Alternative mechanisms of interaction between homotypic and heterotypic parainfluenza virus HN and F proteins

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Cell fusion by human parainfluenza virus (HPIV) type 2 or type 3 requires the coexpression of both the fusion (F) and haemagglutinin–neuraminidase (HN) glycoproteins from the same virus type, indicating that promotion of fusion requires a type-specific interaction between F and HN. In this report we have further investigated the interaction of the ectodomains of the F and HN glycoproteins from HPIV2 and HPIV3. We constructed mutants of the HPIV2 F and HPIV3 F proteins (F-KDEL) lacking a transmembrane anchor and a cytoplasmic tail, and containing a C-terminal signal for retention in the endoplasmic reticulum (ER). The PI2 and PI3 F-KDEL proteins were both found to be retained intracellularly, and neither could induce cell fusion when co-expressed with homotypic HN proteins. Qualitative and quantitative cell-fusion assays also showed that both the PI2 F-KDEL and PI3 F-KDEL proteins have inhibitory effects on PI2 F- and HN-induced cell fusion. However, the F-KDEL mutants were found to inhibit cell fusion by two distinct mechanisms. An interaction between PI2 F-KDEL and PI2 HN results in intracellular retention of HN, and a block in its transport to the cell surface. In contrast, PI3 F-KDEL was found to suppress the steady-state intracellular expression levels of HPIV2 HN. These results support the conclusion that fusion involves an interaction between the HN and F proteins, and suggest that an association between F and HN may occur in the ER.

Introduction

The entry of enveloped viruses into cells requires binding of the virus to one or more cellular receptors and the subsequent fusion of the viral envelope with a cellular membrane. With the paramyxoviruses, the functions of receptor binding and fusion are mediated by two glycoproteins, one having haemagglutinin and neuraminidase activity (HN). The haemagglutinin activity reflects the attachment function, allowing the virion to bind to sialic acid-containing cell-surface receptors. The neuraminidase activity cleaves sialic acid from soluble and membrane-bound glycoconjugates. Recently, a third function of the HN protein has been described: fusion promotion activity (Ebata et al., 1991; Heminway et al., 1994; Hu et al., 1992; Lamb, 1993; Merz & Wolinsky, 1983; Miura et al., 1982; Shibuta et al., 1983). The other glycoprotein (F) is directly involved in fusion activity, which is responsible for virus penetration of the host cell membrane and plays a pivotal role in cell-to-cell fusion (Choppin & Scheid, 1980; Scheid & Choppin, 1974).

The F protein, a type I integral membrane protein, is synthesized as a biologically inactive precursor molecule (F₀) in the rough endoplasmic reticulum (ER), and is subsequently cleaved by a host protease resulting in the biologically active disulfide bond-linked subunits F₁ and F₂ (Homma & Ohuchi, 1973; Scheid & Choppin, 1974). This active fusion glycoprotein is likely assembled into noncovalently associated homotrimers (Russell et al., 1994) and is then transported through the exocytic pathway to the plasma membrane. In the late stages of infection, the HN and F proteins accumulate on the cell membrane, resulting in fusion between neighbouring cells to produce syncytia, which is a typical cytopathogenic effect of the paramyxoviruses.

With most paramyxoviruses, evidence indicates that efficient cell fusion induced by paramyxovirus glycoproteins requires expression of both HN and F proteins (Bousse et al., 1994; Heminway et al., 1994; Hu et al., 1992). It was also shown that cell fusion induced by a paramyxovirus, such as human parainfluenza virus (HPIV) type 2 or 3, requires a type-specific interaction between the HN and F proteins (Horvath et al., 1992; Hu et al., 1992; Tsurudome et al., 1995; Yao & Compans, 1995). Since the promotion of fusion requires that...
the HN and F proteins be derived from the same virus, it was proposed that the HN and F proteins must communicate in a virus-specific manner, allowing a functional molecular interaction between homotypic HN and F proteins (Lamb, 1993). Several reports have provided evidence that specific regions on the F and HN proteins are involved in this type-specific interaction. For instance, a study with Sendai virus and HPIV3 HN protein chimeras indicated that an 82-amino-acid region in the ectodomain adjacent to the transmembrane domain is involved in specific F–HN interaction (Tanabayashi & Compans, 1996). Furthermore, peptides corresponding to a heptad repeat sequence adjacent to the transmembrane domain of the Sendai virus, HPIV2, HPIV3 and other F proteins are type-specific inhibitors of paramyxovirus-induced cell fusion (Lambert et al., 1996; Rapaport et al., 1995; Yao & Compans, 1996).

We have extended the observations reported above by examining the interaction of the ectodomain of the F protein with HN glycoproteins from HPIV2 and HPIV3. For this purpose, we constructed mutants of HPIV2 F and HPIV3 F proteins (F^−KDEL) lacking a transmembrane anchor and a cytoplasmic tail, and containing a C-terminal signal for retention in the endoplasmic reticulum (ER). Furthermore, we carried out a series of experiments which examined potential interactions between HPIV2 F and HN proteins.

### Methods

- **Cells, viruses and antisera.** CV-1, HeLa and HeLa-T4 cells were grown in Dulbecco’s modified Eagle’s medium (DMEM) with 10% foetal calf serum (HyClone Laboratories). The recombinant vaccinia virus vTF7-3 and wild-type vaccinia virus HDJ-I were provided by Bernard Moss (NIIH, Bethesda, MD, USA). Both vaccinia virus stocks were propagated on HeLa cells and titrated by plaque assay on CV-1 cells. Polyclonal rabbit antibodies against HPIV2 were generated as described by Hu et al. (1992). Monoclonal antibodies against PI3 F were kindly provided by K. Orvell (SBL Karolinska Institute, Stockholm, Sweden).

- **Plasmid constructions and mutagenesis.** A DNA fragment encoding a mutant PI2 F gene, lacking the transmembrane anchor and cytoplasmic tail and containing an ER retention signal (KDEL) appended to the C terminus, was amplified by PCR. The sense primer (ACTTTC-AATGCTACGTACGTG) consisted of an oligonucleotide corresponding to nucleotides 1465–1685 of pGEM-3-PI2 F plasmid with a modification to include a SfiI restriction site. The antisense primer (ACCGGTC-GACTTACAGCTCCTTCTTGCTGTCCCTGCGCTGTGATTAGG) corresponds to nucleotides 1620–1612 of pGEM-3-PI2 F plasmid with the introduction of nucleotide sequences encoding the ER retention signal KDEL followed by a stop codon and a signal KDEL. The cloned DNA was propagated in bacteria and purified. The plasmid was digested with SfiI and NotI, resulting in plasmid pGEM-3-PI2 F-KDEL. The 675-bp DNA fragment was ligated into the SfiI and NotI sites of pGEM-3 and resulted in the plasmid designated pGEM-3-PI2 F-KDEL. The clone encoding a PI3 F mutant, lacking the transmembrane anchor and cytoplasmic tail and containing an ER retention signal (KDEL) appended to the C terminus, was constructed by methods similar to those described above. Two synthetic oligonucleotides were used to PCR amplify the mutant PI3 F protein. The forward primer was 5' TCCAAGATATGATTTTGCTAAATG 3', corresponding to nucleotides 1312–1325 of pGEM-3-PI3 F plasmid with a modification to include a NsiI site, and the reverse primer was 5' ACCGCGTCGCA-TACAGCTCCTGCTCCCGCTGATTAGG 3', corresponding to nucleotides 1672–1655 of pGEM-3-PI3 F plasmid and sequences encoding KDEL followed by a stop codon and a SfiI site. The 963-bp DNA fragment was digested with NsiI and SfiI, then ligated into the pGEM-3-PI3 F-KDEL, resulting in plasmid pGEM-3-PI3 F-KDEL. The clone encoding a PI3 F mutant, lacking the transmembrane anchor and a cytoplasmic tail and containing an ER retention signal (KDEL) appended to the C terminus, was constructed by methods similar to those described above. Two synthetic oligonucleotides were used to PCR amplify the mutant PI3 F protein. The forward primer was 5' TCCAAGATATGATTTTGCTAAATG 3', corresponding to nucleotides 1312–1325 of pGEM-3-PI3 F plasmid with a modification to include a NsiI site, and the reverse primer was 5' ACCGCGTCGCA-TACAGCTCCTGCTCCCGCTGATTAGG 3', corresponding to nucleotides 1672–1655 of pGEM-3-PI3 F plasmid and sequences encoding KDEL followed by a stop codon and a SfiI site. The 963-bp DNA fragment was digested with NsiI and SfiI, then ligated into the pGEM-3-PI3 F-KDEL. All products were sequenced to ensure that correct mutations were present and no further mutations had occurred during the PCRs.

- **Transfection and transient expression.** Subconfluent monolayers of HeLa-T4 cells were infected with vTF7-3 virus at an m.o.i. of 10 p.f.u. per cell. After 1 h at 37 °C, cells were washed with PBS three times and then transfected with the indicated mixtures of plasmids and
Interaction of F and HN glycoproteins

Fig. 2. Inhibition of PI2 F/HN-induced cell fusion. HeLa-T4 cells were cotransfected with (a) PI2 F (3 µg) + PI2 HN (3 µg); (b) PI2 F*KDEL (3 µg) + PI2 HN (3 µg); (c) PI2 F (3 µg) + PI2 HN (3 µg) + PI2 F*KDEL (9 µg); (d) PI2 F (3 µg) + PI2 HN (3 µg) + PI3 F*KDEL (9 µg). pGEM-3 DNA was added to some cultures to keep the total amount of transfected DNA per dish constant as detailed in Methods. Cells were photographed at 16 h post-transfection.

10 µl of Lipofectin (GIBCO BRL) in 1 ml DMEM. The total amount of transfected DNA per dish was kept constant, by adjustment as necessary with pGEM-3 plasmid to avoid differences in transfection efficiency. At 16 h post-infection and post-transfection, cells were starved in DMEM lacking methionine and cysteine for 1 h, pulse-labelled with 100 µCi/ml [35S]methionine/cysteine for the indicated time at 37 °C and then chased with DMEM containing 10% foetal calf serum. The radiolabelled cells were lyed with Lysis buffer containing 50 mM Tris (pH 7–4), 150 mM NaCl, 1% Triton X-100, 1% Nonidet P-40 and 1 mM EDTA and clarified by centrifugation. The PI2- or PI3-specific F glycoproteins were immunoprecipitated with polyclonal rabbit antibodies against PI2 or monoclonal antibodies against PI3 F, and with protein A–agarose beads (ImmunoPure; Pierce) as described by Ray et al. (1989). The precipitates were analysed on 8% acrylamide gels containing SDS under reducing conditions and subjected to autoradiography.

Chemical cross-linking. Chemical cross-linking on NP-40-solubilized cell lysates was performed by a method similar to that described by Paterson & Lamb (1993). Cross-linking reactions were carried out with concentrations from 1 to 3 mM of 3,3′-dithiobis sulosuccinimidylpropionate (DTSSP) (Pierce) at 4 °C for 60 min. After cross-linking, proteins were immunoprecipitated and analysed by SDS–3.5% PAGE under nonreducing conditions.

Quantitative fusion assay. Cell fusion was quantified according to the fusion-dependent reporter gene activation method described by Nussbaum et al. (1994), with slight modifications. One population of HeLa-T4 cells was infected with recombinant vaccinia vTF7-3 and transfected with the indicated plasmids. A second population of HeLa-T4 cells was infected with vaccinia IHD-J and transfected with plasmid pGINT7β-gal, which contains the β-galactosidase gene under control of the T7 promoter. Fourteen hours post-transfection, the two cell populations were trypsinized and mixed by adding 100 µl of each cell population (10⁶ cells/ml) in a 96-well tissue culture plate. The plate was incubated at 37 °C for 5 h, and cell fusion was measured at the indicated times by the colorimetric lysate assay as described previously (Yao & Compans, 1995). Fusion activity was expressed as a percentage of the amount of β-galactosidase production in the cells transfected with wild-type PI2 F/HN genes.

Cell-surface expression. (i) Cell-surface biotinylation was carried out essentially as described by Le Bivic et al. (1989). The radiolabel-chased cells were incubated with a 0.5 mg/ml solution of sulfo-
Results

HPIV2 F–KDEL and HPIV3 F–KDEL are transport-defective and are retained in the ER

To characterize the mutant proteins, the truncated PI2 F and PI3 F genes, which encode proteins that lack the transmembrane anchor and cytoplasmic tail and contain a C-terminal ER retention signal (KDEL), were expressed in HeLa-T4 cells using the vaccinia virus–T7 RNA polymerase expression system. As shown in Fig. 1, both the F0 precursor and proteolytically processed F1 were detected in lysates of wild-type PI2 F- or PI3 F-transfected cells, and a significant fraction was cleaved to F1 during a 3 h chase (Fig. 1a, lanes 1 and 3). However, in the case of the PI2 F–KDEL- and PI3 F–KDEL-transfected cells, unlike wild-type PI2 F and PI3 F, the major polypeptide immunoprecipitated in cell lysates from a 3 h chase was the F0 precursor. Only a faint band corresponding to F1 was identified in PI2 F–KDEL-transfected cells (Fig. 1a, lane 2) and no F1 was detected in PI3 F–KDEL-transfected cells (Fig. 1a, lane 4). As expected, both truncated PI2 F–KDEL and PI3 F–KDEL proteins were detected only in the cell lysate but not on the cell surface, nor were they secreted into the medium; however, soluble PI2 F’ and PI3 F’ proteins identical to the F–KDEL proteins, but lacking the ER retention signals, were found to be secreted into media (data not shown). These results indicate that protein transport from the ER to the cell surface and protein processing are prevented by the addition of the KDEL signals to the C termini.

To examine whether PI2 F–KDEL and PI3 F–KDEL were able to undergo the normal oligomerization process, the cross-linking reagent DTSSP was used. In the absence of DTSSP, the monomer species was mainly detected for all the proteins (data not shown). As shown in Fig. 1(b), with addition of DTSSP to cells in the presence of 0.5% Nonidet P-40, we observed that both PI2 F and PI3 F were resolved as three predominant species (1, 2 and 3), consistent with a previous report that paramyxovirus F forms trimers (Russell et al., 1994). Treatment of PI2 F–KDEL- or PI3 F–KDEL-transfected cells with DTSSP yielded a pattern of oligomers which was very similar to those observed for the wild-type proteins. These results indicate that the wild-type and mutant F proteins are similarly folded into oligomeric structures.

Both HPIV2 F–KDEL and HPIV3 F–KDEL can suppress HPIV2 F/HN-induced syncytium formation

The results described above indicate that the HPIV2 F–KDEL protein is transport-defective and is retained in the ER. If F and HN physically interact, then the mutant F protein might bind with the HN protein and retain HN intracellularly, resulting in reduction of cell fusion. As can be seen in Fig. 2(a), wild-type HPIV2 F protein coexpressed with HPIV2 HN mediated rapid and extensive cell fusion while the PI2 F–KDEL (Fig. 2b) or PI3 F–KDEL proteins (data not shown) coexpressed with PI2 HN did not induce cell fusion. When PI2 F and HN as well as PI2 F–KDEL were coexpressed in HeLa-T4 cells, the extent of cell fusion was significantly reduced (Fig. 2c). With increasing ratios of input PI2 F–KDEL plasmid DNA during transfection, 1:1, 1:2, 1:3, a progressive decrease in cell fusion was observed.

To further determine the specificity of the F–KDEL effect on glycoprotein trafficking, PI2 F and HN were coexpressed with heterotypic PI3 F–KDEL at a ratio of F:F–KDEL of 1:3. Interestingly, as shown in Fig. 2(d), the PI3 F–KDEL protein was also found to inhibit PI2 F- and HN-induced cell fusion. Plasmid pGINT7β-gal, which contains the β-galactosidase gene under the control of the T7 RNA polymerase promoter, and plasmid pGEM-3-PIV3F, which encodes wild-type PI3 F (as shown in Fig. 3) were examined as controls in our study.
Neither of these constructs showed any inhibitory effect on PI2 F- and HN-induced cell fusion.

These observations were also confirmed by a quantitative cell-fusion assay. Fig. 3 shows the results of colorimetric assays, expressed as percentages of the β-galactosidase production in cells transfected with wild-type PI2 F and HN. When PI2 F and HN were coexpressed in the presence of PI2 F⁻KDEL or PI3 F⁻KDEL at an F:F⁻KDEL ratio of 1:2, only 31% or 24% of the levels of wild-type PI2 F- and HN-induced fusion activity were detected, but when PI2 F and HN were coexpressed with heterotypic wild-type PI3 F, the extent of cell-fusion activity was similar to that of wild-type PI2 F- and HN-induced fusion. These results demonstrate that both PI2 F⁻KDEL and PI3 F⁻KDEL inhibit PI2 F- and HN-induced cell fusion, whereas coexpression of wild-type PI3 F has no effect.

**HPIV2 F⁻KDEL interferes with the transport of HPIV2 HN to the cell surface**

To further investigate the mechanism by which PI2 F⁻KDEL inhibits PI2 F- and HN-induced cell fusion, we analysed the expression of PI2 F and HN glycoproteins on the cell surface. HeLa-T₄ cells were cotransfected with PI2 F and HN and PI2 F⁻KDEL at increasing F:F⁻KDEL ratios (1:1, 1:2, 1:3), and then labelled for 30 min at 16 h post-transfection. After a chase period of 3 h, the cell-surface proteins were biotinylated at 4 °C and detected as described in Methods. We observed a progressively decreasing level of surface expression of PI2 HN, with increasing amount of input plasmid PI2 F⁻KDEL (Fig. 4a). A lower level of PI2 F on the cell surface was also observed when compared to cells transfected with PI2 F and HN alone, but the levels of PI2 F on the cell surface did not decrease as much as PI2 HN did. The surface expression in the presence of PI2 F⁻KDEL was also quantitatively determined (Fig. 4c). Since decreased surface expression was correlated with the level of PI2 F⁻KDEL plasmid, further studies on the intracellular expression of PI2 F or HN in the presence of PI2 F⁻KDEL were performed. The synthesis of PI2-specific glycoproteins was analysed in a pulse–chase experiment. As shown in Fig. 5(a), we observed no apparent difference in the intracellular PI2 HN level between cells expressing HN alone and cells coexpressing HN and PI2 F⁻KDEL. There appeared to be a slight suppression
of 2F protein levels (Fig. 5B), relative to expression of PI2 F alone. However, a reduced amount of PI2 F was also detected in HeLa-T4 cells cotransfected with plasmid pGIN7pβ-gal which did not show any reduction in cell-fusion activity (data not shown), indicating that the slight suppression of F levels is nonspecific and does not inhibit the function of the viral proteins.

Taken together, the results support the conclusion that physical association between PI2 F-KDEL and PI2 HN results in intracellular retention of HN and a block in its transport to the cell surface, resulting in the reduced cell-fusion activity.

**HPIV3 F'-KDEL suppresses HPIV2 F/HN expression**

In a similar manner, the possible mechanism involved in down-regulation of PI2 cell fusion by heterotypic PI3 F'-KDEL was examined. Interestingly, a significant reduction in the amount of PI2 HN and F expressed on cell surfaces was observed by a cell-surface biotinylation assay (Fig. 4b, d) on cells cotransfected with PI3 F'-KDEL, which was also confirmed by FACS assay (not shown). The reduction of HN on cell surfaces was found to be dose-dependent, since a decreasing level of HN was correlated with an increasing amount of input PI3 F'-KDEL plasmid. In contrast to cells cotransfected with PI2 F'-KDEL, however, the level of HN detected intracellularly was also significantly reduced (Fig. 5a). In order to determine whether the reduced HN cell-surface level when coexpressed with PI3 F'-KDEL could result from the reduction of intracellular HN levels, we compared the reduction of cell surface and cell lysate HN levels in cells coexpressing HN with PI2 F'-KDEL or PI3 F'-KDEL. Fig. 6 shows that similar decreases in levels of HN detected on cell surfaces and in cell lysates were...
observed with increasing amounts of input PI3 F'-KDEL plasmid. In contrast, a progressively decreasing level of HN on cell surfaces but not in cell lysates was seen with increasing amounts of PI2 F'-KDEL. Together, these data indicate that unlike PI2 F'-KDEL, PI3 F'-KDEL inhibits PI2 F/HN-induced cell fusion by an inhibitory effect on the steady-state level of HN expression or folding, rather than by specifically preventing HN transport to the cell surface.

Discussion

A number of reports have suggested that an interaction occurs among paramyxovirus F and HN which could induce a conformational change in the F protein, resulting in exposure of the hydrophobic fusion peptide and triggering the fusion process. Malvoisins & Wild (1993) reported that measles virus F and HA proteins coexpressed in HeLa cells infected with vaccinia virus recombinants can be coimmunoprecipitated at the cell surface after exposure to a cross-linking reagent. We have observed that anti-PI2 HN antiserum coprecipitated PI2 F when the homotypic PI2 F and PI2 HN were coexpressed, but did not precipitate the F proteins from other paramyxoviruses when heterotypic F genes were coexpressed with PI2 HN (Yao et al., 1997). More recently, Stone-Hulslander & Morrison (1997) reported that an interaction between the HN and F proteins can occur before or after HN protein attachment in Newcastle disease virus-infected cells. Tanaka et al. (1996) reported a possible intracellular interaction not only between homotypic PI3 F and HN, but also between PI3 F and heterotypic HN proteins from Sendai virus and simian parainfluenza virus 5. The objective of the present project was to further examine the possible intracellular association of F and HN. To this end, we constructed genes which encode two mutant fusion proteins from HPIV2 and HPIV3, designated PI2 F'-KDEL and PI3 F'-KDEL, which contain the tetrapeptide KDEL attached to the C termini of F proteins lacking the cytoplasmic tail and transmembrane domain. We found that both PI2 F'-KDEL and PI3 F'-KDEL mutants were only localized intracellularly, and were not proteolytically cleaved efficiently. These data support the idea that the KDEL signal sequence marks proteins that are to be retained in the ER (Jackson et al., 1990). Consequently, the KDEL sequence causes inhibition of the normal proteolytic processing of the F₀ precursor protein, which occurs in the Golgi complex (Morrison et al., 1985).

We have obtained evidence for the interaction of the external domain of the PI2 F glycoprotein with PI2 HN by showing an inhibitory effect of PI2 F'-KDEL on PI2 F- and HN-induced cell fusion. Consistent with previous reports, coexpression of homotypic PI2 F and HN in HeLa-T₄ cells resulted in extensive syncytium formation. However, the level of cell fusion was significantly reduced by coexpression of PI2 F'-KDEL, as was also shown by use of a quantitative cell-fusion assay. Further analysis of the mechanism of inhibition indicated that the level of PI2 HN expressed on cell surfaces was greatly decreased when compared with expression of HN alone. However, the intracellular levels of PI2 HN were not significantly different from that seen upon expression of HN alone. These results indicate that the PI2 F'-KDEL mutant protein interacts with PI2 HN and retains it intracellularly. Retention of HN by a homotypic F'-KDEL mutant has also been reported by Tanaka et al. (1996) in studies with HPIV3 HN and F proteins. These results may reflect a transient intracellular F–HN interaction. No significant inhibitory effect by PI2 F'-KDEL was found on the level of PI2 F biosynthesis. Although a reduction in cell-surface PI2 F levels was observed, F'-KDEL proteins may form heterooligomers with wild-type F. We found that F'-KDEL mutant proteins can form homooligomers, which can interfere with wild-type F exiting the ER and transiting through the Golgi complex to the cell surface. Because a similar pattern of reduction in PI2 F cell-surface expression levels was also observed upon coexpression of PI2 F with plasmid pGINT7/β-gal (not shown), which did not show any inhibition of PI2 F- and HN-induced cell fusion, the lower level of PI2 HN expressed on the cell surface is most likely responsible for the inhibition of cell fusion.

The inhibition of syncytium formation induced by PI2 F and HN by heterotypic PI3 F'-KDEL appears to occur by a different mechanism. Although we observed decreased PI2 HN cell-surface expression, we also found a corresponding decrease in intracellular levels of HN, which was not found in cells cotransfected with PI2 F'-KDEL. The suppression of heterotypic HN expression by PI3 F'-KDEL was also observed by Tanaka et al. (1996), who reported that PI3 F'-KDEL can down regulate not only PI3 HN, but also simian parainfluenza virus 5 and Sendai virus HN and measles virus H expression by causing instability of the heterotypic HN proteins. They have proposed that the down-regulation of heterotypic HN expression by the F protein may be responsible for the observed serotypic-specific restriction of syncytium formation. However, this is unlikely since we found no inhibitory effect of wild-type PI3 F on PI2 HN expression. Similarly, coexpression of Sendai virus F did not suppress the expression of HPIV1 HN (Bousse et al., 1997). Previous studies have also shown that coexpression of full-length PI3 F did not block cell fusion induced by PI2 F plus HN (Hu et al., 1992).

Our results therefore indicate that inhibition of PI2 F- and HN-induced cell fusion by PI2 F'-KDEL and by PI3 F'-KDEL is mediated by two distinct mechanisms. PI2 F'-KDEL prevents transport of PI2 HN to the cell surface, presumably via F–HN interaction, while PI3 F'-KDEL suppresses the steady-state level of intracellular PI2 HN expression. The mechanism by which PI3 F'-KDEL affects the steady-state levels of heterotypic HN expression could involve either an early biosynthesis block, interfering with HN folding or glycosylation, or could be a result of causing instability of heterotypic HN proteins. Since paramyxovirus HN proteins are folded very slowly into their final conformation in the ER.
(Parks & Lamb, 1990), it is possible that PI3 F'-KDEL may interact with unfolded PI2 HN and interfere with its folding into a conformation recognizable by antibody. In addition, since pulse-chase experiments did not show a progressive reduction in HN levels with longer chases up to 3 h (not shown), it appears that PI3 F'-KDEL is more likely to block PI2 HN biosynthesis or folding rather than affect PI2 HN stability. Previous work has shown that cell fusion caused by HPIV2 and HPIV3 strictly requires coexpression of F and HN proteins from the same virus type. This high level of specificity, along with the low level of sequence identity between PI2 F and PI3 F, also suggests that PI3 F'-KDEL suppresses PI2 HN steady-state expression levels via a different type of interaction than the one between PI2 F'-KDEL and PI2 HN. Since both PI2 F'-KDEL and PI3 F'-KDEL mutant proteins can oligomerize and form homotrimers, it does not appear that they are malfolded and form inappropriate disulfide bonds with other molecules in the ER. This conclusion is also supported by observations that PI2 F'-KDEL and PI3 F'-KDEL do not interfere with either murine leukaemia virus envelope protein- or human immunodeficiency virus gp160 envelope protein-induced cell fusion (data not shown). The question why only the PI3 F'-KDEL mutant protein but not wild-type PI3 F has an inhibitory effect on steady state levels of HN expression is still not resolved. One possibility is that the PI3 F'-KDEL mutant protein might be more effective due to its retention in the ER.

In conclusion, the results presented here confirm previous observations that HPIV2 glycoprotein-induced cell fusion requires the coexpression of both PI2 F and HN surface glycoproteins. Studies of the inhibition of PI2 F- and HN-induced cell fusion by PI2 F'-KDEL suggest an intracellular association between the PI2 F and HN glycoproteins and that the interactive domain which associates with HN resides in the ectodomain of F. Further, our results indicate that the interaction of PI2 F'-KDEL and HN may occur in the ER, which is consistent with other results that HN-F protein interaction can occur before HN interacts with receptors (Stone-Hulslander & Morrison, 1997).

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