Measles virus M and F proteins associate with detergent-resistant membrane fractions and promote formation of virus-like particles

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Assembly and release of particles comprise a late step in virus–host cell interactions. Though it may share major biological properties with its orthologues in related viruses, trafficking and oligomerization of the matrix (M) protein of Measles virus (MV) and its relative contribution to assembly and budding of particles from particular host cells have not been addressed in more detail. Plasmid-driven expression of authentic and mutant M proteins revealed that the amino acid at position 89, an important adaptation determinant for growth of attenuated strains in Vero cells, influences the electrophoretic mobility but not the intracellular distribution of M proteins, nor their ability to oligomerize or migrate as a doublet band in SDS-PAGE. M proteins were found to co-float with detergent-resistant membrane fractions (DRM) and this was enhanced upon co-expression of the F protein. In contrast to their DRM association, the ability of M proteins to promote release of virus-like particles (VLPs) was not affected by the presence of F proteins, which on their own also efficiently promoted VLP production. Thus, DRM recruitment of MV F and M proteins and their ability to drive particle formation are not correlated.

INTRODUCTION

Measles virus (MV) causes an acute, self-limiting infection which is associated with high morbidity/mortality rates worldwide due to its ability to induce a profound, transient immunosuppression (Clements & Cutts, 1995). Both its receptor usage and its ability to replicate in particular host cells are important in MV pathogenesis (Schneider-Schaulies et al., 2001; Vincent et al., 2002; Yanagi et al., 2002). Whilst MV replicates efficiently in many cell types originating from humans or non-human primates, particular restrictions in these and in most rodent cells have been described. These can arise spontaneously or depend on cellular differentiation and occur at various levels, also including viral budding and/or production of infectious virus particles (Helin et al., 1999; Niewiesk et al., 1997; Schneider-Schaulies et al., 1993, 1995; Vincent et al., 1999, 2002).

As a member of the order Mononegavirales, MV contains a negative-stranded non-segmented RNA genome tightly encapsidated by nucleocapsid (N) proteins and associated with the viral polymerase complex, consisting of the large (L) and phospho (P) proteins (Horikami & Moyer, 1995; Rima, 1996). The ribonucleoprotein complex is surrounded by a host cell-derived lipid bilayer from which two glycoproteins project. The haemagglutinin (HA) protein binds to the cellular receptors CD46 and/or CD150 (Dorig et al., 1993; Naniche et al., 1993; Tatsuo et al., 2000), and the proteolytically activated, disulphide bridge-linked fusion (F) protein mediates membrane fusion at neutral pH (Wild & Buckland, 1995). The interaction of the cytoplasmic tail of the MV F protein with the matrix (M) protein has been documented both physically and biologically (Cathomen et al., 1998b; Moll et al., 2002; Naim et al., 2000). In infected or transfected cells, the M protein has an intrinsic property to associate with cellular membranes, probably through its hydrophobic surface (Manie et al., 2000; Riedl et al., 2002). Both in infected and transfected cells, the M protein associates with detergent-resistant membrane (DRM) fractions, which are thought to provide platforms for assembly and budding (Manie et al., 2000; Vincent et al., 2000). Apparently, neither budding from these sites nor the cooperation of M protein with MV glycoproteins are essential since infectious particles are produced from recombinant MVs expressing vesicular stomatitis virus (VSV) G protein
instead of the MV glycoproteins (Spiliohofer et al., 1998). Interestingly, MV budding does not fully rely on M protein expression either, as infectious MV can also be released in the absence of this protein (Cathomen et al., 1998a). However, this only occurs at very low levels and the nature of the infectious particle released is not clear. This suggests that the M protein, similarly to its orthologues in related viruses, is a driving force for MV budding (Hartlieb & Weissenhorn, 2006; Timmins et al., 2004). Many mutated M proteins have been described and this has been considered as important for the lack of particle formation in and maintenance of persistent infections (reviewed by Billeter et al., 1994). In order to evaluate its function in MV budding, we have studied the ability of M protein to drive formation of virus-like particles (VLP). We noted that the amino acid residue at position 89, which is known to be an important determinant for MV growth in Vero cells (Miyajima et al., 2004; Tahara et al., 2005), affects the electrophoretic mobility of this protein. M proteins were found to oligomerize and to partially partition into DRM fractions. We found that both MV M (a fraction of which is modified by ubiquitination) and F proteins individually promote the formation of VLPs, although they do not act in synergy. Thus we propose that M and F act separately in MV particle morphogenesis and release.

METHODS

Cells and viruses. HeLa and 293 cells were maintained in minimal essential medium (MEM) containing 5% or 10% fetal calf serum (FCS), respectively, and 293 cells transfected to stably express the Edmonston (ED)- or wild-type (WT)-derived F protein [293-F(ED) and 293-F(WTF)] were maintained in MEM 10% FCS supplemented with 0.5 mg G418 ml⁻¹. MV vector strain Edmonston (ED) was grown in Vero cells in MEM 5% FCS and titrated on marmoset (Saimiri sciureus) cells. For titration, a 10⁻¹ dilution of 24 h supernatant of 293-F(WTF) cells was added to each well of a 48-well plate (NUNC) containing confluent Vero cells (or HeLa cells) in MEM 5% FCS and incubated for 3 days. Supernatants were collected 48 h post-transfection or -infection of 293 or HeLa cells and then analysed by SDS-PAGE (15% gel) and M protein was detected by immunoblotting.

Indirect immunofluorescence, flow cytometry and transmission electron microscopy (TEM). For indirect immunofluorescence, cells were fixed in 0.05 M cacodylate buffer containing 2.5 % glutaraldehyde, (pH 7.2), dehydrated, embedded in Epon and ultrathin-sectioned. For electron microscope immunolocalization, cells were fixed using 4% paraformaldehyde, treated with 50 mM NH₄Cl, dehydrated and embedded in LR-White. Ultrathin sections were incubated with MAB8910 and a secondary goat a-mouse antibody conjugated to 12 nm colloidal gold (Dianova), and analysed with an EM10 transmission electron microscope (LEO, now Zeiss).

Plasmid constructions, transfections and detection of proteins. The plasmids used were pCG-M(ED), pCG-M(ED89K→E), pCG-M(WTF) and pCG-M(WTF89E→K). They were generated in pCG(ED), a modified eukaryotic expression vector which contains the cytomegalovirus immediate-early promoter and Dnal and Csp1 restriction sites. These sites facilitate the directional cloning of full-length MV M genes which were amplified by RT-PCR from viral RNA or by PCR from existing clones using primers primMVuniM+ (5'–GCAGGTTAAAGTGATTGCGTCCCAAATTC-3') and primMVuniM– (5’–CTACCTCGTGTTGCGTCTCGTTG-3') containing equivalent restriction sites (underlined). Site-directed mutagenesis using a QuickChange kit (Stratagene) was used to introduce an A→G point mutation into pCG-M(ED) to generate pCG-M(ED89K→E) and a G→A point mutation into pCG-M(WTF) to produce pCG-M(WTF89E→K). M proteins were expressed by transient transfection into HeLa cells using Superfect reagent (Qiagen) or 293, 293-F(ED) and 293-F(WTF) cells using Saint-Mix (Synvolux) prepared according to the manufacturer’s instructions. Lysates were prepared according to standard procedures and analysed by SDS-PAGE (15% gel). For separation of the M-specific bands into doublets, long-distance separation, low current and low temperature conditions were used, while separated bands could be shifted back into one by short-run and short-distance electrophoresis. For immunoblot analysis, MAB8910 and a horseradish peroxidase-conjugated goat a-mouse antibody (Dianova) (for detection of M proteins) or a polyclonal rabbit antiserum raised against the cytoplasmic tail of F protein followed by a horseradish peroxidase-conjugated goat a-rabbit antibody (Cell Signaling) (for detection of the MV F protein) were used. Secondary conjugates were detected by using enhanced chemiluminescence (ECL) reagent (Amersham) and, when indicated, signals were quantified by using AIDA software (Raytest).

Co-immunoprecipitation. Plasmid pH5-HA-UB (which encodes ubiquitin fused with the influenza virus HA epitope tag, kindly provided by Dirk Lindemann, Technical University Dresden, Germany) (Strack et al., 2002) was either singly or co-transfected with pCG-M(ED) into HeLa cells. Prior to lysis, cells were treated with MG132 (10 μM in DMSO for 4 h), and lysed 48 h post-transfection in RIPA buffer [0.15 M NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 0.05 M Tris (pH 8.0), EDTA-free protease inhibitor cocktail (Roche), 5 mg pepstatin ml⁻¹] supplemented with 10 mM N-ethylmaleimide (Sigma-Aldrich). Immune complexes, obtained after precipitation using monoclonal antibody F-7 which is specific for the HA epitope tag (Santa Cruz Biotechnology), were analysed by SDS-PAGE (15% gel) and M protein was detected by immunoblotting.

Floatation assays. Cells were lysed in cold lysis buffer containing 0.1% Brij 98 in NTE buffer [25 mM Tris (pH 7.5), 150 mM NaCl, 5 mM EDTA, 1 mM NaF] and EDTA-free protease inhibitor cocktail (Roche) for 20 min on ice. After mixing with 3 vols 60% sucrose in NTE buffer, lysates were overlaid with 30% sucrose in NTE buffer (12.5 vols) and 2.5% sucrose in NTE buffer (4 vols). Samples were centrifuged at 300 000 g for 16 h at 4 °C. Fractions were collected from the bottom to the top of the gradient and proteins were precipitated with 2 vols cold acetone, and analysed by SDS-PAGE (15% gel) followed by immunoblotting. DRM fractions were detected using H-319, a rabbit polyclonal serum raised against CD55 (Santa Cruz Biotechnology) followed by a secondary goat a-rabbit antibody (Cell Signaling).

Detection of viral and virus-like particles. Supernatants were collected 48 h post-transfection or -infection of 293 or HeLa cells...
(3 \times 10^8 \text{ or } 7.5 \times 10^5 \text{ cells, respectively}) \text{ and subjected to low-speed centrifugation (10 min, 4 °C at 3000 g) followed by ultracentrifugation through a 20% sucrose cushion [in 10 mM Tris (pH 7.4), 0.1 M NaCl, 1 mM EDTA] for 2 h, 4 °C at 150,000 g. Pelleted material was resuspended in SDS sample buffer. Cell lysates were also obtained from the cultures and 1/60 of the total lysate was subjected, together with the pelleted material, to SDS-PAGE (15% gel) followed by immunoblotting to detect F or M proteins. Relative accumulation levels of M or F proteins in particles were calculated after determining the total amount of the respective protein in each lysate and its corresponding supernatant (together set to 100%) using AIDA software (Raytest).

RESULTS

The amino acid at position 89 affects electrophoretic mobility but not subcellular distribution or oligomerization of MV WT- and vaccine strain-derived M proteins

Exchange of the conserved asparagine (E) residue in MV WT strains for lysine (K) (found in attenuated MV strains) was recently found to be important for MV growth on Vero cells (Miyajima et al., 2004; Tahara et al., 2005). To establish whether exchange of this residue affects the general biological properties of M proteins, we generated pCG-based constructs to drive expression of the authentic [pCG-M(ED) and pCG-M(WTF)] and point-mutated [pCG-M(ED89K–E) and pCG-M(WTF89E–K)] M proteins. When transfected into HeLa cells, all constructs gave rise to proteins detectable by two monoclonal M protein-specific antibodies (MAB8910 and B347) by flow cytometry (Fig. 1a). Indirect immunofluorescence analysis revealed that they did not differ with regard to their intracellular distribution and localized mainly to the cytosol, but also to the plasma membrane (Fig. 1b). Next we tested whether aa 89 affected the ability of MV M proteins to oligomerize, which has been found important for membrane trafficking and budding in related viruses (Gomis-Ruth et al., 2003; Panchal et al., 2003; Timmins et al., 2003). Immunoblot analysis performed with transfected HeLa cell extracts, which were left unboiled prior to SDS-PAGE separation, revealed that all M proteins were able to oligomerize at similar efficiencies (Fig. 1c). As particularly visible when monomers are expressed at high levels [M(ED89K–E) and M(WTF)], M proteins did not migrate as discrete bands, indicating they might carry a post-translational modification (Fig. 1c, and see below). When our standard SDS-PAGE conditions were used for separation, M proteins detected in transfected and ED-infected HeLa or 293 cells reproducibly migrated as double bands, irrespective of the amino acid at position 89. Exchange of E for K at this position affected the overall electrophoretic migration pattern of these proteins (Fig. 1d). Thus, 89K–E shifted M(ED) to a higher and 89E–K M(WTF) to a lower

Fig. 1. Amino acid 89 influences the electrophoretic mobility but not the subcellular distribution and oligomerization of MV M proteins. Expression of M(ED), M(WTF), M(ED89K–E) and M(WTF89E–K) was detected 24 h after transfection of the corresponding expression constructs into HeLa cells by flow cytometry using MAB8910 (red lines) or B347 (blue lines) with a mouse isotype IgG (black lines) serving as control (a), by indirect immunofluorescence (magnification: 40×) (b) or by immunoblot in extracts left unboiled prior to SDS-PAGE (c). Extracts were prepared 24 h following transfection of HeLa or 293 cells [with lysates of ED-infected (ED) or uninfected (co) cells used as controls] and analysed by immunoblot using standard separation conditions (d).
apparent molecular mass (by about 2 kDa) than the authentic proteins [M(ED), 37 kDa and M(WTF), 39 kDa]]. Apparently, the established difference in electrophoretic migration for M proteins derived from attenuated and wild-type MV strains (Rima, 1983; Rima et al., 1979, 1995) is largely, but not entirely, determined by aa 89 since the mutant M proteins migrated similarly, but not identically, to the authentic proteins (Fig. 1d). Since, apart from the overall migration pattern, aa 89 had no detectable impact on the biological properties of M proteins assayed so far, we confined all subsequent analyses to the authentic M(ED) and M(WTF) proteins.

**MV M protein associates with DRM fractions and this is enhanced upon co-expression of F protein**

In HeLa cells infected with MV or recombinant vaccinia viruses encoding MV proteins, association of a fraction of M and F proteins with and preferential budding of virus from DRM domains containing the glycosylphosphatidylinositol (GPI)-anchored CD55 have been described (Manie et al., 2000; Vincent et al., 2000). In agreement with these studies, we observed that approximately 10% of the total M protein pool associates with CD55-containing DRM fractions obtained from extracts of ED-infected or pCG-M-transfected 293 cells [Fig. 2a, b; shown for ED(M)]. In agreement with previous findings in HeLa cells, the majority of the F protein was also found to co-float with CD55 in 293 cells stably or transiently expressing this protein (not shown). When our standard separating conditions were used (Fig. 2a, b, d), M-specific doublets equivalent to those described (Fig. 1d) were also detectable, yet neither of these protein species associated preferentially with the DRM or the detergent-soluble fractions. To investigate whether co-expression of F protein would alter DRM association of M proteins, pCG-M(ED) was transfected into 293 cells stably expressing authentic (ED-derived) (Fig. 2c) or heterologous (WTF-derived) (Fig. 2d) F proteins. Though accumulation levels of M protein in both stable transfectant cell lines were lower than in parental 293 cells, co-expression of either F protein detectably enhanced the levels of DRM-associated M protein, in contrast to what has been described in vaccinia-driven expression systems (Vincent et al., 2000). This increase was about fourfold as determined after quantification of the percentage of total M protein amounts in the respective extracts, thus indicating that the presence of F protein allows M protein accumulation at these sites.

**M and F proteins promote, but do not cooperate in, formation of VLPs**

The ability of MV M and/or F proteins to promote particle formation was analysed directly by TEM on ED-infected and pCG-M(ED)-transfected 293 or 293-F(ED) cells. Vesicular structures budding from the surface were clearly detectable in 293 and 293-F(ED) cells transfected to express M(ED) protein and in 293-F(ED) cells alone (Fig. 3b-e, upper row), but not in untransfected cells or cells transfected to express N protein (Fig. 3a and not shown). VLPs released after expression of M or F proteins alone or in combination tended to be smaller than viral particles (Fig. 3b-e). Particles released from MV-infected (Fig. 3g) and pCG-M(ED)-transfected 293 (Fig. 3h) and 293-F(ED) cells (Fig. 3i) did contain M protein, as confirmed by MAB8910 binding followed by detection by a secondary antibody conjugated to colloidal gold particles. The ability of M protein orthologues to promote VLP formation is often linked to transient mono-ubiquitination of these proteins (Hartlieb & Weissenhorn, 2006; Martin-Serrano et al., 2005). To investigate whether at least a fraction of MV M proteins carries this modification, 293 cells were co-transfected with pCG-M(ED) and pBJ5-HA-UB, from which HA-tagged ubiquitin (HA-Ub) is expressed, and exposed to a proteasome inhibitor, MG132, prior to extract preparation to stabilize potentially ubiquitin-modified proteins. Indeed, M(ED) protein was precipitated by the HA-specific antibody from lysates of doubly transfected cells. The only marginally slower electrophoretic mobility than that seen for the majority of M protein in the cell lysate suggests that it is most likely mono-ubiquitinated (Fig. 4a).

To assess VLP formation driven by M proteins on a quantitative basis, their relative amounts in supernatants of 293 or HeLa cells transfected with pCG-M(ED) or pCG-M(WTF) were determined. They comprised, on average, up to 2% of the total M protein pools of the transfected cultures (Fig. 4b, d). This percentage did not differ between
M(E) and M(WTF) [Fig. 4d, and not shown for M(WTF)]. Accumulation levels of M protein in supernatants of infected 293 cells were almost identical to those determined for M-driven VLPs, confirming that particle production by MV is very inefficient (Fig. 4d). Notably, the M protein released separated into doublets (Fig. 4b, c). However, in contrast to its association with DRM fractions (Fig. 2c), accumulation of M protein in supernatants was not enhanced by the presence of F protein provided by 293-F(ED) cells (Fig. 4d). This was also seen upon transient co-expression of either ED- or WTF-derived F protein and in 293-F(WTF) cells [which stably expresses the F(WTF) protein], indicating that there is no obvious correlation between M protein-driven VLP production and the amount of protein associated with DRMs (not shown). Surprisingly, F protein was found to be released from 293-F cells rather efficiently (Fig. 4c, upper panel), accumulation levels comprising on average 6% of the total F protein pools, independently of stable or transient expression of F protein or of co-expressed M protein (Fig. 4c, and not shown). Thus, the M and F proteins seem not to act cooperatively in driving VLP production (Fig. 4d). Again, the amount of F proteins pelleted from supernatants of infected cells corresponded to that seen for the VLPs (Fig. 4d).

**DISCUSSION**

MV M proteins have been studied in detail since, about two decades ago, they were found to be extensively mutated in persistently infected brain cells. Such mutations were shown to cause aberrant expression, functional impairment, instability or complete absence of these proteins (reviewed by Rima & Duprex, 2005; Schneider-Schaulies et al., 1995, 2003). Although abrogation of M protein expression or function has been considered as causatively linked to the lack of virus production in the central nervous system, the role of this protein in promoting particle assembly and release has not been satisfactorily addressed.

M proteins of vaccine and WT strains have long been known to differ with regard to their electrophoretic mobility in SDS-PAGE (Rima et al., 1995, 1979). We have now established that the amino acid residue at position 89 largely accounts for this phenomenon (Fig. 1d) and that, although other residues distinctive for WT strains (such as aa 64 and aa 209) may also play a part, their contribution is much less significant. Substitution of E by K at aa 89 was recently shown to greatly increase the ability of a WT recombinant MV to replicate in Vero cells, as did substitution of aa 64 (P→S) (Miyajima et al., 2004; Tahara et al., 2005). However, the consequences of these particular substitutions in functional terms have not been further addressed in these studies and thus remain to be established. Clearly aa 89 does not detectably affect the biological properties of M proteins further, for example, their ability to oligomerize (Fig. 1c) and thus, similarly to its functional analogue Ebola virus VP40 protein, is likely to be of critical importance in driving particle formation.
Although M proteins transiently expressed in Madin–Darby canine kidney (MDCK) cells did not associate with the plasma membrane (Riedl et al., 2002), a substantial fraction of all M proteins analysed in our study were readily detectable on the cell surface (Fig. 1b). Since MV M proteins contain all of the signals for budding, as is evident from their ability to promote VLP formation independently (Figs 3, 4), they would be expected to accumulate at the cell membrane. Thus, the failure of M proteins to associate with the plasma membrane in MDCK cells might relate to the polarized phenotype of these cells, which could influence protein trafficking. It might be interesting to determine whether this affects VLP production as well.

We have shown that M proteins can reproducibly be separated into doublets (Figs 1, 2, 4) and that this is independent of both their origin and the specific amino acid at position 89. This critically depended on the SDS-PAGE conditions and the doublet could be converted into a single band when acrylamide concentration, separation distance or running conditions were altered, for example, when we modified the separation distance deliberately to enable documentation of the ubiquitinated M protein fraction (Fig. 4a). Although it is tempting to speculate that the two M protein species detected arose from differential post-translational modifications, it is unclear what these could be. Lipidation, as typically observed for membrane-associated proteins, including human immunodeficiency virus gag protein, would be the most likely post-translational modification. As yet, only F protein has been found to be palmitoylated in MV-infected cells (Caballero et al., 1998). Although they cannot be excluded, modification by phosphorylation or ubiquitin conjugation is unlikely to account for the two M protein species; the first has never been evidenced for MV M protein, and the ease of detection of the doublet as compared to the difficulty to detect the minor fraction of ubiquitinated M proteins only after addition of MG132 (Fig. 4a) argues against the second one.

As seen for their orthologues, MV M proteins are apparently transiently mono-ubiquitinated (Fig. 4a) and this can only be detected for a minor fraction of this protein, due to the highly transient nature of this modification (Demirov & Freed, 2004; Hartlieb & Weissenhorn, 2006; Martin-Serrano et al., 2005, 2004; Strack et al., 2000). The latter is consistent with the requirement of this class E factor-dependent modification for initiation of sequential recruitment of ESCRT (endosomal sorting complex required for transport) complexes and subsequent sorting and vesicle budding into late endosomal compartments (also referred to as multivesicular bodies) or, for viruses, sorting to and ‘pinching off’ from plasma membranes. Whether M protein trafficking and/or budding of MV VLPs...
or entire particles indeed involves a retrograde endosomal transport, as shown for Ebola virus VP40, needs to be further investigated (Kolesnikova et al., 2004a, b). Classical L domains, which are known to be crucial for recruitment of ESCRT complexes and subsequent sorting in retroviral gag and M proteins of filo- and rhabdoviruses (Freed, 2002; Timmins et al., 2004), have not as yet been identified in MV M proteins. Our finding that M proteins promote VLP formation suggests the presence of at least one functional L domain; the highly conserved YMFL (aa 52–55) or PSVP (aa 311–314) motifs could be candidates for interaction with ALIX/AIP-1 or Tsg101, respectively. A recently described L domain for paramyxoviruses (FPIV) has not been detected within the MV M protein sequence (Schmitt et al., 2005).

Consistent with earlier findings (Manie et al., 2000; Vincent et al., 2000), a fraction of the total individually expressed M and F proteins co- floated with DRMs (Fig. 2). Whilst in these studies F protein failed to recruit M protein into DRMs, we reproducibly observed about a fourfold increase in DRM-resident M protein upon stable or transient co-expression of either an autologous or heterologous F protein (Fig. 2c, d). The extent to which the cytoplasmic tail of F protein contributes to this recruitment could not be determined at present, since the only antibody able to detect this protein by immunoblot is directed against its cytoplasmic tail and thus does not recognize F proteins lacking this particular domain. The reasons for the differences in the study by Manie et al. (2000) and ours are unclear. They may, however, reflect the use of different expression systems. Although M and F proteins apparently cooperate in terms of DRM recruitment, they do not with regard to VLP formation. Whilst both proteins can promote VLP formation on their own, the amount of M or F protein, respectively, released from co-transfected cultures does not significantly increase, and, remarkably, corresponds almost exactly to that seen in virions (Fig. 2c).

Firstly, this indicates that the ability of MV proteins to promote particle formation and their DRM association are not correlated; this may be influenced by the fact that DRMs isolated by standard procedures contain both plasma and internal membrane fractions, while particle formation should be confined to plasma membrane-associated proteins only. However, it is clear that MV budding is not strictly confined to DRMs (Vincent et al., 2000). Secondly, in contrast to Ebola virus where glycoprotein co-expression markedly enhances VP40-driven particle production (Licata et al., 2004), MV F fails to enhance M protein-driven VLP formation. Due to a current lack of reagents it was not possible to determine whether VLPs released from doubly transfected cells contained M or F proteins alone or both. Similar to their orthologues in MV, simian virus 5 M and F proteins can induce budding which results in the production of VLPs containing M and F proteins (Takimoto & Portner, 2004). A sequence motif (TYTLE), conserved within the cytoplasmic domains of Sendai virus and human parainfluenza virus 1 F proteins, was found to be required for particle formation (Takimoto et al., 2001). However, this is not contained within the C terminus of MV F protein. Thus, both domains are important for this activity and its mechanism needs to be addressed in future experiments. Remarkably, the M proteins, but even more frequently, the cytoplasmic tails of F proteins are loaded with mutations in persistent MV brain infections. The experimental setups established in our manuscript enable, amongst other follow-up studies, to evaluate to what extent these mutations might restrict particle production and thus contribute to establishment of persistence.

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