European Journal of Cell Biology 94 (2015) 316–322

Contents lists available at ScienceDirect

European Journal of Cell Biology

journal homepage: www.elsevier.com/locate/ejcb

Nipah virus fusion protein: Importance of the cytoplasmic tail for endosomal trafficking and bioactivity

Michael Weis, Andrea Maisner*

Institute of Virology, Philipps University of Marburg, Marburg, Germany

ARTICLE INFO

Keywords:
Nipah virus
Fusion protein
Endocytosis
Cleavage
Fusion activity
Cytoplasmic tail

ABSTRACT

Nipah virus (NiV) is a highly pathogenic paramyxovirus which encodes two surface glycoproteins: the receptor-binding protein G and the fusion protein F. As for all paramyxoviruses, proteolytic activation of the NiV-F protein is an indispensable prerequisite for viral infectivity. Interestingly, proteolytic activation of NiV-F differs principally from other paramyxoviruses with respect to protease usage (cathepsins instead of trypsin- or furin-like proteases), and the subcellular localization where cleavage takes place (endosomes instead of Golgi or plasma membrane). To allow efficient F protein activation needed for productive virus replication and cell-to-cell fusion, the NiV-F cytoplasmic tail contains a classical tyrosine-based endocytosis signal (Y_{525}RSL) that we have shown earlier to be needed for F uptake and proteolytic activation. In this report, we furthermore revealed that an intact endocytosis signal alone is not sufficient for full bioactivity. The very C-terminus of the cytoplasmic tail is needed in addition. Deletions of more than four residues did not affect F uptake or endosomal cleavage but downregulated the surface expression, likely by delaying the intracellular trafficking through endosomal-recycling compartments. Given that the NiV-F cytoplasmic tail is needed for timely and correct intracellular trafficking, endosomal cleavage and fusion activity, the influence of tail truncations on NiV-mediated cell-to-cell fusion and on pseudotyping lentiviral vectors is discussed.

© 2015 Elsevier GmbH. All rights reserved.

1. Epidemiology of NiV infections

Nipah virus (NiV) is a fruit bat-derived virus that can infect a variety of mammalian species including pigs, dogs, cats, guinea pigs, hamsters, and humans (Chua et al., 1999; Hooper et al., 2001; Wong et al., 2003). In the first documented NiV outbreak in 1998 in Malaysia, NiV was transmitted from its natural reservoir, pteropid bats, to pigs. The infection efficiently spread within the pig population and was also transmitted to humans with close contacts to sick animals. Most of the infected humans developed severe multifocal encephalitis, and 39% of them died (Chua, 2003; Chua et al., 1999). Since 2001, small NiV outbreaks are reported nearly every year in Bangladesh causing encephalitic diseases with mortality rates up to 70%. During these outbreaks, NiV is mostly transmitted directly from fruit bats to humans. Consumption of NiV-contaminated raw date palm sap is regarded as the primary route of transmission (Luby and Gurley, 2012). Importantly, several of these sporadic outbreaks have included short chains of human-to-human transmission which were associated with contact to secretions from Nipah patients (Luby et al., 2009). Due to its high zoonotic potential and its high pathogenicity in humans, NiV is classified as biosafety level 4 (BSL-4) pathogen.

2. NiV structure and fusion processes

NiV, together with the Australian Hendra and Cedar viruses (HeV; CedPV), form the *Henipavirus* genus within the *Paramyxoviridae* family (Marsh et al., 2012; Wang et al., 2000). Its negative-stranded RNA genome encodes six structural proteins and is encapsidated by the nucleoprotein (N), the phosphoprotein (P) and the polymerase (L). The matrix protein (M) lines the inner side of the lipid envelope in which the two viral surface glycoproteins, tetrameric G and trimeric F proteins, are inserted (Fig. 1A). Glycoprotein G is responsible for binding to ephrin-B2/-B3 on host cells (Bonaparte et al., 2005; Negrete et al., 2005, 2006) and complexes of G and F mediate fusion between viral and cellular membranes during virus entry and cell-to-cell spread (reviewed in Diederich and Maisner, 2007). Aside of their incorporation into infectious NiV particles, NiV-F and -G can efficiently pseudotype lentiviral vectors thereby allowing specific targeting of ephrin-positive cells (Khetawat and Broder, 2010; Palomares et al., 2013; Witting et al., 2013).

* Corresponding author. Tel.: +49 64212865360; fax: +49 6421 2868962.
E-mail address: maisner@staff.uni-marburg.de (A. Maisner).

http://dx.doi.org/10.1016/j.ejcb.2015.05.005
0171-9335/© 2015 Elsevier GmbH. All rights reserved.

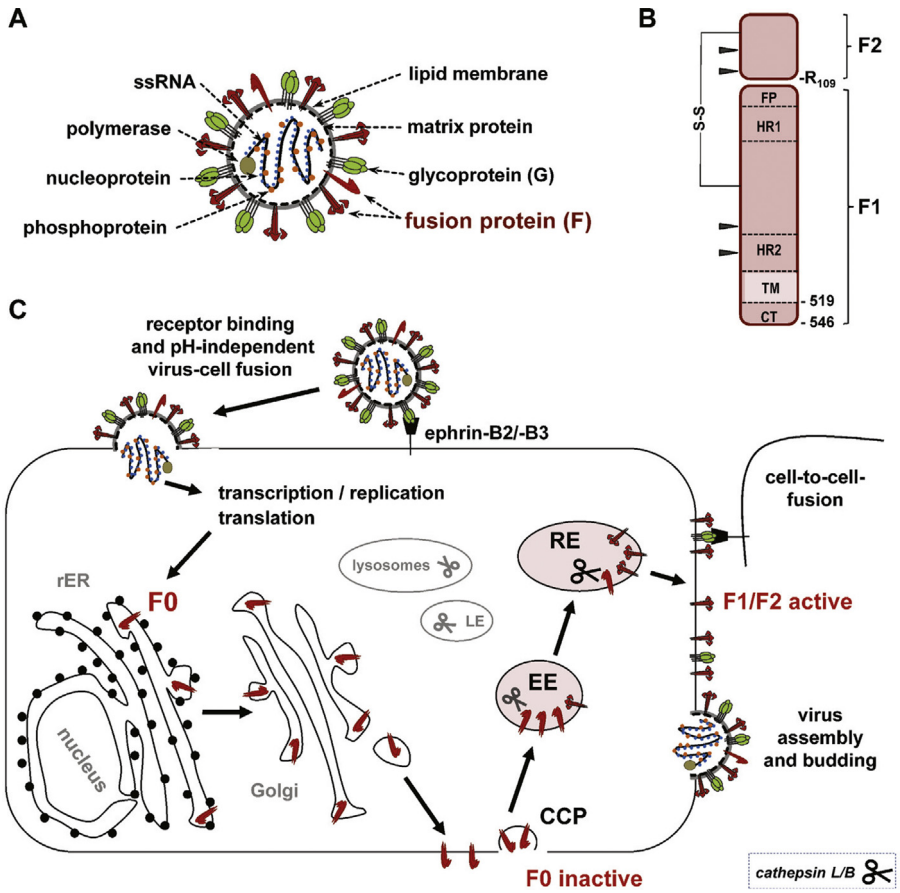

Fig. 1. Overview on NiV structure and replication. (A) Structural components of a NiV particle. The viral ribonucleocapsid (RNP) consists of the single-stranded negative RNA genome (ssRNA) enwrapped by the nucleoprotein N, the phosphoprotein P and the viral polymerase L. The RNP is connected with the surrounding lipid membrane via the matrix protein. The envelope contains receptor-binding G proteins (tetramers) and trimeric F proteins in a cleaved form (fusion-active F1/F2 subunits) and, to lower amounts, as uncleaved forms (precursor F0). (B) Schematic representation of functionally important regions in fusion-active NiV-F protein (F1/F2 subunits). FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. Arrowheads indicate N-glycan attachment sites at asparagines N67, N99, N414, N464 (Moll et al., 2004b). (C) NiV replication cycle illustrating the F activation pathway. CCP, clathrin-coated pits; EE, early endosomes (EEA-1 and Rab5-positive); RE, recycling endosomes (Rab4 or Rab11-positive); LE, late endosomes (CD63 and Rab7-positive).

As a typical class I fusion protein, NiV-F forms homotrimers and is composed of the N-terminal F2 and the C-terminal membrane-anchored F1 subunit. The F1 subunit contains the hydrophobic fusion peptide (FP) followed by two heptad repeat regions (HR1, HR2), a transmembrane domain (TM) and a C-terminal cytoplasmic tail (Fig. 1B). In infected cells, NiV-F is synthesized as fusion-inactive precursor F0 which is then processed by host cell proteases into its fusion-active form F1/F2. Proteolytic activation at the monobasic cleavage site (R_{109}) leads to the release of the hydrophobic FP at the N-terminus of the F1 subunit, which is needed to insert into target membranes during fusion processes.

During such cell-to-cell or virus–cell fusion processes, NiV-G and F proteins must act in concert. Only after G binding to cellular receptors (ephrins), conformational changes in the F1/F2 trimers are triggered. These finally lead to re-folding of the HR1 and 2 regions into coiled-coil domains building up a stable six-helix bundle that allows the formation of a fusion pore between virus and host, or two host cell membranes (reviewed in Chang and Dutch, 2012; Jardetzky and Lamb, 2014; Lee and Ataman, 2011).

3. Proteolytic activation of the NiV-Fusion protein

As for all paramyxoviruses, proteolytic activation of the NiV-F protein by host cell proteases is an indispensable prerequisite for viral infectivity and thus for pathogenicity. Interestingly, the NiV-F activation mechanism completely differs from the commonly used fusion protein cleavage pathways of other paramyxoviruses involving furin- or trypsin-like proteases in the Golgi or at the plasma membrane (Klenk and Garten, 1994). In NiV-infected cells, newly synthesized precursor F0 proteins are transported along the secretory pathway. After arrival at the cell surface, fusion-inactive F0 precursors are endocytosed via clathrin-coated pits (CCP) and are subsequently cleaved into F1/F2 subunits by pH-dependent endosomal cathepsin L or B (Diederich et al., 2005; Diederich et al., 2012; Meulendyke et al., 2005; Moll et al., 2004a; Pager et al., 2006; Vogt et al., 2005). After trafficking back to the plasma membrane (recycling), fusion-active F1/F2 subunits can induce cell-to-cell fusion and can be incorporated into budding virus particles (illustrated in Fig. 1C).

Due to the fact that NiV needs endosomal cathepsins for activation, it was speculated that NiV, similar to SARS-CoV or Ebola virus requires endocytosis and cleavage steps during virus entry (Chandran et al., 2005; Simmons et al., 2005). But though macropinocytosis was proposed as potential entry pathway for NiV (Pernet et al., 2009), neither endocytosis nor cathepsin cleavage steps are essentially required for entry and infection of new host cells (Diederich et al., 2008). Proteolytic activation of the NiV-F protein occurs before incorporation into budding virions but not after virus entry. Thus, NiV particles released from infected cells contain sufficient amounts of cleaved and fusion-competent F proteins to initiate the infection of new host cells by pH-independent fusion with the plasma membrane.

Download English Version:

https://daneshyari.com/en/article/2178356

Download Persian Version:

https://daneshyari.com/article/2178356

Daneshyari.com