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

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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.

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).

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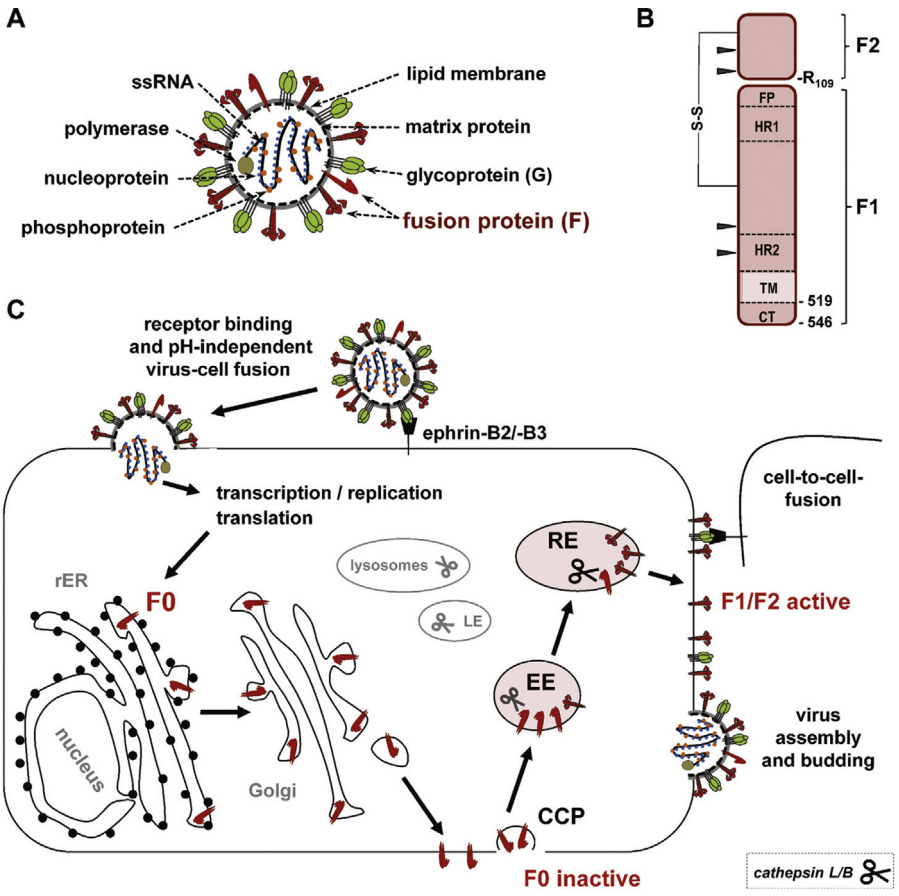

Fig. 1. Overview on NiV structure and replication. (A) Structural components of a NiV particle. The viral ribonucleocapsid (RNP) consists of the single-stranded negative RNA genome (ssRNA) enwrapped by the nucleoprotein N, the phosphoprotein P and the viral polymerase L. The RNP is connected with the 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.

Cathepsin-mediated cleavage of NiV-F occurs ubiquitously in all cell types tested so far. There are, however, cell-type specific differences in the protease usage. While cathepsin L activates the protein in Vero cells, endosomal cathepsin B was found to be responsible for F cleavage in several epithelial cell types such as MDCK cells or primary bronchial epithelial cells (Diederich et al., 2012; Pager et al., 2006). The finding that NiV can use two broadly expressed cathepsins for fusion activation suggests that protease expression does not restrict NiV spread in vivo. Interestingly, furin, an ubiquitous Golgi protease known to process fusion proteins of other systemically spreading paramyxoviruses, cannot activate NiV-F even if the amino acid sequences around the cleavage site were mutated to respective consensus sequences (Diederich et al., 2009). This further highlights the peculiarity of the proteolytic activation of NiV-F proteins not only with respect to protease usage (cathepsins), but also to the subcellular localization where cleavage takes place (endosomes).

4. Cytoplasmic tail motifs influencing NiV-F protein trafficking and fusion activity

As proteolytic activation of the NiV-F takes place after endocytosis, it essentially depends on the functional Y_{525}RSL internalization signal located in the F protein cytoplasmic tail (Fig. 2A). Both, inhibition of clathrin-mediated endocytosis as well as mutation of the critical tyrosine at position 525 (Y_{525}) disrupting the YxxΦ endocytosis motif, interfered with F cleavage and the generation of infectious NiV particles (Diederich et al., 2005; Diederich et al., 2008; Vogt et al., 2005). From the pronounced colocalization of endocytosed F proteins with EEA-1, Rab4-, and Rab11-positive endosomes, and the independence of F activation on trafficking through late endosomal (Rab7-positive) compartments, it was concluded that cathepsin-mediated F cleavage predominantly occurs within the endosomal-recycling compartment (Diederich et al., 2012; Fig. 1C).

Aside of its central function in F endocytosis, the Y_{525}RSL in the NiV-F cytoplasmic tail acts as a basolateral targeting signal in polarized epithelial cells. Thus, mutation of the critical Y_{525} not only affected endocytosis and proteolytic activation, but also F protein surface distribution in both, polarized epithelial as well as in polarized endothelial cells (Erbar and Maisner, 2010; Lamp et al., 2013; Weise et al., 2010). In addition to the canonical Y_{525}RSL motif, the di-tyrosine motif ($Y_{542/543}$) at the very C-terminus of the cytoplasmic domain (see Fig. 2A) was found to be required for F protein sorting in polarized endothelial cells and neurons (Erbar and Maisner, 2010; Mattera et al., 2014).

In addition to the two tyrosine-based motifs in the cytoplasmic tail that influenced endocytosis and/or polarized F trafficking, the membrane-proximal tribasic KKR motif (residues 520–522; Fig. 2A) was shown to be of functional importance for the fusogenicity of the protein. These basic residues in the cytoplasmic domain are thought to affect the conformation of the F protein ectodomain by a so-called inside-out signaling. Mutations in the KKR motif therefore modulated the fusogenic activity by affecting the kinetics of six-helix bundle formation or the affinity of F–G interactions (Aguilar et al., 2007).

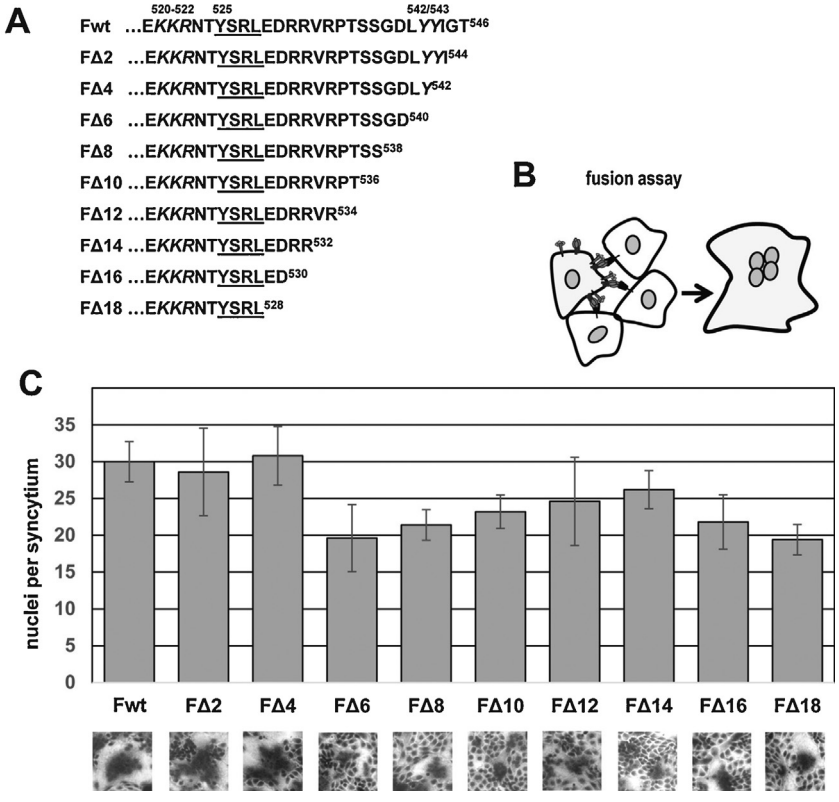

Fig. 2. Fusion activity of NiV-F tail truncation mutants with an intact endocytosis signal. (A) Amino acid sequence of the cytoplasmic domains of wildtype and mutant NiV-F proteins. The Y_{525}RSL endocytosis signal known to be important for uptake and proteolytic cleavage by endosomal cathepsins is underlined (Diederich et al., 2005; Vogt et al., 2005). Italics indicate the tribasic KKR motif (520–521) shown to modulate fusogenic activity (Aguilar et al., 2007) and the di-tyrosine motif ($Y_{542/543}$) shown to partially affect polarized F sorting in endothelial cells and neurons (Erbar and Maisner, 2010; Mattera et al., 2014). (B) Principle of the fusion assay method. Cells were cotransfected with plasmids encoding the two NiV surface glycoproteins. Upon functional coexpression of receptor-binding G and fusion-active F proteins, cell-to-cell fusion with neighboring ephrin-positive cells result in syncytia formation. (C) Fusion activity of F tail truncation mutants. MDCK cells transiently expressing NiV-G together with either wildtype or truncated F proteins. 24 h after transfection, the cells were fixed and stained with Giemsa's staining solution. Magnification, ×100. Syncytium formation was quantified by counting and averaging the number of nuclei per syncytium of at least twenty randomly chosen syncytia.

5. Length of the cytoplasmic tail influences NiV-F fusion activity

Disruption of the Y_{525}RSL signal in NiV-F impairs endocytic uptake and thus proteolytic activation and fusion activity of the protein (Diederich et al., 2005; Diederich et al., 2008; Vogt et al., 2005). Though the need for an intact endocytosis signal is evident, the importance of the residual C-terminal residues in the cytoplasmic tail is less clear. Their role is difficult to predict since truncations of cytoplasmic domains of paramyxovirus fusion proteins have been described to result in varying effects, ranging from not affecting fusion activity at all, to severe influences on fusogenicity either by downregulating surface expression, impeding fusion pore enlargement, or blocking transition from hemifusion to complete fusion (Bagai and Lamb, 1996; Branigan et al., 2006; Dutch and Lamb, 2001; Moll et al., 2002; Tong et al., 2002). The influence of the 18 cytoplasmic amino acids (residue 529–546) downstream of the essential Y_{525}RSL endocytosis signal on NiV-F bioactivity is poorly understood. An earlier paper analyzing point mutants suggested a minor importance of the di-tyrosine motif ($Y_{542/543}$) for rapid F endocytosis, slightly downregulating the endocytosis rate from 2% to 1.6% per min (Vogt et al., 2005). However, this mutation was not seen to obviously affect fusion activity. Three other papers describing tail truncation mutants with or without deletion of the Y_{525}RSL endocytosis signal gave inconsistent results, ranging from no effect of certain deletions to increased or decreased expression and fusion activity (Aguilar et al., 2007; Khetawat and Broder, 2010; Witting et al., 2013). The lack of a clear picture for tail truncation mutants might be due to different cell types or expression systems, or usage of tail deletion mutants containing an AU tag (DTYRYI) adding a bona fide endocytosis signal to the cytoplasmic domain. To assess the need for a full-length tail to generate a functional NiV-F protein more systematically, we generated untagged F proteins with an intact Y_{525}RSL motif lacking increasing numbers of downstream amino acids (Fig. 2A). In the shortest tail truncation mutant, FΔ18, the endocytosis motif is located at the very C-terminus of the protein. All truncation mutants were cloned into the eukaryotic expression vector pCAGGS and were transfected into MDCK cells using lipofectamine 2000 reagent as described previously (Lamp et al., 2013). After similar total expression levels for all truncation mutants were confirmed by immunostaining, their bioactivity was analyzed by standard fusion assays (Weise et al., 2010; see model in Fig. 2B). To this end, MDCK cells were transfected with NiV-G in combination with wildtype or mutant F proteins. At 24 h after transfection, cells were fixed and stained with Giemsa's staining solution to visualize the competence of truncated F proteins to support fusion. Fig. 2C shows that all mutants except for FΔ2 and FΔ4 showed reduced syncytia formation activity compared to wildtype F protein (F_{wt}). The same was observed in fusion assays using Vero cells (not shown). The finding that all tail mutants lacking more than 4 amino acids showed a significantly reduced fusion activity supports the view that either the C-terminal di-tyrosine motif ($Y_{542/543}$) or a cytoplasmic tail length of more than 24 residues is needed for full bioactivity of NiV-F.

6. Tail truncation neither affected F protein endocytosis nor endosomal cleavage but downregulated constitutive surface expression of fusion-active F proteins

As F activation is only mediated within the endosomal-recycling compartment, reduced fusion activity of tail-truncated F proteins might be due to a defective endocytic uptake of the proteins. To test this idea, we performed an antibody uptake experiment (Moll et al., 2001; Vogt et al., 2005). For this, cells were transfected with either F_{wt} or mutant F proteins. Surface expressed F proteins were then labeled with NiV-specific antibodies at 4 °C and either kept on ice, or were incubated for 5 min or 30 min at 37 °C to allow endocytosis to occur. Primary antibodies that remained at the surface after the incubation period were then blocked by incubation with horseradish-peroxidase (HRP)-conjugated secondary antibodies on ice. Then, cells were permeabilized and endocytosed primary antibodies were stained with AlexaFluor 568-conjugated secondary antibodies. The results were the same for all fusion-defective mutants (FΔ6, FΔ8, FΔ10, FΔ12, FΔ14, FΔ16 and FΔ18). Fig. 3A therefore exemplarily shows the uptake of F_{wt} and FΔ18 as representative truncation mutant (F_{trunc}). Fluorescent endocytic vesicles containing F and F_{trunc} were already found after 5 min of endocytosis throughout the cell. After incubation for 30 min, full-length and truncated F proteins accumulated in larger perinuclear patches, likely representing recycling endosomes. Supporting this idea, all tail truncation mutants were found to colocalize with Rab11-positive compartments as did the wildtype F protein (Fig. 3B). Compared to wildtype F, the mutants did not show an increased colocalization with Rab-7 positive late endosomes (not shown). We thus conclude that tail-truncation does not principally alter NiV-F distribution in endocytic compartments.

To determine if reduced fusion activities of tail-truncated F proteins are caused by decreased protein synthesis or cleavage rates, we performed a pulse-chase experiment. As recently described by Weis et al. (2014), F-expressing cells were metabolically labeled with [^{35}S]cysteine/methionine for 45 min. After pulse labeling, the medium was removed and cells were incubated further for 1, 2 or 4 h in chase medium. The F proteins were then immunoprecipitated and subjected to SDS–PAGE under reducing conditions to separate uncleaved precursor F0 from cleavage products F1 and F2. As with the uptake assay, we did not see a difference between all fusion-defective F truncation mutants. Therefore, Fig. 3C only shows the result of the pulse-chase analyses for wildtype F and one representative mutant. Total F amounts (F0 + F1 + F2) at any chase time were the same, indicating similar protein synthesis and degradation rates for wildtype and tail-truncated F proteins. There was neither an obvious difference in the cleavage kinetics since similar amounts of F1 cleavage products were found for F_{wt} and F_{trunc} proteins after 1, 2 and 4 h of chase time (Fig. 3C). This indicates that newly synthesized (pulse-labeled) full-length and tail-truncated F had reached the early-recycling endosomal cleavage compartment with comparable kinetics.

As cell-to-cell fusion can only be mediated by cleaved F proteins expressed on the cell surface, the reduced fusion activity of efficiently synthesized, endocytosed and cleaved tail-truncated F proteins must be assumed to be the result of a reduced steady state surface expression of fusion-active F proteins. This idea was tested with a surface biotinylation experiment. As described by Krüger et al. (2014), F-expressing cells were surface-labeled at 4 °C with non-membrane permeating sulfo-*N*-hydroxysuccinimido-LC-Biotin. After cell lysis, biotinylated proteins were precipitated with NeutrAvidin agarose and F proteins were detected by western blot analysis using NiV-specific antibodies. As depicted in Fig. 3D, F_{trunc} showed a slightly reduced expression of uncleaved (F0) on the cell surface. More importantly, cleaved forms (F1) of F_{trunc} were not at all detected at this exposure time. As total cleavage rates of F_{trunc} were found to be as efficient as that of wildtype F (Fig. 3C), reduced surface expression (Fig. 3D) indicates a less efficient transport from the endosomal cleavage compartment back to the cell surface. We thus conclude that the reduced fusion activities of F proteins with tail truncations of more than 4 C-terminal amino acids were mainly the result of a slower trafficking through endosomal compartments delaying F recycling back to the plasma membrane, thereby downregulating the expression of fusion-active F1/F2 proteins on the cell surface.

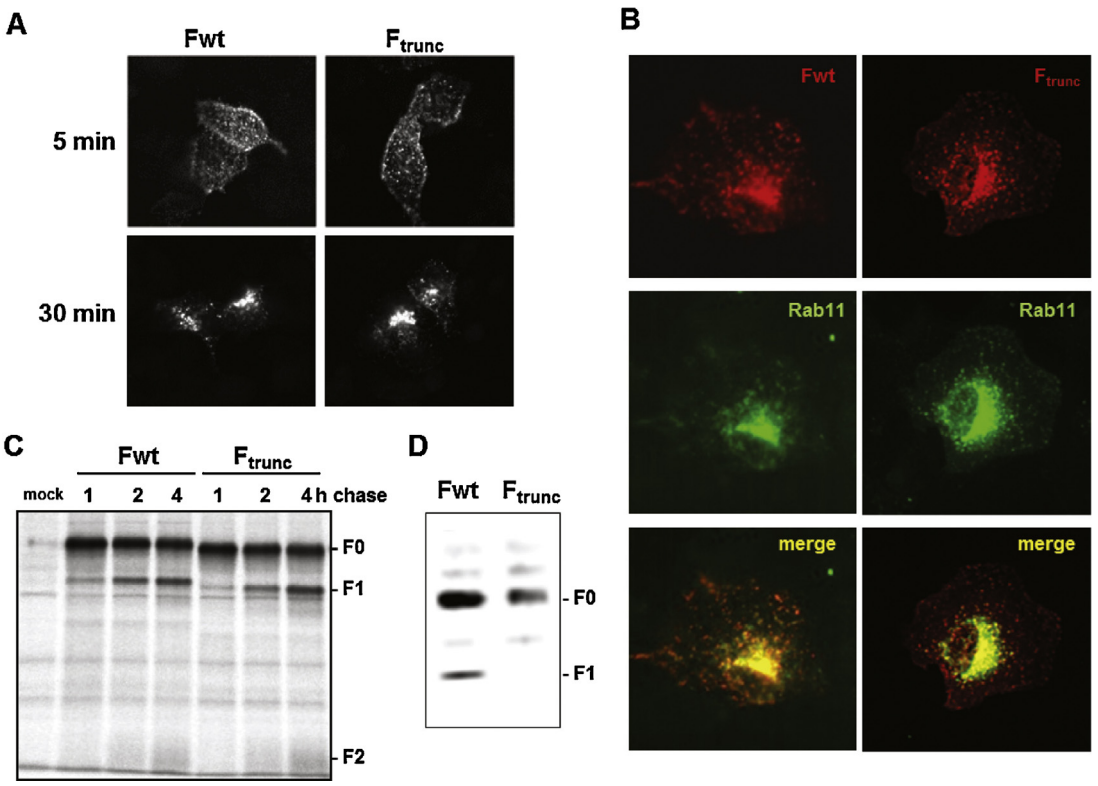

Fig. 3. Endocytosis, cleavage and surface expression of wildtype and tail-truncated F proteins. As all fusion-defective truncation mutants FΔ6, FΔ8, FΔ10, FΔ12, FΔ14, FΔ16 and FΔ18 showed the same phenotype, only the results for FΔ18, the mutant with largest tail deletion, are exemplarily shown. (A) Endocytosis of wildtype and tail-truncated NiV-F analyzed by an antibody-uptake experiment (Vogt et al., 2005). MDCK cells expressing either wildtype F (F_{wt}) or FΔ18 (F_{trunc}) were incubated with NiV-specific guinea pig antiserum at 4 °C for 30 min and then warmed to 37 °C for either 5 min or 30 min to allow endocytosis to occur. Surface-bound antibodies were blocked by HRP-conjugated secondary antibodies. Endocytosed primary antibodies were stained after permeabilization with methanol–acetone by AF 568-conjugated secondary antibodies. (B) Colocalization with Rab11-positive endosomes. Vero cells were transfected with wildtype or truncated F genes in combination with a Rab11-CFP encoding plasmid. 20 h after transfection, cells were fixed with 2% PFA and permeabilized with 0.2% TX-100 as described in (Diederich et al., 2012). F proteins were visualized with NiV-specific antibodies and AF 568-labeled secondary antibodies. (C) Cleavage kinetics of wildtype and tail-truncated NiV-F. At 24 h after transfection, F-expressing MDCK cells were radio labeled with [^{35}S]cysteine/methionine for 45 min and then incubated in chase medium for the indicated times as described by Weis et al. (2014). After lysis, the F proteins were immunoprecipitated and separated by 15% SDS–PAGE, followed by autoradiography using a BioImager. (D) Cell surface expression of wildtype and tail-truncated NiV-F. To determine the amount of surface-expressed F proteins, the cells were surface labeled with biotin using a protocol described in Weis et al. (2015). After cell lysis, biotinylated proteins were precipitated with NeutrAvidin agarose and separated by SDS–PAGE, blotted to nitrocellulose and detected by western blotting using a NiV-specific guinea pig antiserum and IRdye800-labeled secondary antibodies. Fluorescent signals were recorded with a LI-COR Odyssey imaging system.

7. How C-terminal tail truncation can affect F trafficking within the endosomal compartment

Our data suggest that changes in surface expression of tail-truncated NiV-F are due to changes in vesicular transport kinetics. This is most likely the consequence of an altered interaction of the F cytoplasmic tail with cellular sorting proteins, slowing down the constitutive protein recycling (for review see Grant and Donaldson, 2009; Maxfield and McGraw, 2004). Truncation appeared not to cause a "missorting" to late endosomal/lysosomal compartments because we did not see differences in the colocalization of wild-type and tail-truncated F proteins with Rab11-positive recycling endosomes (see Fig. 3B). We neither observed an increased accumulation in Rab7-positive late endosomes or an increased degradation of tail-truncated F proteins (see Fig. 3C). Truncations most likely changed the affinity of the tail to interact with cellular sorting or adaptor proteins thereby affecting the kinetics of the trafficking within the endosomal-recycling compartment and delaying F recycling back to the plasma membrane. There are two possible reasons for these changes: The first is that C-terminal truncations of more than 4 residues alter the conformation of the cytoplasmic tail thereby modulating the affinity of the Y_{525}RSL motif to interact with cellular sorting proteins. In this respect, it has to be noted that the NiV-F cytoplasmic tail was recently shown to interact with μ subunits of adaptor proteins AP-1, AP-2, AP-3 and AP-4. As all μ subunits generally interact with YxxO motifs, mutations in the critical Y and L position of the Y_{525}RSL caused a drastic reduction with all APs (Mattera et al., 2014). A second explanation for a less efficient interaction of truncated F tails with the cellular transport machinery could be the removal of the di-tyrosine motif $Y_{542/543}$. This second tyrosine-based motif in the NiV-F tail might function as a second cytoplasmic sorting signal, or even as a specific cytoplasmic "recycling motif" (Dai et al., 2004; Li et al., 2007; Tanowitz and von Zastrow, 2003). The idea of an individual interaction of the di-tyrosine motif with cellular adaptor or sorting proteins is supported by the findings that point mutations of $Y_{542/543}$ disturbed interaction of the F cytoplasmic tail with adaptor proteins in a yeast-two-hybrid system. Mutations furthermore resulted in a partial loss of polarized distribution in neurons, and slightly down-regulated the endocytosis rate (Mattera et al., 2014; Vogt et al., 2005).

8. Implications of tail-truncated NiV-F proteins to complement lentiviral vectors

During virus assembly, viral surface glycoproteins interact with viral matrix proteins at the plasma membrane. While intact cytoplasmic tails are usually needed for a well-organized interaction with homologous matrix proteins and so for efficient budding of infectious virions, full-length cytoplasmic tails rather restrict

the capacity of glycoproteins to complement/pseudotype lentiviral vectors because they cannot interact with the non-homologous lentiviral gag protein. Therefore, tail truncations often have beneficial effects on the heterotypic incorporation into lentiviral particles.

Lentiviral vectors (LV) are of increasing interest for gene therapeutic approaches because they can transduce nondividing cells and can stably integrate foreign genes into a target cell genome. The restricted cell tropism of LV is normally altered by pseudotyping with glycoproteins derived from other enveloped viruses. The most commonly used glycoprotein is that of vesicular stomatitis virus (Cronin et al., 2005; Reiser et al., 1996). For more specific targeting of LV cell entry to distinct tumor cell populations, tail-truncated measles virus (MV) glycoproteins with modified receptor-binding capacities are frequently used (Frecha et al., 2009; Friedrich et al., 2013; Funke et al., 2008; Nakamura et al., 2005; Ou et al., 2012). Despite promising advances, development of MV-glycoprotein-based LV for in-vivo use is limited by the widespread presence of preexisting neutralizing antibodies in the human population. Such vector neutralization can be circumvented by pseudotyping LV with glycoproteins of less prevalent viruses, for example F and G proteins of henipaviruses.

A first study by Khetawat and Broder (2010) already showed that NiV envelope glycoproteins (F and G) can be used to pseudotype LVs, even if they contain their full-length cytoplasmic tails. LV pseudotyped with F tail truncation mutants still containing the Y_{525}RSL signal but lacking further downstream residues (FΔ5, FΔ13, FΔ15, FΔ18) were less efficiently produced (Khetawat and Broder, 2010). The observation that decreased LV production correlated with a decreased F surface expression and incorporation into LV is in full agreement with our finding here that tail truncation (mutants FΔ6–FΔ18) partially downregulated steady state surface expression, likely as a result of a delayed trafficking within the endosomal-recycling compartment.

Besides the finding that truncation mutants lacking up to 18 of 28 residues less efficiently pseudotyped LV, Khetawat and Broder (2010) demonstrated that F mutants with larger tail deletions no longer containing the Y_{525}RSL endocytosis signal (FΔ24, FΔ28) supported a 3–10-fold increased LV production. A similar finding was made by Palomares et al. (2013) for FΔ23. Though Witting et al. (2013) reported that G tail truncation had a more pronounced effect on LV production as did F tail deletions, they also observed that F proteins with a large tail deletion (FΔ25) improved the LV titers.

So far, all studies showed that LV pseudotyping with F deletion mutants lacking almost the complete tail is the most efficient. This is likely because tail-deleted NiV-F proteins do not hinder the interaction with the gag protein of the LV capsid and are therefore more easily incorporated. Incorporation is furthermore facilitated by the increased cell surface expression of these deletion mutants all lacking the Y_{525}RSL signal. However, due to the lack of rapid endocytosis, the deletion mutants are barely cleaved by endosomal cathepsins and are therefore mostly expressed as fusion-inactive F0 precursors (Fig. 1C). Endocytic uptake and recycling by normal membrane turnover only give raise to little amounts of cathepsin-cleaved F1/F2 forms on the cell surface and incorporated in LV particles (Khetawat and Broder, 2010; Witting et al., 2013). As must be expected, such low amounts of fusion-active F proteins only allow some residual cell-to-cell fusion activity upon coexpression with NiV-G. However, in the context of LV, the limited amount of F1/F2 forms is obviously sufficient. This suggests different functional requirements of the cytoplasmic tail for efficient NiV glycoprotein-mediated cell-to-cell fusion and LV infectivity, the latter depending on NiV glycoprotein-mediated fusion of the lentiviral envelope and the target cell membrane. As fusion needs the cooperation of multiple fusion-active F1/F2 protein trimers, the distinct requirements for efficient NiV G/F-mediated cell-to-cell and (lenti)virus–cell fusion events are likely linked with the different sizes of the contact regions between the membranes to fuse, and the differences in the densities of fusion-active F proteins. It might be speculated that in the LV envelope, F proteins are more densely packed, so that the low amount of F1/F2 forms expressed from endocytosis-defective F tail deletion mutants is sufficient to initiate small fusion pores. This might allow effective LV entry and infection. In contrast, concentration of fusion-active F tail deletion mutants on cell membranes is likely too low to support efficient cell-to-cell fusion.

9. Concluding remark

The 28-amino acid long NiV-F cytoplasmic tail contains several important residues and motifs in the membrane proximal and the very C-terminal region. Consequently, most alterations or deletions in the tail affect intracellular trafficking, endosomal cleavage and/or fusion activity. We have shown here that a tail truncation of more than 4 amino acids already negatively impacts NiV-F surface expression and fusion activity upon coexpression with NiV-G. Nevertheless, tail deletions up to 25 residues were not only tolerated but even needed for effective complementation of LV. As the functional requirements obviously depend on the viral system, changes in the cytoplasmic tail influencing NiV-F trafficking and bioactivity need to be individually evaluated in the respective context.

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