Secretion of a murine retroviral Env associated with resistance to infection

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Fv4 is an endogenous defective murine leukaemia virus (MuLV) which expresses high levels of an envelope protein (Env) closely related to that of the ecotropic class of MuLVs. Mice bearing the natural Fv4 gene or a transgenic version are resistant to infection by ecotropic MuLVs. Fv4 mice secrete the surface peptide (SU) of the Fv4 Env in their serum and this secreted Env can block infection of NIH3T3 cells. To study the secretion of Fv4, we metabolically labelled cells expressing Fv4 Env or Env from infectious MuLVs and followed synthesis, glycosylation, proteolytic processing and secretion of Env species. We found no difference in the kinetics of synthesis or processing of Fv4 Env compared to the envelopes of infectious MuLVs, but Fv4 Env associated more weakly with its transmembrane anchor and was shed from the surface of cells.

Introduction

Retroviral envelope glycoproteins have amino-terminal signal sequences which direct nascent Env peptides to the lumen of the endoplasmic reticulum (ER) where they are glycosylated. The Env precursors are then transported to the Golgi complex where sugars are modified and the Env precursor is cleaved by a cellular protease to generate the Golgi complex where sugars are modified and the Env precursor is cleaved by a cellular protease to generate the glycoprotein (SU), responsible for binding to the ecotropic receptor, and the transmembrane anchor (TM), involved in viral-cell fusion and anchoring SU to virus and cell membranes (Van Zaane et al., 1976; Hunter & Swanstrom, 1990). SU and TM are held together by non-covalent bonds and possibly also by disulfide bonds (Pinter & Honnen, 1983; Thomas & Roth, 1995).

In the case of murine leukaemia viruses (MuLVs), the initial Env gene product found in the ER is a molecule with N-linked oligosaccharides, mostly mannose sugars, with an apparent molecular mass of 80–90 kDa, depending on virus strain (Witte & Wirth, 1979). This ‘gp85’ molecule, which is sensitive to digestion by endocellulosidase H (EndoH) and PNGase F (peptide: N-glycosidase F), is transported to the Golgi where the high-mannose sugars are converted to ‘complex sugars’, which are resistant to cleavage by EndoH but sensitive to PNGase. Also in the Golgi, the Env precursor is cleaved to form the hydrophilic SU gp70 and hydrophobic TM p15E moieties (Pinter et al., 1978). During virus budding, the viral protease cleaves p15E to generate p12E and p2E molecules (Van Zaane et al., 1976), thereby activating the Env protein for fusion (Rein et al., 1994).

The Fv4 provirus, located on mouse chromosome 12 (Odaka et al., 1981), resembles the 3’ half of a MuLV with an intact env gene and 3’ LTR, but a deletion of the 5’ LTR, gag and most of the pol gene (Ikeda et al., 1985). Transcription of the Fv4 mRNA is driven by a cellular promoter and this mRNA encodes an Env protein ~75% identical (at the nucleotide level) to ecotropic MuLV Env proteins (Ikeda et al., 1985). The Fv4 locus was first identified as a gene causing resistance to Friend murine leukaemia virus (FrMLV) (Suzuki, 1975; Gardner et al., 1980). Mice carrying the dominant allele of Fv4 (corresponding to the defective provirus) resist infection with ecotropic MuLVs. When transfected with the Fv4 gene, NIH3T3 cells express the Fv4 Env on the cell surface and, if they express large amounts of this Env, are resistant to infection with ecotropic retroviruses (Ikeda & Sugimura, 1989). Mice that are transgenic for the Fv4 gene are also resistant to infection, depending on the level of expression of Fv4 Env (Limjoco et al., 1993). Fv4 resistance can be transferred to non-Fv4 mice by bone marrow transplantation (Ikeda & Odaka, 1979; Kitagawa et al., 1994; Limjoco et al., 1995).

Fv4 causes resistance to ecotropic MuLVs by interference, a virological phenomenon that involves non-virion Env binding to virus receptor inside cells, during synthesis and transport of these molecules, or on the cell surface, thereby blocking receptor-mediated virus uptake. In cells expressing
the ecotropic Env and the ecotropic receptor, glycosylation of the ecotropic receptor is altered, implying that these molecules interact intracellularly (Kim & Cunningham, 1993). Fv4 mice also secrete Fv4 SU in their serum and the secreted SU can block binding of FrMLV to the ecotropic receptor on other cells (Kitagawa et al., 1995; Nihrane et al., 1996). Thus, interference from without may also play a role in resistance. Fv4 may also alter the immune response to MuLV by preventing immunosuppression associated with FrMLV infection (Morrison et al., 1986), or by specifically altering the immune response to viral envelope antigens as a result of prior exposure to the inherited Fv4 envelope protein (Limjoco et al., 1995; A. Nihrane & J. Silver, unpublished).

Fv4 Env is first synthesized as a gp85 precursor molecule which is subsequently processed to SU gp70 and TM p15E moieties (Ikeda & Odaka, 1983, 1984; Nihrane et al., 1996). We previously showed that transgenic Fv4 mice secrete gp70 as a soluble glycoprotein and that this gp70 is derived largely, but not exclusively, from haematopoietic cells (Nihrane et al., 1996). Here, we show that gp70 secretion is quantitatively much greater for cells expressing Fv4 Env than for cells expressing Moloney murine leukaemia virus (MoMLV) Env; that the Fv4 Env is secreted in the absence of p15E TM; that Fv4 Env is synthesized, glycosylated and cleaved with the same kinetics as other MuLV envelopes; and that secretion of Fv4 gp70 can be explained in part by dissociation of Fv4 SU from the cell surface.

Methods

Mice, cells, antibodies, plasmid and reagents. FVB/N.Fv4-2/2+ mice have been described previously (Limjoco et al., 1993). All cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM; BioWhittaker) with 10% foetal calf serum (FCS). NIH3T3 cells were purchased from ATCC. FrMLV was obtained from Janet Hartley (National Institutes of Health, Bethesda, Md., USA). Tail cultures from 10 to 15-day-old mice were established as previously described (Nihrane et al., 1996). Briefly, tails were dipped for 40 s in 70% alcohol, cut and minced in collagenase solution (150 U/ml) with the antibiotics penicillin (10000 U/ml) and streptomycin (10000 µg/ml) to 15-day-old mice were established as previously described (Nihrane et al., 1996). Tail cells (1–2 x 106) in 60 mm diameter dishes were starved for 10 min at 37 °C in 1 ml methionine- and cysteine-free DMEM medium containing 5% FCS and then labelled for 30 min with 200 µCi TRANSIPPS label (ICN Biomedical). The cells were then washed, placed in 1:5 ml fresh DMEM, 10% FCS, and incubated for various times at 37 °C, after which they were disrupted in lysis buffer (100 mM NaHPO4, H2O, 100 mM NaCl, 0.5% sodium deoxycholate, 0.01% BSA, 0.1% SDS). The cell lysate and the corresponding filtered cell supernatants (0.45 µm pore size) were pre-clear by incubating with protein A beads (Life Technologies) for 1 h at 4 °C. After removing the beads by centrifugation, samples were incubated for 1 h with 50 µl protein A beads previously treated with 5 µl polyclonal goat anti-Rauscher gp70 antibody. The beads were then washed three times with 0.1% Triton X-100, 300 mM NaCl, 50 mM Tris–HCl (pH 7.5). The labelled proteins and beads were split into three parts. One part was eluted at 100 °C in 60 µl reducing sample buffer (Laemmli, 1970) and analysed by SDS–12% PAGE and autoradiography. The other two parts were used in deglycosylation experiments.

PNGase and EndoH treatment. Labelled proteins were recovered from the protein A beads by incubating at 100 °C for 10 min in 25 µl denaturing solution (0.5% SDS, 1% β-mercaptoethanol). For PNGase digestion, samples were incubated for 2 h at 37 °C in the above denaturing solution plus 3.3 µl 10% NP40, 3.3 µl 10 x G7 buffer (New England Biolabs) and 2000 U PNGase. For EndoH treatment, samples were incubated for 1 h at 37 °C in the denaturing solution plus 3 µl 10 x G5 buffer and 2000 U EndoH. Proteins were subsequently characterized by SDS–PAGE and autoradiography.

Cell surface fluorescence imaging and flow cytometry assay for supernatant Fv4 Env. Tail cells (1–2 x 106) in 60 mm diameter dishes were incubated with 1 ml of a 0.5 mg/ml solution of NHS-fluorescein at 4 °C for 30 min as described by Vigers et al. (1988). The cells were then washed once with DMEM and three times with ice-cold PBS containing calcium and magnesium (PBS/CM), and cultured at 37 °C in 1:5 ml complete DMEM. Some samples were assessed for viability by staining with Trypan Blue. Supernatants were recovered at various time points, filtered and assayed for FITC-labelled Fv4 Env capable of binding the ecotropic receptor as follows. Approximately 106 trypsinized NIH3T3 cells were incubated for 45 min at 37 °C with 1:5 ml of recovered supernatants. After washing three times with HBSS, 0.1% BSA, 0.1% sodium azide, the cells were analysed using an EPICS Profile Cytometer (Coulter). As receptor-negative controls, we used NIH3T3 cells that were chronically infected with FrMLV or uninfected NIH3T3 cells that were blocked with 100 µl 20% Fv4 serum (obtained from FVB/N.Fv4-2 transgenic mice) for 45 min at 37 °C.

Establishment of MoMLV envelope cell lines. The MoMLV envelope expression plasmid DNA (Matano et al., 1993) was transformed into E. coli DH5α competent cells (Life Technologies), grown overnight and purified by QIAGEN Plasmid Maxi Kit (Qiagen). One day before the transfection, NIH3T3 cells were seeded at 104 cells per T25 flask in DMEM, 10% FCS. The following day, 20 µg purified plasmid DNA was transfected into the cells by calcium phosphate precipitation (Graham & Van der Eb, 1973) and the cells were incubated overnight at 37 °C in 5% CO2. Clones were selected in hygromycin (500 µg/ml) and characterized by flow cytometry and immunoprecipitation for envelope expression.

Pulse-chase labelling and immunoprecipitation. Cells (1–2 x 106) in 60 mm diameter dishes were starved for 10 min at 37 °C in 1 ml methionine- and cysteine-free DMEM medium containing 5% FCS and then labelled for 30 min with 200 µCi TRANSIPPS label (ICN Biomedical). The cells were then washed, placed in 1:5 ml fresh DMEM, 10% FCS, and incubated for various times at 37 °C, after which they were disrupted in lysis buffer (100 mM NaHPO4, H2O, 100 mM NaCl, 0.5% sodium deoxycholate, 0.01% BSA, 0.1% SDS). The cell lysate and the corresponding filtered cell supernatants (0.45 µm pore size) were pre-clear by incubating with protein A beads (Life Technologies) for 1 h at 4 °C. After removing the beads by centrifugation, samples were incubated for 1 h with 50 µl protein A beads previously treated with 5 µl polyclonal goat anti-Rauscher gp70 antibody. The beads were then washed three times with 0.1% Triton X-100, 300 mM NaCl, 50 mM Tris–HCl (pH 7.5). The labelled proteins and beads were split into three parts. One part was eluted at 100 °C in 60 µl reducing sample buffer (Laemmli, 1970) and analysed by SDS–12% PAGE and autoradiography. The other two parts were used in deglycosylation experiments.
After washing, the proteins were eluted from the beads for 10 min at 100 °C in 20 µl 10% SDS and diluted in 1 ml PBS. The boiled beads were removed by centrifugation and the supernatants were incubated with 50 µl streptavidin–agarose beads (Life Technologies) for 4 h at 4 °C. The beads were then washed and the proteins eluted and characterized by SDS–PAGE and autoradiography.

Results

Gp70 secretion is peculiar to Fv4

To see if secretion of SU by Fv4-expressing cells was peculiar to the Fv4 Env, we transfected NIH3T3 cells with a vector encoding the MoMLV Env (Matano et al., 1993), isolated three Env-expressing clones and compared the synthesis and processing of envelope glycoproteins in these cells to that in tail cells from Fv4-transgenic mice and FrMLV-infected NIH3T3 cells. Cells were metabolically labelled with [35S]methionine and [3H]cysteine for 30 min and ‘chased’ with non-radioactive amino acids for various times, after which the cells were lysed and cell lysates and supernatants were immunoprecipitated with a polyclonal goat antibody to MuLV gp70. An Env precursor of about 85 kDa was detected in the cell extract immediately after labelling (Fig. 1a, lane C0). This precursor was cleaved into species of approximately 70 kDa (gp70) and 15 kDa (p15E), easily seen at the 2 and 5 h chase points (Fig. 1, lanes C2 and C5). The small amount of p15E in the immunoprecipitate of gp70 shows that some p15E remains associated with gp70 during immunoprecipitation, as has been noted many times before. In the supernatant of the MoMLV env cell line, only a faint band corresponding to gp70 was detected (Fig. 1, lane S0), indicating that only a small amount of MoMLV envelope was released from the cells. Comparable data were obtained with the other two MoMLV envelope cell lines. Several other groups have reported that ecotropic MuLV SU glycoproteins are only weakly released from envelope-expressing cells (Heard & Danos, 1991; Battini et al., 1995).

In contrast, tail cells from Fv4-transgenic mice released a large amount of gp70 into the supernatant (Fig. 1b, lower panel, lanes S1–S5). We used Fv4 tail cells in these experiments rather than NIH3T3 cells transduced with Fv4 Env because the former produce more Fv4 Env (Nihrone et al., 1996). Our previous work showed that the ratio of secreted SU to cell-associated SU was about 1:1 in Fv4-NIH3T3 cells (Nihrone et al., 1996), as is the case in the Fv4 tail cells examined here (Fig. 1b, lanes S4–S5 versus lanes C4–C5), compared to less than 1:10 in the MoMLV Env cell lines (Fig. 1a, lane S5 versus C5).

NIH3T3 cells infected with FrMLV also produce a large amount of gp70 in the supernatant (Fig. 1b, upper panel), but this is expected since these cells produce viral particles.

Fv4-gp70 is secreted free of p15E

We previously showed that p15E was detected in immunoprecipitates of gp70 from cell extracts of Fv4 tail cells, whereas p15E was not cross-immunoprecipitated from the supernatants of these cells despite the presence of large amounts of gp70 (Nihrone et al., 1996). We interpreted these results as indicating that Fv4 SU is secreted in the absence of TM. However, another possibility is that both Fv4 TM and SU are secreted but for some reason are not associated with one another in the supernatant. To address this directly, we immunoprecipitated cell lysates and supernatants with an anti-p15E MAb (Chesebro et al., 1981). The MAb detected p15E in the cell lysate after 1 h of chase (Fig. 2a, lanes C1, C2 and C5). However, no p15E or p12E (the cleaved form of p15E present in virions) was found in the supernatant of Fv4 cells showing that it is not secreted (Fig. 2a, lanes S0–S5).

The anti-p15E MAb detected the gp85 Env precursor weakly in cell lysates (Fig. 2a, lanes C1–C5). The absence of this band in the C0 lane is believed to be due to a technical problem with loss of sample since the background smear is also reduced in this lane; plenty of gp85 was present in the C0 sample since it was immunoprecipitated with anti-gp70 antiserum (Fig. 2a, left side, C0 lane). The weakness of the gp85 signal using the anti-p15E MAb suggests that the epitope detected by this MAb is partially ‘buried’ in the gp85 precursor. It is also of interest that the p15E MAb did not cross-immunoprecipitate Fv4 gp70 from the cell lysate, suggesting that binding of the p15E MAb may disrupt the association of p15E with gp70.

We performed the same studies with chronically infected NIH3T3 cells and the NIH3T3-MoMLV envelope cell lines. In the extracts of FrMLV-infected cells, the MAb immunoprecipitated p15E and gp85 as well as some background bands (Fig. 2b, left side, lanes C0–C5), whereas in the supernatants of these cells it immunoprecipitated a p12E species consistent with cleavage of p15E in viral particles (Fig. 2b, lanes S1–S5). The anti-p15E MAb did not cross-immunoprecipitate gp70 from the supernatant, despite its abundance (cf. Fig. 1b upper panel). As a negative control, uninfected NIH3T3 cells are shown on the right side, along with a positive control of FrMLV-NIH3T3 cells from the same experiment. In the MoMLV Env cell line, p15E and gp85 were detected in the cell extracts. No p15E species were detected in the supernatant, as expected, since this cell line does not secrete viral particles or Env proteins (Fig. 2c, lanes C1–C5 and S1–S5).

Fv4 tail cells, FrMLV-infected NIH3T3 cells and NIH3T3-MoMLV Env cells show no difference in the kinetics of synthesis, glycosylation or cleavage of envelope glycoproteins

The fact that Fv4 Env was peculiar in its propensity to secrete SU raised the possibility that differences might exist in the kinetics of synthesis, glycosylation or cleavage of Fv4 Env compared to FrMLV and MoMLV Env. To address this question, we analysed metabolically labelled cells at short times after a 30 min pulse. The envelope glycoproteins were immunoprecipitated with goat anti-gp70 antibody and digested with PNGase or EndoH enzymes to evaluate their glycosylation state. PNGase is believed to cleave all N-linked
where no other bands are present (Fig. 3b, lower panel), but it is also true for the gp70 species in the cell lysate (Fig. 3b, top panel). EndoH may remove some sugars from some of the gp70 molecules since it slightly reduced the intensity of the gp70 band and made it appear somewhat ‘fuzzy’ (Fig. 3b, lower panel). The fact that essentially all of the gp85 molecules were cleaved by EndoH while most of the gp70 molecules were resistant implies that cleavage of gp85 to gp70 occurs after the high-mannose sugars have been modified in the Golgi complex. This pattern has been observed for other MuLV envelopes (Pinter & Honnen, 1983, 1984).

In the MoMLV Env cell line, the gp85 precursor was similarly sensitive to digestion with PNGase and EndoH and was reduced to about 60 kDa by both enzymes (Fig. 3c). Interestingly, the mobility of the PNGase-deglycosylated form of gp85 appears to be slightly retarded after longer chase times (compare the mobility of the ~60 kDa band in the PNGase lanes at 60’ and 35’ versus 15’ and 0’). Careful inspection of Fig. 3(a) shows the same phenomenon for the Fv4 Env. The mobility of the EndoH deglycosylated form of gp85 also appears to shift up with increasing chase times. A likely explanation for these slight mobility shifts is O-glycosylation since PNGase and EndoH do not remove O-linked sugars. Gp85 is known to be O-glycosylated, and the mobility difference attributed to O-glycosylation is about 1 kDa (Pinter & Honnen, 1988). Our data are therefore consistent with O-glycosylation occurring 30–60 min after synthesis.

As in the case of the Fv4 tail cells, the MoMLV gp70 species was detected after 15 min chase, and was reduced to about 50 kDa by PNGase (Fig. 3c). EndoH had a subtle effect on gp70, increasing its mobility slightly and making it appear somewhat diffuse. To a first approximation, the kinetics of glycosylation and processing of Env in the MoMLV cell lysates was the same as in the Fv4 tail cells.

The results with FrMLV-infected NIH3T3 cells were similar except that these cells have duplicated species of ‘gp85’, ‘gp70’ and their deglycosylation products (Fig. 3d, e). These cells were derived by infecting NIH3T3 cells with a spleen extract from a FrMLV-infected mouse. Such extracts are known to contain recombinant mink cell focus viruses which can have Env proteins with slightly different mobilities (Famulari & Cieplensky, 1984). The kinetics of glycosylation and cleavage of the Env species in these cells are grossly the same as for the Fv4 and MoMLV Env cells.

**Secretion of Fv4 gp70 can be explained in part by its dissociation from the cell surface**

To investigate whether Fv4 gp70 was released from the surface of cells, we performed two types of surface-labelling. In the first, we reacted cells with NHS-fluorescein, a reagent that attaches fluorescein groups to free amines on the cell surface (Vigers et al., 1988). We collected supernatant at various times after fluoresceination and assayed the supernatant for Fv4 Env using an ectropic receptor-binding assay as follows. Super-

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**Fig. 1.** Comparative pulse-chase and immunoprecipitation of Env glycoproteins synthesized in Fv4 tail cells, NIH3T3 cells infected with FrMLV and NIH3T3 cells transfected with a MoMLV Env expression vector. Cells were metabolically labelled for 30 min with [35S]methionine and [35S]cysteine and grown for various times in the presence of cold methionine and cysteine. Cell lysates and supernatants were precipitated with goat anti-Rauscher gp70 antibody and analysed by SDS–PAGE under reducing conditions. Chase time in hours is given after the designations ‘C’ (for cell lysate) or ‘S’ (for supernatant).

oligosaccharides. EndoH removes N-linked oligosaccharides from high-mannose glycoproteins, but it does not cleave complex oligosaccharide side-chains. As such, resistance to EndoH serves as a marker for transport to the Golgi complex where high-mannose sugars are converted to complex oligosaccharides.

In the Fv4 system, the gp85 Env precursor was sensitive to PNGase and EndoH since it was entirely converted by these enzymes to a species of approximately 60 kDa. This is most easily seen immediately after pulse labelling when no other bands are present (Fig. 3a, b, lanes C0’), but it is also true at later time points (lanes C15’–C60’). This indicates that gp85 does not contain complex sugars.

After 15 min chase, a small amount of gp70 can be seen in the cell lysate, and this species is easily detectable in the cell extract and supernatant after 35 min chase (Fig. 3a, top and bottom panels, lane C35’). The gp70 moiety was completely converted by PNGase to a species with a molecular mass of about 50 kDa. In contrast, digestion with EndoH had little effect on gp70. This is most clearly seen in the supernatant
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Fig. 2. Comparative analysis of p15E transmembrane proteins in Fv4 tail cells, NIH3T3 cells infected by FrMLV and NIH3T3 cells transfected with a MoMLV Env expression vector. Cells were metabolically labelled for 30 min with [35S]methionine and [35S]cysteine and grown for various times in the presence of cold methionine and cysteine. Cell lysates and supernatants were precipitated with MAb anti-p15E and analysed by SDS–PAGE under reducing conditions. Chase time in hours is given after the designations ‘C’ (for cell lysate) or ‘S’ (for supernatant). (a) Fv4 tail cells. For comparison, polyclonal antibody anti-gp70 was used with cell lysate directly after pulse (C0) or with supernatant 5 h after chase (S5). Arrow indicates position where p12E species would be expected if it were present. (b) NIH3T3-FrMLV cells. For comparison, uninfected NIH3T3 cells are shown in the right hand two lanes, along with a positive control of FrMLV-NIH3T3 cells from the same experiment. (c) NIH3T3-MoMLV Env cell line.

Discussion

Our data show clearly that compared to FrMLV and MoMLV, the Fv4 Env is characterized by weak association between SU and TM. Thus, while the two molecules are associated inside cells, SU is secreted from Fv4 cells without TM, whereas it is not released from MoMLV Env cells and is released from FrMLV-infected cells in association with TM on viral particles. Two types of interactions have been proposed to hold SU and TM together, interchain disulfide bonds and non-covalent interactions. The literature concerning interchain disulfide bonds is controversial, probably because the formation or disruption of these bonds depends on experimental conditions (Pinter et al., 1978; Pinter & Honnen,
Fig. 3. Comparative analysis of synthesis, cleavage and transport of Env glycoproteins in Fv4 tail fibroblasts, NIH3T3-FrMLV cells and NIH3T3 cells expressing the MoMLV Env. Cells were metabolically labelled for 30 min with [35S]methionine and [35S]cysteine and grown for various times in the presence of cold methionine and cysteine. Cell lysates and supernatants were
in vivo gp70–p15E complex (Lostrom et al., 1979). The simplest interpretation of the results is that the SU–TM interaction is non-covalent and weak, especially for Fv4 SU–TM, and that it is destabilized by the anti-p15E MAb 372.

Site-specific mutagenesis has shown that mutations in the carboxy-terminal half of SU including the proline-rich ‘hinge’ region weaken the association of SU with TM (Lobel & Goff, 1984; Gray & Roth, 1993; Thomas & Roth, 1995). Substitution of the carboxy-terminal half of Fv4 for the equivalent segment of MoMLV led to a 1000-fold drop in virus titre (Masuda & Yoshikura, 1990), suggesting that Fv4 is defective in this region. Fv4 has 11 amino acid differences in the carboxy-terminal half of SU when compared to sequenced infectious MuLV clones (Masuda & Yoshikura, 1990). Among these differences are the amino acid changes non-conservative: a lysine (versus isoleucine, glutamic acid or proline in other viruses) at position 328, a lysine (versus serine or asparagine) at position 382 and an arginine (versus glycine or alanine) at position 393. These amino acid differences are good candidates for the cause of weaker association of Fv4 SU with TM.

Changes in TM could also contribute to weak association. TM sequences are highly conserved with only four differences between Fv4 and other viruses, two of which are conservative (an arginine for lysine at position 552 and an aspartic acid for glutamic acid at position 686). The two non-conservative changes in TM are an arginine for glycine at position 525 and a glutamic acid for lysine at position 545. The former is in the highly conserved hydrophobic amino terminus of TM, a region where non-hydrophobic amino acid substitutions in other viruses block virus-induced fusion (Freed et al., 1992). The inability of Fv4 Env to cause fusion in the XC assay and the loss of infectivity and XC fusogenicity when the Fv4 TM is substituted for the MoMLV TM (Masuda & Yoshikura, 1990)
is probably a consequence of the arginine to glycine change at position 525 in the TM. The second non-conservative TM difference between Fv4 and other viruses (position 545) may contribute to the poor association between Fv4 SU and TM.

Given the lack of tight association between Fv4 SU and TM, we felt it was important to characterize the kinetics of synthesis and processing of Fv4 Env to see if it was atypical in any other way. Our experiments showed no significant difference between Fv4 Env and the Env of other MuLVs in terms of glycosylation or cleavage. As in the case of other MuLVs, the Fv4 Env precursor contains EndoH-sensitive sugars, which are converted to EndoH-resistant sugars at the time the precursor is cleaved into SU and TM (Van Zanne et al., 1976; Pinter & Honnen, 1988; Hunter & Swanstrom, 1990). These glycosylation changes and cleavage are detected within 15–30 min of synthesis (Fig. 3).

Our studies with fluorescein and biotin labelling of surface Fv4 SU imply that a portion of the secreted SU is membrane-bound prior to secretion. Our studies do not rule out that some SU is secreted directly and it will be of interest to try to make the surface-labelling studies more quantitative by determining the fraction of secreted Fv4 SU that is membrane-associated prior to secretion. The membrane form of Fv4 SU is presumably associated with TM, albeit weakly, although some could also be bound to the ectropic receptor.

The fact that Fv4 SU dissociates easily from TM could contribute to the potency of the interference phenomenon in vivo by blocking viral receptor on the outside of cells. In an in vivo system, secreted Fv4 Env binds to receptor on NIH3T3 cells (Kitagawa et al., 1995) and at high concentration can block infection of these cells (Nihrane et al., 1996). Secreted forms of amino-terminal portions of other SU molecules have also been shown to block infection in vitro (Heard & Danos, 1991; Battini et al., 1995). Secreted Fv4 Env may protect non-Fv4 cells from infection in vivo in bone marrow chimera containing a mixture of Fv4 and non-Fv4 cells (Kitagawa et al., 1994; Limjoco et al.; 1995). Experiments with HIV show that soluble CD4 can block infection in vitro but this strategy fails in vivo because the amount of soluble CD4 necessary to block infection is too high (Deen et al., 1988; Fisher et al., 1988; Spouge, 1994). Quantitative studies of the amount of Fv4 Env necessary to block MuLV infection are needed to better evaluate the significance of serum Fv4 Env. Serum Fv4 Env could also play a role in modulating the immune response to MuLV (Kearny et al., 1994).

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References


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