525–528) perfectly fits the YXXO-type endocytosis signal consensus motif (where Y is a tyrosine, X can be any amino acid, and O is a large hydrophobic amino acid). These motifs are recognized by the cytosolic adaptor complex 2, which selectively concentrates proteins within clathrin-coated vesicles (25). In addition, the NiV F cytoplasmic domain contains a YY motif, which further increases the YRSL motif-mediated endocytosis rate to 2%/min (15). By exchanging the three tyrosine residues in the F cytoplasmic domain against alanines (Fig. 1, FYA), we generated a mutant that was internalized with a rate of <0.1%/min (15). To confirm that endocytosis is required for F cleavage, proteolytic activation of the endocytosis-negative FYA mutant was analyzed. The surface biotinylation of FYA-expressing cells showed that the mutant protein was abundantly expressed on the cell surface but was not cleaved at a detectable level (Fig. 5A, NiV FYA). Consequently, FYA was barely able to induce cell-to-cell fusion (Fig. 5B, control). Cleavage deficiency of the endocytosis-negative mutant is likely due to the fact that the F protein no longer encounters the activating protease in the endosomal compartment. Nevertheless, it could not be ruled out that the protease is a cell surface resident protease that cannot cleave non-endocytosed F protein because the protein must pass through the endosomal-recycling pathway to undergo a low pH-dependent conformational change before it can be activated on the cell surface. To test whether an acidic step can restore the fusion activity, FYA-transfected cells were subjected to an external pH 5 shift (26). At 24 h posttransfection, medium adjusted to pH 5 was added for 10 min at 37 °C. Then the medium was again exchanged against normal culture medium (pH 7). When fusion was analyzed at 31 h posttransfection, cells expressing the NiV FYA protein together with the NiV G protein did not show increased syncytia formation (Fig. 5B, + pH 5 shift). This indicates that the deficient FYA cleavage on the cell surface is due to the lack of a low pH-induced conformational change but to the lack of active protease on the cell surface.

**DISCUSSION**

Class I fusion proteins, which are present in paramyxoviruses, orthomyxoviruses, filoviruses, and retroviruses, are proteolytically processed at basic cleavage sites either in the TGN by ubiquitous furin-like proteases or after arrival at the cell surface by exogenous trypsin-like proteases (9). In contrast to what is reported for other class I fusion proteins, activation of the NiV F protein is neither mediated during transport along the secretory pathway by a ubiquitous Golgi protease nor accomplished by an exogenous protease at the cell surface. Using intracellular transport inhibitors and the endocytosis-negative FYA mutant, we clearly demonstrated that activation of the NiV F protein occurs after internalization of the protein by clathrin-mediated endocytosis, showing for the first time that proteolytic activation of a paramyxoviral F protein can occur within the endosomal compartment. In addition to endocytosis, acidification of the endosome is required for NiV proteolytic activation. Fusion activity of the FYA mutant could not be restored by decreasing the extracellular pH, indicating that the activating protease is a low pH-dependent protease that is not present on the cell surface in an active form.

In full agreement with the finding that endocytosis is an essential prerequisite for proteolytic activation, the surprising observation that endocytosis-deficient F mutants showed a decreased fusion activity despite an increased surface expression (15) can now be conclusively explained by the defective proteolytic processing of the surface-expressed mutant F proteins. The idea that proteolytic activation of the NiV F protein is completely different from what is known for other paramyxoviruses was already suggested by our earlier findings that cleavage of the NiV F monobasic cleavage site occurs ubiquitously and does not even require a basic residue at the cleavage site (8). Thus, activation cannot be mediated by a trypsin- or furin-like enzyme such as those known to process other paramyxoviruses. We now know not only that activation of the NiV F protein is exceptional with respect to protease usage but also that the subcellular localization where proteolytic cleavage takes place is strikingly different. Similar to other F proteins with monobasic cleavage sites, the NiV F protein is not activated by the ubiquitous Golgi protease furin during transport and, thus, reaches the cell surface as fusion-inactive protein. However, whereas other paramyxoviral F proteins depend on exogenous trypsin-like proteases, making their activation on the cell surface restricted, the NiV F protein is ubiquitously internalized, activated within the endosomal compartment, and subsequently recycled to the cell surface as fusion-active protein. As recycled NiV F protein has not entered late endosomes or lysosomes, it must be concluded that activation occurs within early or recycling endosomes. The minor colocalization of endocytosed F protein with lamp-1 further supported the idea that only a small amount of the protein is transported to the late endosomal/lysosomal compartment. As proteins that are recycled to the cell surface and proteins that are targeted to lysosomes have similar sorting signals, it must be assumed that the relative position of the signal within the NiV F cytoplasmic tail mediates internalization and recycling rather than lysosomal targeting (27, 28).

Although endosomes were originally thought to only function
as a major sorting station of the endocytic pathway and receive endocytosed late endosomes from the cell surface for recycling or transport to late endosomes and lysosomes, it is now well accepted that endosomes also represent a biologically important processing compartment within cells (29, 30). Endosomal cathepsins, metalloproteases, and secretases mediate cleavage or limited proteolysis of a variety of cellular proteins, leading either to degradation or activation of the respective proteins (31–38). Furthermore, proteases of the endosomal compartment are known to be involved in the proteolytic processing of a few viral proteins such as the outer capsid proteins of reoviruses (39). However, the NiV F protein is the first viral surface protein reported to be endocytosed upon activation. This raises the interesting possibility that pharmacological drugs that block clathrin-mediated endocytosis might be potential tools for therapeutic treatment in the early phase of a NiV-induced encephalitis.

In agreement with our findings, Pager et al. (40) recently reported that cleavage of the Hendra virus F protein is also sensitive to increases in intracellular pH levels. The Hendra virus F protein also contains a YSRL endocytosis motif in its cytoplasmic tail (6), our results strongly suggest that the closely related Hendra virus F protein is also cleaved by a pH-dependent endosomal protease. Thus, activation in the endosomal compartment is likely to be a common property of the highly pathogenic henipaviruses, making these viruses unique not only within the Paramyxoviridae but also when compared with all enveloped viruses encoding for class I fusion proteins.

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REFERENCES
1. Chua, K. B., Goh, K. J., Wong, K. T., Kamarulzaman, A., Tan, P. S., Ksiazek, T. G., Zaki, S. R., Paul, G., Lam, S. K., and Tan, C. T. (1999) Lancet 354, 1257–1259
2. Chua, K. B., Roh, C. L., Hoei, P. S., Wee, K. F., Khong, J. H., Chua, B. H., Chan, Y. P., Lim, M. E., and Lam, S. K. (2002) Microbes Infect. 4, 145–151
3. Choi, C. (2004) Sci. Am. 291, 21A, 22
4. Hsu, V. P., Hossain, M. J., Parashar, U. D., Ali, M. M., Ksiazek, T. G., Kuzmin, I., Niezgoda, M., Rupprecht, C., Breese, J., and Breiman, R. F. (2004) Emerg. Infect. Dis. 10, 2082–2087
5. Harcourt, B. H., Tamin, A., Ksiazek, T. G., Rollin, P. E., Anderson, L. J., Bellini, W. J., and Rota, P. A. (2000) Virology 271, 334–349
6. Wang, F. F., Yu, M., Hansson, E., Pritchard, L. I., Shiel, B., Michalski, W. P., and Eaton, B. T. (2000) J. Virol. 74, 9972–9979
7. Tamin, A., Harcourt, B. H., Ksiazek, T. G., Rollin, P. E., Bellini, W. J., and Rota, P. A. (2002) Virology 296, 190–200
8. Moll, M., Diederich, S., Klenk, H. D., Crab, M., and Maisner, A. (2004) J. Virol. 78, 9705–9712
9. Klenk, H. D., and Garten, W. (1994) Cellular Receptors for Animal Viruses (Winner, E., ed) pp. 241–280, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
10. Maisner, A., Mrkic, B., Herrler, G., Moll, M., Biller, M. A., Cattaneo, R., and Klenk, H. D. (2000) J. Gen. Virol. 81, 441–449
11. Tashiro, M., Yokogoshi, Y., Tohiba, K., Seto, J. T., Rott, R., and Kido, H. (1992) J. Virol. 66, 7211–7216
12. Murakami, M., Towatari, T., Ohuchi, M., Shiota, M., Ako, M., Okumura, Y., Parry, M. A., and Kido, H. (2001) Eur. J. Biochem. 288, 2847–2855
13. Bossart, K. N., Wang, L. F., Flora, M. N., Chua, K. B., Lam, S. K., Eaton, B. T., and Broder, C. C. (2002) J. Virol. 76, 11186–11198
14. Moll, M., Kaufmann, A., and Maisner, A. (2004) J. Virol. 78, 7274–7278
15. Vogt, C., Eickmann, M., Diederich, S., Moll, M., and Maisner, A. (2005) J. Virol. 79, 3865–3872
16. Moll, M., Klenk, H. D., Herrler, G., and Maisner, A. (2001) J. Biol. Chem. 276, 17877–17894
17. Moll, M., Klenk, H. D., and Maisner, A. (2002) J. Virol. 76, 7174–7186
18. Watanabe, M., Hirano, A., Stengelin, S., Nelsen, J., Thomas, G., and Wang, T. C. (1995) J. Virol. 69, 3206–3210
19. Dems, R. W., Russ, G., and Yewdell, J. W. (1989) J. Cell Biol. 109, 61–72
20. Griffiths, G., Quinn, P., and Warren, G. (1983) J. Cell Biol. 96, 835–850
21. Barr, F. A., Leyte, A., Moliner, S., Pfeuffer, T., Tooze, S. A., and Huttner, W. B. (1991) FEBS Lett. 294, 239–243
22. Wang, L. H., Rothberg, R. G., and Anderson, R. G. (1993) J. Cell Biol. 123, 1107–1117
23. Marsh, E. W., Leopold, P. L., Jones, N. L., and Maxfield, F. R. (1995) J. Cell Biol. 129, 1509–1522
24. Oka, K., and Ikehara, Y. (1985) Eur. J. Biochem. 152, 605–609
25. Bonifacino, J. S., and Traub, L. M. (2003) Annu. Rev. Biochem. 72, 395–447
26. Huang, R. T., Rott, R., and Klenk, H. D. (1981) Virology 110, 243–247
27. Rohrer, J., Schweizer, A., Russell, D., and Kornfeld, S. (1996) J. Cell Biol. 132, 565–576
28. White, J. R., Lee, J. M., Young, P. R., Hertzberg, R. P., Jurewicz, A. J., Chaikin, M. A., Widlowski, K., Foley, J. J., Martin, L. D., Griswold, D. E., and Sarau, J. H. (1998) J. Virol. 72, 7274–7278
29. Authier, F., Posner, B. I., and Bergeron, J. J. (1996) FEBS Lett. 389, 55–60
30. Slagsvold, T., and Broder, C. C. (2002) J. Virol. 76, 11186–11198
31. Marsh, E. W., Leopold, P. L., Jones, N. L., and Maxfield, F. R. (1995) J. Cell Biol. 129, 1509–1522
32. Hooper, N. M., Karran, E., and Turner, A. J. (1997) Biochem. J. 321, Pt. 2, 285–297
33. Chapman, H. A. (1998) Curr. Opin. Immunol. 10, 93–102
34. Hutlas, I., and Reudelhuber, T. L. (1999) FEBS Lett. 443, 48–52
35. Schlondorff, J., and Broder, C. C. (1999) J. Cell Sci. 112, Pt. 2, 3603–3617
36. Autier, P., Metouli, M., Fabrega, S., Kouach, M., and Briand, G. (2002) J. Biol. Chem. 277, 9437–9446
37. Wang, X., Ma, D., Keski-Oja, J., and Pei, D. (2004) J. Biol. Chem. 279, 9331–9336
38. Chyung, J. H., Raper, D. M., and Selkoe, D. J. (2005) J. Biol. Chem. 280, 4383–4392
39. Ebert, D. H., Deussing, J., Peters, C., and Dermody, T. S. (2002) J. Biol. Chem. 277, 24609–24617
40. Pager, C. T., Wurth, M. A., and Dutch, R. E. (2004) J. Virol. 78, 9154–9163