Transfer of endoplasmic reticulum and Golgi retention signals to human immunodeficiency virus type 1 gp160 inhibits intracellular transport and proteolytic processing of viral glycoprotein but does not influence the cellular site of virus particle budding

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In this study, specific signals known to mediate endoplasmic reticulum or Golgi localization of transmembrane proteins have been transferred to the human immunodeficiency virus type 1 (HIV-1) env gene product. The intracellularly retained recombinant glycoproteins were not proteolytically processed to gp120 and gp41, which is further evidence that this process occurs at a later stage in the transport pathway, presumably within or near the trans-Golgi network. Since the subcellular localization of the viral glycoproteins of enveloped viruses can be one of the factors determining the cellular site of particle assembly and release, experiments were performed to determine if this property was altered by coexpression of the recombinant HIV-1 glycoproteins. When wild-type virus was compared to mutant virus encoding the intracellularly retained glycoproteins, the extent of HIV-1 particle release into the extracellular medium remained unaffected, and electron-microscopic analysis did not reveal any significant alteration in the cellular sites of particle assembly and budding. Thus, in COS-7 cells, altered subcellular localization of the viral glycoprotein does not exert a dominant influence on the assembly site of the HIV-1 particle.

Introduction

Human immunodeficiency virus type 1 (HIV-1) particle assembly and budding take place at the plasma membrane of the host cell and require the expression of only the internal (Gag) structural proteins. However, when the viral glycoproteins (Env) are also present, these are incorporated into virions, which only then are infectious. The viral matrix (MA) domain of the precursor Pr55$^ag$ plays a central role both in particle formation and glycoprotein incorporation, and MA mutants have been generated which result in normal release of particles lacking Env, although Env had been present in the transfected cell (Yu et al., 1992; Dorfman et al., 1994). Retroviral transmembrane glycoprotein (TM) and MA are in close spatial proximity in virions (Gebhardt et al., 1984) and it appeared reasonable to assume that a direct or indirect interaction between MA and TM could be of functional importance. In polarized epithelial cells, expression of the Gag proteins alone leads to particle budding from both the apical and basolateral membranes. However, when the HIV-1 glycoprotein, which itself has an intrinsic basolateral localization in these cells (Owens & Compans, 1989), is coexpressed, particle budding is redirected predominantly to the basolateral membrane (Owens et al., 1991). This polarized release of particles depends on the presence of both wild-type MA and Env. MA mutants, mentioned above, which fail to result in glycoprotein incorporation, as well as env mutants truncated within the cytoplasmic C terminus, again result in particle budding from both membranes (Lodge et al., 1994). Thus, it is conceivable that Env can determine the exact localization of particle release by an early direct or indirect interaction with the viral MA domain.

Studies on other enveloped viruses have also provided evidence for a direct or indirect interaction between MA and Env. For example, G glycoprotein of vesicular stomatitis virus (VSV), embedded in liposomes, forms more stable oligomeric structures when the MA protein is also present (Lyles et al., 1992). Sendai virus MA protein, when expressed alone, is not membrane-bound but demonstrates an altered subcellular...
localization to membranous structures when the glycoproteins HN or F are coexpressed (Sanderson et al., 1993). In these latter two cases (VSV and Sendai virus) and also with lentiviruses and type C retroviruses, electron-microscopic examination of infected or transfected cells reveals assembling and budding structures only at the plasma membrane. It is, however, conceivable that a putative direct or indirect interaction between MA and Env occurs intracellularly and leads to the generation of submicroscopic complexes, which are subsequently cotransported to the assembly site at the plasma membrane. An interaction between internal structural proteins and Env within the cell and not at the plasma membrane would also occur in the case of those viruses which assemble and bud from intracellular membranous compartments (e.g. coronaviruses, Hepatitis B virus, bunyaviruses and others). In these cases, it is assumed that the intrinsic subcellular localization of one of the viral glycoproteins determines the budding site (for review see Hobman, 1993).

Proteolytic processing of HIV-1 gp160 to gp120 and gp41 occurs during intracellular transport and is essential for the attainment of membrane-fusion ability and viral infectivity (McCune et al., 1988; Willey et al., 1988; Bosch & Pawlita, 1990). There is a considerable amount of evidence suggesting that this process occurs at a late stage during intracellular transport and predominantly involves the cellular protease furin, which is located within the trans-Golgi network (TGN) (Hallenberger et al., 1992; Bosshart et al., 1994; Molley et al., 1994; Schäfer et al., 1995; Kantanen et al., 1995). However, this issue is not completely resolved and results indicating that proteolytic cleavage could occur at earlier intracellular sites have also been obtained (Stein & Englemann, 1990; Raja et al., 1993).

In this paper, we have transferred signals reported to mediate residency within the endoplasmic reticulum (ER) and Golgi complex (for review see Nilsson & Warren, 1994) to the HIV-1 glycoprotein. We have subsequently determined the proteolytic cleavage status of the intracellularly retained recombinant molecules and examined whether the altered subcellular localization of the viral glycoproteins has an influence on the site of particle assembly and budding.

Methods

Expression plasmids. PNL4-3H11env (Bosch & Pawlita, 1990; Wilk et al., 1992), a derivative of pNL4-3 (Adachi et al., 1986), is the wild-type construct used in these analyses and will be referred to as pNL-Wt. The nucleotide numbering employed is that of the BH10 strain (Myers et al., 1995). A derivative of PNL4-3H11env, encoding an Env gene product mutated at the proteolytic cleavage site [R 508 to S, K 510 to N (Bosch & Pawlita, 1990)] such that proteolytic processing no longer occurs, will be referred to here as pNL-Env/NC (NC for ‘non-cleavable’).

Generation of recombinant proviral constructs. Insertion of nucleotide sequences encoding the appropriate signals was performed using the oligonucleotide-directed mutagenesis protocol according to Kunkel (1985) on a single-stranded DNA template generated from a subgenic Bluescript plasmid (Stratagene) containing the HindIII–Xhol fragment (nucleotide positions 8170–8926) from pNL-Wt. The 22- amino-acid sequence of the first transmembrane domain of mouse infectious bronchitis virus, reported to mediate residency in the Golgi compartment (Swift & Machamer, 1991), was inserted in place of the 22-amino-acid-long membrane-anchor domain of gp160, located between amino acids 684 and 705, by inserting the appropriate nucleotide sequence 3’ of nucleotide 8302 (to yield Env-TM/Golgi). The heterologous nucleotide sequence was flanked 5’ and 3’ with complementary env sequences. The amino acid sequence reported to mediate residency in the ER, DEKKMP (Jackson et al., 1990, 1993; Lee & Donaghe, 1992), followed by a translational stop codon, was generated at the C terminus of gp160 by inserting the appropriate nucleotide sequence 3’ of nucleotide 8821 (to yield Env856ER). The 21 nucleotides encoding the six inserted amino acids plus the stop codon were flanked 5’ and 3’ with complementary env sequences. The net result was that 15 env nucleotides were deleted and 21 heterologous nucleotides inserted. Specifically, the mutagenizing oligonucleotides had the sequences: Env-TM/Golgi, 5’TAAAGGAGTTCTATTCAATACACAAAAAGGATATTAATAATATTTTACTGCTACATTTTCTTGTTGTTCTTAACCATAATTCATCTGAATGCTGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA; Env856ER, 5’TAAAAGGAGTTCTATTCAATACACAAAAAGGATATTAATAATATTTTACTGCTACATTTTCTTGTTGTTCTTAACCATAATTCATCTGAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA«GAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA«GAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA; Env856ER, 5’TAAAAGGAGTTCTATTCAATACACAAAAAGGATATTAATAATATTTTACTGCTACATTTTCTTGTTGTTCTTAACCATAATTCATCTGAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA«GAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA«GAATGCTGCTGCTGCTGATGGCTATGCAACCCAGAATATTGGATATGACAGCGGAAATATTTTATTTA. In each case, the numbers refer to the positions of the env sequences and the underlined sequences show the heterologous nucleotides. The completely sequenced HindIII–BanHI fragment (nucleotides 8170–8504) containing the transmembranal Golgi residency signal was, via a subcloning step, used to replace the same fragment in pNL-Wt, yielding pNL-Env/TM/Golgi.

The completely sequenced BamHI–Xhol fragment (nucleotides 8504–8926) containing the C-terminal ER residency signal was used to replace the same fragment in pNL-Wt, yielding pNL-Env856ER.

Transfection of proviral DNA and infectivity of released virus particles. Most transfections were performed in COS-7 cells. However, metabolic labelling of recombinant viral glycoproteins for immunoprecipitation (Fig. 4b, c) was performed in transfected 293T cells (Pear et al., 1993). Provirial vectors (10 μg DNA) were transfected using standard calcium phosphate procedures. For analysis of the fusion activity of cell-surface glycoprotein, transfected COS-7 cells on glass cover-slips were cocultured with an excess of CD4-expressing HeLa cells, (Magi-cells; Maddon et al., 1986; Kimpton & Emerman, 1992) for 24–48 h post-transfection (p.t.). For initial analysis of antibody specificities, lysates from HeLa cells infected with recombinant vaccinia virus expressing the HIV-1 Env gene product (Owens & Compans, 1989) were employed. Infection of MT-4 cells with cell-free supernatants from transfected COS-7 cells and analysis of infected cells by indirect immunofluorescence were performed as previously described (Wilk et al., 1992).

Analysis of protein expression in cells and virus particles. Transfected cells on glass cover-slips were fixed and permeabilized in acetone for indirect immunofluorescence employing rabbit antisera to gp160 as previously described (Bosch & Pawlita, 1990). Cell lysates for Western blot analyses were prepared 48 h p.t. by scraping washed cell monolayers into 0.5% Triton X-100 in PBS containing a mixture of protease inhibitors (Protease inhibitor cocktail tablets, Boehringer Mannheim). The cell lysates were incubated for 10 min on ice before clarification in a bench centrifuge. In order to concentrate virus particles, clarified culture supernatants were precipitated with polyethylene glycol and subsequently taken up in sample buffer for Western blot analysis. Western blot analyses of viral proteins in cell and virus lysates were performed employing rabbit anti-gp120 and anti-p24 sera as previously described (Krausslich et al., 1993). The relative amounts of virus particles released into the culture supernatants were quantified by ELISA detecting p24 (CA) (Mediproc). Transfected 293T...
cells were metabolically labelled for 5 h at 48 h p.t. with 100 µCi/ml [35S]methionine and [35S]cysteine (Pro-mix, Amersham) and subsequently lysed in 0.5% Triton X-100 in PBS as above. For metabolic labelling of viral glycoproteins expressed from recombinant vaccinia virus, HeLa cells were infected with a vaccinia virus inoculum sufficient to infect all cells and labelled with 100 µCi/ml [3H]glucosamine (Amersham) for 2–20 h p.t. Again, cell lysates were prepared in 0.5% Triton X-100 in PBS as above. A portion of the labelled HeLa cell lysate was adjusted to 1% SDS, heated for 5 min at 65 °C and subsequently adjusted to 1% Triton X-100, 0.5% deoxycholate and 0.1% SDS (RIPA buffer). Prior to specific immunoprecipitation, aliquots of the labelled cell lysates were pre-precipitated with normal rabbit serum (NRS) and protein A Sepharose (Pharmacia). The supernatants obtained after preprecipitation were then incubated overnight at 4 °C, either with NRS or anti-Env (a 1:1 mixture of rabbit anti-gp120 and anti-gp160 (Bosch & Pawlita, 1990)) plus protein A Sepharose, or with 10 µg CD4-H3 or 1 ml cell culture supernatant from Chessie 8 or from H902 hybridoma cell lines plus protein G Sepharose (Pharmacia). CD4-H3 is a recombinant soluble CD4, consisting of CD4 domains 1 and 2 chimerized to constant domains of human IgG3 (Traunecker et al., 1991). Chessie 8 hybridoma cells produce mouse monoclonal antibody specific for gp41 (Abacioglu et al., 1994), and H902 hybridoma cells produce mouse monoclonal antibody specific for the V3 loop of gp120 (Chesebro & Wehrly, 1988; Pincus et al., 1989).

In these latter two cases, 1 ml culture supernatant contains approximately 10 µg IgG. After three washes with 0.5% Triton X-100 in PBS (or with RIPA buffer in those cases in which the lysates had been in this buffer), proteins bound to Sepharose were subjected to PAGE and autoradiography. For digestion with endoglycosidase H, labelled glycoproteins immunoprecipitated with a 1:1 mixture of Chessie 8 and H902 supernatants were solubilized from the protein G Sepharose with 0.13% SDS at 95 °C and subsequently adjusted to 1% Triton X-100 and 0.1% SDS. Samples were then incubated with or without 2 mU endoglycosidase H (Boehringer Mannheim) for 16 h at 37 °C.

Expression and cell surface fusion activity of recombinant glycoproteins from pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER

Expression of the recombinant glycoproteins was first analysed by indirect immunofluorescence of permeabilized transfected COS-7 cells employing antisera against gp160. As has previously been observed (Bosch & Pawlita, 1990), the fluorescence signal for wild-type glycoprotein was predominantly present at membraneous structures within the cell, with only weaker immunofluorescence extending out towards the cell surface. This reflects the fact that in transfected and infected cells, a large portion of wild-type HIV-1 glycoprotein remains within the ER before being rapidly degraded within lysosomes (Willey et al., 1988). Only the remaining amount of gp160, which is normally transported and further processed, can be affected by the intracellular retention signals present on Env-TM/Golgi and Env856ER. Presumably as a result of this, the intracellular immunofluorescence patterns with the two recombinant glycoproteins did not significantly differ from that of Env-Wt (Fig. 2).

When pNL-Wt-transfected COS-7 cells were cocultivated with CD4+ HeLa cells, multinucleated syncytia that were positive in indirect immunofluorescence with anti-gp160 serum were formed (Fig. 2d), showing that fusion-competent glycoprotein had reached the cell surface. This did not occur with Env-TM/Golgi- or Env856ER-transfected COS-7 cells, which remained as single cells, equivalent to those seen in Fig. 2 (a–c), on the lawn of cocultivated COS-7 and CD4+ HeLa cells (not shown). This demonstrates that in these cases, membrane-fusion competent glycoprotein had not reached the cell surface.

Results

Viral glycoproteins with putative signals for localization to the ER or Golgi complex

A schematic representation of the recombinant glycoproteins encoded by pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER is shown in Fig. 1. In pNL-Env-TM/Golgi the membrane anchor of gp160, located between amino acids 684 and 705, has been replaced by the first membrane-anchor domain of the mouse infectious bronchitis virus M protein (Swift & Machamer, 1991), which has been reported to mediate residency within the Golgi compartment. The sequence DEKKMP, derived from the C terminus of the adenovirus E3/19K protein and reported to mediate localization to the ER (Nilsson et al., 1989; Jackson et al., 1990, 1993; Lee & Donaghy, 1992), is present at the C terminus of Env856ER.
glycoproteins have not been transported to the cellular compartment at which proteolytic processing occurs, but rather have been retained within cellular compartments.

It was important to confirm that the changes introduced into pNL-Env-TM/Golgi and pNL-Env856ER were not resulting in gross malfolding of the encoded glycoproteins with associated retention and degradation in the ER. In order to examine this point, immunoprecipitations employing rabbit polyclonal anti-Env serum, CD4-H3, reacting with the gp120-receptor-binding site (Traunecker et al., 1991), and two different mouse Env monoclonal antibodies were performed (Fig. 4a, b). Chessie 8 monoclonal antibody is directed against an epitope in gp41 (aa 727–732 of HIV LAI gp160) (Abacioglu et al., 1994) and H902 monoclonal antibody binds to the hypervariable loop of gp120 (Chesebro & Wehrly, 1988; Pincus et al., 1989). In an initial experiment (Fig. 4a), the extents to which wild-type HIV-1 glycoproteins could be immunoprecipitated in 0±5% Triton X-100 (left panel) and after heating in SDS in RIPA buffer (right panel), were examined. Whereas all four reagents could immunoprecipitate wild-type env gene products in 0±5% Triton X-100, only anti-Env and Chessie 8 could do so in RIPA buffer. This shows that CD4-H3 and H902 monoclonal antibodies require ‘native’ conformation for recognition. In Fig. 4(b), the wild-type and recombinant glycoproteins have been immunoprecipitated in 0±5% Triton X-100. A further recombinant Env-glycoprotein (EnvNC), which, due to mutation at the proteolytic cleavage site, can no longer be processed to gp120 and gp41 (Bosch & Pawlita, 1990), has also been examined. All of the tested glycoproteins were specifically immunoprecipitated in 0±5% Triton X-100. A further recombinant Env-glycoprotein (Env/NC), which, due to mutation at the proteolytic cleavage site, can no longer be processed to gp120 and gp41 (Bosch & Pawlita, 1990), has also been examined. All of the tested glycoproteins were specifically immunoprecipitated in 0±5% Triton X-100.
can be observed in the cases of Env-TM/Golgi and Env856ER is further proof that these components have not been intracellularly transported to cellular compartments in which processing events lead to the generation of larger oligosaccharide moieties. These analyses strongly indicate that the overall folding of the recombinant glycoproteins Env-TM/Golgi and Env856ER is correct and make it likely that the failure of transport to the cleavage compartment, which is presumably in or near the TGN (Hallenberger et al., 1992; Bosshart et al., 1994; Molley et al., 1994; Schäfer et al., 1995; Kantenan et al., 1995), is a result of the presence of the intracellular retention signals.

Endoglycosidase H digestion of Env-TM/Golgi, Env856ER and wild-type gp160 components (wild-type gp120 cannot be observed in this exposure) resulted in the generation of a single predominant species migrating at approximately 90 kD and presumably representing completely deglycosylated ‘gp160’ (Fig. 4c). In the case of Env/NC, an additional species with a slower migration can also be observed. This has been generated by removal of high mannose oligosaccharides from gp160*, which, due to intracellular transport, also contains complex oligosaccharides not removable by endoglycosidase H. The fact that no larger species could be observed in the cases of Env-TM/Golgi and Env856ER is compatible with their subcellular localizations to an early Golgi compartment and endoplasmic reticulum, respectively. In these compartments oligosaccharides still remain sensitive to digestion with endoglycosidase H.

**Viral proteins in cells and released virions after transfection with pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER**

Coexpression of the recombinant glycoproteins had no effect on the overall expression levels or the processing patterns of p24 (CA) and of CA-containing Gag precursor intermediates in transfected cells. These were not reproducibly different for pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER (Fig. 3, lower left).

It was now important to determine if coexpression of the recombinant glycoproteins could influence the intracellular site of virus assembly and release. Employing an ELISA detecting p24 (CA), it could be demonstrated that the total amounts of virus particles released into the extracellular medium of cells transfected with pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER were equivalent. In addition, the Gag-processing patterns of released particles were similar in all cases (Fig. 3, bottom right). However, as shown in Fig. 3 (top right), although Gp120 could be observed in particles released from pNL-Wt-transfected cells, this was not the case with pNL-Env-TM/Golgi- and pNL-Env856ER-transfected cells. This is presumably due to the failure of these glycoproteins to be proteolytically processed and adequately transported to the assembly site at the plasma membrane.
The released virus particles were further analysed by examining their infectivities in MT-4 cells, which are permissive for HIV-1. Whereas wild-type virus resulted in complete infection of the culture within a few days, virus particles released from cells transfected with pNL-Env-TM/Golgi and pNL-Env856ER were completely non-infectious and
no immunofluorescence-positive MT-4 cells could be observed even after 7 days post-infection.

**Electron-microscopic analysis of released and budding virus particles**

The results described until now support the view that the transferred retention signals have resulted in redirection of the recombinant glycoproteins to intracellular localizations, presumably to the ER (pNL-Env856ER) and the cis-Golgi (pNL-Env-TM/Golgi). However, despite this, the amounts of released virus particles were equivalent in all cases. In order to determine whether released virus had reached the extracellular medium by assembly and budding from the same location, or if this has been redirected to a novel subcellular compartment with subsequent intracellular transport and release, thin sections of COS-7 cells, transfected with pNL-Wt, pNL-Env-TM/Golgi and pNL-Env856ER were analysed by electron microscopy. In all cases, virus particles were predominantly observed extracellularly and budding structures were seen only at the plasma membrane (Fig. 5). Since the glycoprotein spikes of HIV-1 are difficult to visualize by thin-section electron microscopy, no difference in the outer surfaces of particles released from pNL-Wt, which incorporates viral glycoprotein, in comparison to pNL-Env-TM/Golgi and pNL-Env856ER, which do not incorporate viral glycoprotein (Fig. 3), could be observed. Occasionally, released particles could be seen in what appeared to be intracellular membraneous structures (Fig. 5b, e), but this observation was not specifically increased with any construct tested. This shows that, despite the localization of the glycoproteins encoded by pNL-Env-TM/Golgi and pNL-Env856ER being intracellular, particle assembly and budding have not been redirected to a different subcellular compartment.

**Discussion**

The aim of the experiments described in this paper was to generate recombinant glycoproteins which would be retained at altered subcellular localizations and examine if this affects proteolytic processing and the subcellular site of virus particle assembly and budding. The retention signals employed to generate Env-TM/Golgi and Env856ER with altered subcellular localizations have previously been demonstrated to confer localization to the cis-Golgi complex and ER, respectively (Swift & Machamer, 1991; Jackson et al., 1990, 1993; Lee & Donaghue, 1992), and the evidence presented in this paper supports the view that this is also the case here.

Env-TM/Golgi and Env856ER were not proteolytically processed, were not present in a fusion-competent form at the cell surface, and the recombinant glycoproteins were not incorporated into released virions. Immunoprecipitation with a chimeric soluble CD4 molecule and with a monoclonal antibody against the immunodominant hypervariable domain of gp120, both of which require correct conformation for recognition, confirmed that the recombinant glycoproteins were correctly folded. It is very unlikely that the changes introduced into Env-TM/Golgi and Env856ER have resulted in these molecules no longer being substrates for the processing enzyme, since HIV-1 glycoproteins with significantly greater alterations than those in Env-TM/Golgi and Env856ER can still be proteolytically processed and induce syncytium formation (Wilk et al., 1992, 1996). These results together point to the recombinant molecules having a native conformation, but being retained within the Golgi and ER due to the presence of the retention signals. Lack of proteolytic processing of Env-TM/Golgi and Env856ER points to the cellular cleavage compartment being localized beyond the sites to which these molecules are transported. However, some studies in the past have supported the view that cleavage could occur within early Golgi compartments (Stein & Engleman, 1990). In this context, it is also of note that Raja et al. (1993) reported proteolytic processing occurring in the case of HIV-1 Env which had been retained in the ER as a result of complex formation with coexpressed recombinant CD4, which itself carried an ER retention signal. The results reported in this paper do not support the conclusion that cleavage occurs in the ER or in an early Golgi compartment. Rather, they are compatible with the favoured view that proteolytic cleavage occurs at or near the TGN (Kantanen et al., 1995) and is carried out predominantly by the cellular protease furin (Hallenger et al., 1992), which localizes to this compartment ( Bosshart et al., 1994; Molley et al., 1994; Schäfer et al., 1995).

In comparison with wild-type, expression of pNL-Env-TM/Golgi and pNL-Env856ER did not result in any significant change in the amount or composition of the internal structural proteins in cell lysates, nor in the amounts of virus particles released into the culture supernatant. However, in these cases no glycoprotein was present in extracellular particles. Again in comparison with wild-type, electron-microscopic analysis of transfected cells revealed no differences in the patterns of particle assembly and release. This shows that the recombinant glycoproteins were not capable of redirecting Gag protein assembly to those intracellular membranes to which they were localized.

Targeting of the retroviral structural proteins to cellular membranes is mediated by the MA domain of the Gag precursor, Pr55agg. Treatment of cells with monensin, which blocks intracellular vesicular transport, has been shown to inhibit retroviral gag precursor transport and release (in the case of MuLV; Hansen et al., 1990) or result in increased budding into intracellular membranes (in the case of Rous sarcoma virus; Bosch & Schwarz, 1984). MuLV particles have also been observed intracellularly (Hansen et al., 1993). Furthermore, a large deletion within the HIV-1 MA domain has been shown to result in an accumulation of virus particles within the ER (Fäcke et al., 1993), indicating possible aberrant Gag intracellular transport in this mutant. Thus, although HIV-1 assembly and budding occur predominantly at the plasma
membrane, it is possible that MA does not directly target the inner face of the cell surface membrane, but rather that there is an initial association with intracellular membranes followed by vesicular transport to the assembly site. This could allow an association of Gag precursor with viral glycoprotein, also located at intracellular membranes, with the possibility of a cotransport of submicroscopic complexes to the assembly site. Under certain circumstances this would allow the intrinsic transport – or localization – properties of the viral glycoproteins to influence the transport and assembly properties of the internal structural proteins. Examples are intracellular assembly and budding of certain enveloped viruses [e.g. coronaviruses, Hepatitis B viruses into the ER, bunyaviruses into an early Golgi compartment (for review see Hobman, 1993)] as well as the mediation of polarized HIV-1 particle release in polarized epithelial cells (Owens et al., 1991). In this latter case, it has been shown that the C terminus of the HIV-1 env gene product is important and may serve as an interacting partner with MA (Lodge et al., 1994).

Env-TM/Golgi and Env856ER failed to redirect particle release to their altered intracellular membrane locations. One possibility for this failure could be that, due to the introduced molecular changes, the potential region of interaction, presumably within the C terminus of Env, is no longer accessible. In Env-TM/Golgi, the introduced molecular change in the membrane-anchor region is spatially distant from the C terminus. In a previous study, we demonstrated that replacing the membrane anchor of HIV-1 Env with that of a cellular glycoprotein does not significantly affect glycoprotein function. This was measured by membrane-fusion activity, incorporation into released particles and retention of infectivity (Wilk et al., 1996). In Env856ER, only six amino acids have been added to the authentic C terminus. Thus, although the possibility of non-accessibility of a putative interaction domain cannot be formally ruled out, it seems unlikely to apply here. The fact that, in spite of this, particle release is unaffected may be interpreted only to mean that in polarized cells, in contrast to COS-7 cells, as yet unknown circumstances allow the HIV-1 glycoproteins to successfully redirect particle assembly and budding.

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References


Intracellular retention of HIV glycoprotein


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