Efficient transport of Semliki Forest virus glycoproteins through a Golgi complex morphologically altered by Uukuniemi virus glycoproteins

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Communicated by L. Kääriäinen

In infected BHK21 cells, the glycoproteins G1 and G2 of a temperature-sensitive mutant (ts12) of Uukuniemi virus (UUK) accumulate at 39°C in the Golgi complex (GC) causing an expansion and vacuolization of this organelle. We have studied whether such an altered Golgi complex can carry out the glycosylation and transport to the plasma membrane (PM) of the Semliki Forest virus (SFV) glycoproteins in double-infected cells. Double-immunofluorescence staining showed that 90% of the cells became infected with both viruses. Almost the same final yield of infectious SFV was obtained from double-infected cells as from cells infected with SFV alone. The rate of transport from the endoplasmic reticulum (ER) via the GC to the plasma membrane of the SFV glycoproteins was analysed by immunofluorescence, surface radiolabelling and pulse-chase labelling followed by immunoprecipitation, endoglycosidase H digestion and SDS-PAGE. The results showed that: (i) the SFV glycoproteins were readily transported to the cell surface in double-infected cells, whereas the UUK glycoproteins were retained in the GC; (ii) the transport to the PM was retarded by 20 min, due to a delay between the ER and the central Golgi; (iii) E1 of SFV appeared at the PM in a sialylated form. These results indicate that the morphologically altered GC had retained its functional integrity to glycosylate and transport plasma membrane glycoproteins.

Key words: Golgi/virus/glycoprotein/transport/membrane

Introduction

In eukaryotic cells, membrane proteins are synthesized in the rough endoplasmic reticulum (ER), where they are cotranslationally transferred through the ER membrane to the luminal side and anchored in the membrane by a hydrophobic transmembrane sequence (Blobel, 1980). From the ER, the membrane proteins are transported via the Golgi complex (GC) to their final destinations, e.g. the plasma membrane (PM) or the lysosomes (Farquhar, 1985; Lodish et al., 1981). Proteins specific for the ER or the GC use only the initial parts of this transport pathway (Anderson et al., 1983; Strous et al., 1983). How proteins are routed from the ER to their correct destinations, or how specific proteins are retained in different compartments, is poorly understood.

Membrane proteins of enveloped viruses maturing at the PM [e.g. Semliki Forest virus (SFV), influenza virus, vesicular stomatitis virus] are important tools in studying the biosynthesis and sorting of PM proteins (Lodish et al., 1981; Kääriäinen and Pesonen, 1982; Simons and Garoff, 1980; Simons and Warren, 1983). Membrane proteins of viruses budding at internal membranes have, on the other hand, served as models for studying proteins, the transport of which is arrested in specific compartments. Examples of such proteins are the VP7 of rotaviruses (ER specific) (Kabencell and Atkinson, 1985) and the E1 of coronaviruses (transitional element- and Golgi-specific) (Tooz et al., 1984; Repp et al., 1985; Sturman and Holmes, 1985).

During recent years, we have studied the transport of the glycoproteins of two different viruses: SFV (an alphavirus) and Uukuniemi virus (UUK) (a bunyavirus), which mature at two different cellular locations, the PM and the GC, respectively. In cells infected with UUK, the viral glycoproteins G1 and G2 (mol. wt 70 000 and 65 000, respectively) accumulate in the GC causing a progressive dilation and vacuolization of this organelle (Kuismanen et al., 1982, 1984). The site of virus maturation is evidently determined by the accumulation of the glycoproteins in the GC. The transport from the ER to the central Golgi (acquisition of endoglycosidase H-resistant glycans) at 37°C is relatively slow (t1/2 ~ 30–45 min) (Kuismanen, 1984). The terminal glycosylation step(s) also occurs slowly, ~90–120 min after synthesis (Kuismanen, 1984). In addition to the typical high-mannose- and complex-type glycans, short endo H-resistant glycans, which presumably are processing intermediates, are found in virions (Pesonen et al., 1982).

We have recently isolated a temperature-sensitive mutant (ts12) of UUK, which at 39°C (non-permissive temperature) does not form virus particles (Gahmberg, 1984). In cells infected with ts12 at 39°C, the glycoproteins accumulate in the GC causing the typical UUK-induced expansion and vacuolization. G1 and G2 remain in the GC up to 6 h in the presence of cycloheximide (Gahmberg et al., 1986). The maturation defect is not reversible since no virus is formed when the infected cultures are transferred from the restrictive to the permissive temperature.

What are the correlations between the morphological changes of the GC, the special features of virus maturation and the slow transport of the viral glycoproteins? Is it possible that the Golgi functions would be impaired due to the infection, thereby preventing G1 and G2 from leaving the GC and making them appear ‘Golgi-specific’? Could the disturbance of the normal organization of the Golgi stacks impair the rate of transport and processing of the glycans of PM proteins?

To answer these questions, we have studied the biosynthesis of the glycoproteins of SFV in cells pre-infected with ts12 of UUK. The SFV glycoproteins E1 and p62 (precursor of E2 and E3) are transported as a complex (Simons and Garoff, 1980). At 37°C the transport from the ER to the GC takes place within 20–25 min (Green et al., 1981) followed by the appearance of the glycoproteins at the PM starting ~30 min after their synthesis (Green et al., 1981; Kääriäinen et al., 1980; Simons and Warren, 1983). During transport, E1 acquires one complex glycan with terminal sialic acid, while the primary high-mannose-type glycans of E2 are trimmed by removal of glucose and 3–4 mannose residues (Kääriäinen and Pesonen, 1982).
sensitive transport mutant, ts1 (Kääriäinen et al., 1980; Saraste et al., 1980). With this mutant, the virus glycoproteins accumulate in the ER at 39°C (Saraste and Hedman, 1983). Upon shift of the cultures to 28°C, the glycoproteins migrate synchronously to the PM. We show here that in double-infected cells the SFV glycoproteins are efficiently transported to the PM and become terminally glycosylated, whereas the UUK glycoproteins are retained in the GC.

Results

Superinfection of UUK ts12-infected cells with SFV

BHK21 cells were infected with UUK ts12 at the restrictive temperature (39°C) for 20 h. Intracellular immunofluorescence staining with antibodies against UUK glycoproteins revealed that all cells had been infected productively and at least 80% of them displayed the typical perinuclear vacuolar staining of late UUK infection like that shown in Figure 1A. As shown previously this staining pattern is due to a dilation and vacuolization of the GC (Kuismanen et al., 1984; Gahmberg et al., 1986). At 20 h after UUK ts12 infection the cells were superinfected with wild-type SFV. Double immunofluorescence staining of the cells 4 h later revealed that both SFV and UUK glycoproteins were found in the same intracellular perinuclear vacuolar structures (Figure 1A and B). Also the cell surface was brightly stained with antibodies against SFV (Figure 1C). Surface staining of double-infected cells with antibodies against UUK glycoproteins gave negligible fluorescence (not shown), similar to that seen in cells infected with UUK ts12 alone, indicating that UUK glycoproteins were not co-transported with those of SFV.

At different times after infection with SFV, virus was harvested from the medium of double-infected cells and of cells infected with SFV alone, and assayed for infectivity. The growth of SFV was slightly retarded in the double-infected cells, ~15–30 min throughout the growth cycle (Figure 2). The final yield of infectious virus at 8–10 h from double-infected cells was ~60–40% of that from the cells infected with SFV alone.

We also studied whether the SFV glycoproteins could be chased out from the disorganized GC occupied with UUK glycoproteins (Figure 3A and B). At 4 h after superinfection with SFV, cells were treated with cycloheximide (50 μg/ml) to prevent further
Fig. 3. Double-immunofluorescence staining of BHK21 cells infected with UUK ts12 for 20 h followed by superinfection with SFV wild-type for 4 h (A and B). Cycloheximide (50 μg/ml) was added 4 h after superinfection with SFV and the incubation was continued for 1 h (C and D) or for 3 h (E and F). Permeabilized cells were stained with monoclonal anti-UUK G1 antibodies and polyclonal rabbit antibodies against SFV glycoproteins as in Figure 1. A, C and E show the intracellular distribution of UUK glycoproteins as revealed by TRITC staining. B, D and F show the intracellular localization of SFV glycoproteins by FITC staining.
protein synthesis, and double-stained with antibodies against UUK and SFV glycoproteins. After 60 min incubation in the presence of the drug most of the fluorescence due to anti-SFV antibodies had disappeared (Figure 3D). Staining of the same cells with antibodies against UUK glycoproteins showed an expansion of the perinuclear fluorescence, prominent already after a 1 h treatment with cycloheximide (Figure 3C and E). We conclude from this experiment that the SFV glycoproteins were transported further from the GC, whereas the UUK glycoproteins were effectively retained there.

**Kinetics of transport of SFV glycoproteins in cells infected with UUK ts12 and SFV ts1**

UUK ts12-infected cells were superinfected at 25 h with a temperature-sensitive mutant ts1 of SFV at 39°C. At this temperature the SFV glycoproteins are synthesized normally but are arrested in the rough ER (Saraste et al., 1980; Saraste and Hedman, 1983). When the cultures are transferred to 28°C (permissive temperature) in the presence of cycloheximide the SFV glycoproteins are transported in a synchronous manner through the GC to the plasma membrane (Kääriäinen et al., 1980; Saraste and Kuismanen, 1984). We have estimated that at least 50% of the pulse-labeled SFV glycoproteins synthesized before the shift to 28°C are transported to the plasma membrane (Pesonen et al., 1981).

At 3.5 h after superinfection with SFV ts1 the cultures were shifted to 28°C in the presence of cycloheximide, and the amount of SFV glycoproteins at the cell surface was measured using a radioimmunoassay (RIA) devised earlier (Kääriäinen et al., 1980). Cells infected with SFV ts1 alone and mock-infected cells served as controls (Figure 4). There was a delay of ~15–30 min in the appearance of SFV glycoproteins at the surface of double-infected cells as compared with cells infected with ts1 alone.

Next we wanted to know whether the delay in the appearance of SFV glycoproteins at the cell surface in double-infected cells was due to a retardation during the early steps in the transport pathway. For this purpose we could take advantage of the fact that SFV E1 has a single complex glycan (Pesonen and Renkonen, 1976; Pesonen et al., 1981). The acquisition of endoglycosidase H resistance was used to measure the transport time of E1 from the ER to the central Golgi (Lodish et al., 1981; Simons and Warren, 1983; Dunphy et al., 1985) in single- and double-infected cells. The cells were pulse-labeled with [35S]methionine for 10 min at 3.5 h after superinfection with SFV ts1 or infection with ts1 alone at 39°C. After the pulse the cultures were transferred to 28°C in the presence of cycloheximide as before, and chased in the presence of an excess of unlabeled methionine. At different times lysates were prepared and SFV E1 was...
Fig. 6. Kinetics of transport of SFV ts1 glycoproteins in single- (C, F, I, L and O) and double-infected (A, B, D, E, G, H, J, K, M and N) BHK21 cells as revealed by immunofluorescence staining for SFV and UUK glycoproteins. Cells infected with UUK ts12 mutant for 25 h were superinfected with SFV ts1 at 39°C for 3.5 h (A and B) followed by a shift to 28°C in the presence of cycloheximide (50 μg/ml) for 10 min (D, E), 20 min (G, H), 30 min (J, K) and 60 min (M, N). SFV ts1-infected cells (single infection) served as controls: 3.5 h after infection at 39°C (C) followed by a shift to 28°C for 10 (F), 20 (I), 30 (L) and 60 (O) min. The intracellular distribution of UUK glycoproteins in double-infected cells is shown in the left most vertical panel (A–M) as revealed by polyclonal rabbit anti-G1 and G2 antibodies followed by TRITC-conjugated swine anti-rabbit IgG. SFV glycoproteins of the same double-infected cells were visualized by polyclonal guinea pig anti-SFV envelope antiserum followed by FITC-conjugated rabbit anti-guinea pig IgG (middle vertical panel, B–N). SFV glycoproteins of the single-infected control cells were stained as in double-infected cells (rightmost panel; C–O).
immonoprecipitated with anti-E1 rabbit antiserum, followed by endo H digestion of a portion of the samples. The samples were then analysed in SDS–PAGE as shown in Figure 5. In double-infected cells (Figure 5B) the endo H resistance was obtained more slowly than in cells infected with SFV ts1 alone (Figure 5A). About half of E1 had become endo H resistant within 75 min (Figure 5A, lane 7) and 105 min (Figure 5B, lane 9) in single- and double-infected cells, respectively. As can be seen in lane 11, part of E1 remained sensitive to endo H treatment (lower bands in Figure 5A and B) even after 120 min chase at 28°C. We have shown previously by immunoelectron microscopy (Saraste and Hedman, 1983) and by glycan analysis (Pesonen et al., 1981) that part of the SFV glycoproteins is permanently arrested in the ER. To estimate the time for acquisition of endo H resistance for E1, which was transported during the chase at 28°C, we had to deduce the proportion of non-transportable E1. For this purpose the fluorograms, shown in Figure 5, were scanned for band intensities and the fraction of endo H-sensitive material after 2 h chase (lower band lane 11, Figure 5A) was deducted from the intensities of the lower bands in all other samples. The results are shown in Figure 5C. By this method we could estimate that half of the transportable E1 became endo H resistant within 50–55 min in single-infected and within 70–75 min in the double-infected cells at 28°C.

To analyse the transport of SFV glycoproteins in individual cells after a shift of the ts1–ts2 double-infected cultures to 28°C we also used double immunofluorescence staining of the glycoproteins of UUK and SFV. Cycloheximide was added to the cultures 5 min prior to the shift to 28°C. At different times cultures infected with SFV ts1 alone or double-infected, were withdrawn, permeabilized and treated with antibodies against UUK and SFV glycoproteins. The vacuolized GC was localized by staining with UUK antibodies, previously shown to stain the GC (Gahmberg et al., 1986).

Before the shift to 28°C, a reticular cytoplasmic fluorescence typical for ER (Kaariainen et al., 1980, 1983) was seen after staining with antibodies against SFV glycoproteins both in double- and single-infected cells (Figure 6B and C), respectively, whereas UUK antibodies decorated a perinuclear region (Figure 6A).

Ten minutes after the shift to 28°C, cells infected with SFV ts1 alone showed a clear but limited perinuclear fluorescence (Figure 6F) which became more prominent in samples taken at 20 and 30 min (Figure 6I and L). In the double-infected cultures the perinuclear fluorescence stained with anti-SFV antibodies was visible with certainty only in samples taken at 30 min or later after the shift to 28°C (Figure 6K and N). This difference between the single- and double-infected cultures suggests that in UUK-infected cells the transport of SFV glycoproteins from the ER to the GC was retarded by ~20 min.

**Terminal glycosylation of SFV E1 in double-infected cells**

UUK ts12-infected cells were superinfected after 25 h with wild-type SFV at 39°C. At 4 h after SFV infection the cells were treated with periodate to oxidize sialic acid residues followed by reduction with tritiated borohydride (Gahmberg and Andersson, 1977). The cells were lysed and antibodies against E1 were used for immunoprecipitation. Cells infected with SFV alone served as controls. The immunoprecipitates were analysed by SDS–PAGE (Figure 7, lanes A and B). E1 proteins from both single- and double-infected cells were labeled. Quantitation of the fluorogram was done by scanning. The intensity of the band from double-infected cells amounted to 82% (Figure 7, lane A) of that from the same number of single-infected cells (Figure 7, lane B) indicating that the terminal glycosylation was not severely affected by UUK infection. The amount of the protein moiety of E1 was also estimated in single- and double-infected cells using lactoperoxidase-catalyzed iodination (Hubbard and Cohn, 1972). Quantitation of the bands showed that the amount of E1 protein labeled in double-infected cells was 75% of that in single-infected cells (Figure 7, lanes C and D) (quantitation scans not shown). We conclude from these experiments that the SFV glycoproteins were transported to the cell surface roughly equal amounts and were terminally glycosylated in a similar manner in single- and double-infected cells.

**Discussion**

The early finding by von Bonsdorff et al. (1970) that UUK buds through smooth-surfaced membranes in a perinuclear region at the GC has been verified by a variety of different methods (Kuismanen et al., 1982, 1984, 1985; Kuismanen, 1984; Gahmberg et al., 1986).

Immunofluorescence and immunoelectron microscope studies have revealed that a progressive expansion and vacuolization of the GC take place in UUK-infected cells concomitantly with the accumulation of G1 and G2 (Kuismanen et al., 1984, 1985; Gahmberg et al., 1986). Is it possible that these morphological changes of the GC impair the exocytotic pathway? Were this the case, it could explain why G1 and G2 are retained in the GC. They would in fact be falsely regarded as ‘Golgi-specific’ components.

These questions cannot be answered only by studying the biosynthesis of UUK glycoproteins. We have therefore, super-infected UUK-infected cells with another enveloped virus, SFV, which normally matures at the plasma membrane. We took advantage of a recently isolated temperature-sensitive mutant of
UUK, ts12. At 39°C the virus glycoproteins are synthesized normally and accumulate in the GC evidently causing its vacuolization in the absence of virus formation (Gahmberg, 1984; Gahmberg et al., 1986).

In the present study, ts12-infected cells were superinfected with SFV at a time when most cells showed the typical vacuolization of the GC. Almost normal yields of wild-type SFV were obtained from the double-infected cells (Figure 2). There was, however, a slight delay of SFV production throughout the growth cycle. It is likely that SFV was maturing at the plasma membrane, since the virus glycoproteins could be demonstrated at the cell surface by immunofluorescence (Figure 1C) and by external labeling (Figure 7). These results suggested that the changes of the GC did not severely impair its functions. The results obtained by surface labeling of the wild-type SFV-infected cells were confirmed using a reversible transport mutant of SFV, ts1.

In UUK ts12-infected cells, the SFV ts1 glycoproteins were also transported to the cell surface after the shift of the double-infected cultures to 28°C. There was a delay of ~20 min as compared with cells infected with ts1 alone. At the same time the UUK ts12 glycoproteins were retained in the vacuolized GC. We conclude from these results that the transport of SFV proteins, although somewhat retarded, took place despite the alterations of the GC.

Almost equal labeling of E1-associated sialic acid residues at the cell surface in single- and double-infected cells showed further that the glycosylation apparatus was largely unaffected by the structural changes of the GC.

Surface immunofluorescence with antibodies against UUK G1 and G2 was negligible in both double- and single-infected cells, indicating that the transport of SFV glycoproteins did not enhance the transport of UUK glycoproteins to the plasma membrane. This means that the sorting function of the GC was not altered by UUK infection. Since the exocytotic function of GC was normal the reason for the arrest of UUK glycoproteins in this organelle was not an artifact. The glycoproteins must share some essential properties of genuine Golgi-specific membrane proteins.

Since there was a delay in the appearance of SFV glycoproteins at the cell surface in UUK-infected cells, we anticipated that this could be due to the disorganization of the GC. We measured first the transport time of SFV E1 glycoprotein from the ER to the GC using as a criterion the kinetics of acquisition of endo H-resistant glycans. There was delay of ~20 min in the double-infected cells. Thus the observed delay in the transport to the cell surface must be between the ER and the central GC (Dunphy et al., 1985). This result was supported by immunofluorescence microscopy (Figure 6).

The reason for the specific delay in the transport of SFV glycoproteins from the ER to the GC is not known. Assuming that there are specific receptors for SFV glycoproteins mediating the transport from the ER to the GC as suggested for secreted proteins (Lodish et al., 1983; Fries et al., 1984; Kelly, 1985) and for some membrane glycoproteins (Williams et al., 1985) the normal function of these receptors could have been modified by UUK glycoproteins. This is unlikely since under our experimental conditions the UUK ts12 glycoproteins had already been transported to the GC during the 20–25 h period preceding superinfection with SFV. Preliminary experiments with pulse-labeled collagen in UUK ts12-infected cells suggest that the secretion of collagen is retarded in the virus-infected cells as compared with uninfected cells (unpublished). Thus, we may be dealing with a more general phenomenon than the delayed transport of SFV glycoproteins.

How can we explain the slow processing of UUK glycoprotein-associated glycans? One possibility would be that G1 and G2 are not transported vectorially like the secretory and plasma membrane proteins, which are typical ‘visitors’ of the GC (Dunphy and Rothman, 1985). If they also lack the specificity for a particular Golgi compartment their transport within the organelle might be randomized. For example G1 and G2 leaving the cis-cisternae might be transported directly to the trans-cisternae. In this case no processing of the glycans could take place. This hypothesis would also explain why mature, purified UUK virions contain immature complex glycans lacking galactose and sialic acid (Pesonen et al., 1982).

To explain the functional integrity of the vacuolated GC in the processing of SFV glycoproteins we have to predict that the structural components of the different Golgi compartments do not participate in a similar circulation as proposed for the UUK glycoproteins. The complicated apparatus, which is responsible for the vectorial transport of secretory and membrane proteins (Rothman and Lenard, 1984; Rothman, 1985; Wattenberg et al., 1986) must have been preserved even in the structurally disorganized GC.

Materials and methods

Viruses and cells

The origin and cultivation of wild-type SFV and the ts1 mutant and their infectivity assays in chick embryo fibroblasts and BHK21 cells have been described previously (Kerrin and Kää riäinen, 1974; Saraste et al., 1980). The isolation and cultivation of UUK ts12 mutant have been described earlier (Gahmberg, 1984). BHK21 cells grown on plastic dishes (NUNC) (35, 60 or 100 mm diameter) were infected with 5 p.f.u./cell of UUK ts12, 10 p.f.u./cell of SFV wild-type or 50 p.f.u./cell of SFV ts1 mutant in single and double infections as indicated for individual experiments.

Immunofluorescence microscopy

Non-confluent monolayers of BHK21 cells grown on glass coverslips were infected with UUK ts12 and superinfected with either wild-type SFV or ts1 mutant. At indicated times the infected cells were fixed with 3% paraformaldehyde and permeabilized with 0.05% Triton X-100. Double staining was carried out with an indirect immunofluorescence method (Kuismanen et al., 1982). Rabbit polyclonal anti-UUK G1–G2 antisera (Kuismanen et al., 1982) or mouse monoclonal antibodies raised against UUK G1 were used (Kuismanen et al., 1984) in combination with polyclonal either rabbit (Saraste et al., 1980) or guinea pig (kindly supplied by J. Saraste, University of Helsinki) SFV anti-envelope antibodies. The second antibody with the polyclonal antisera was either swine anti-rabbit IgG or rabbit anti-guinea pig IgG conjugated to either tetramethylrhodamine isothiocyanate (TRITC) or fluorescein isothiocyanate (FITC) (Dako, Copenhagen, Denmark). The second antibody with the monoclonal antisera was goat anti-mouse IgG conjugated to TRITC (Cappel Laboratories, Cochranville, PA).

The fluorescence was examined with a Polylux microscope (Reichert-Jung, Austria), using 100 × oil immersion objective and filters for TRITC and FITC fluorescence. Photomicrographs were taken on Ilford HP5 film.

Labeling and analysis of viral proteins

BHK21 cells grown on 35-mm plastic dishes were infected with UUK ts12 mutant at 39°C for 25 h followed by infection with SFV ts1 mutant for 3.5 h at 39°C (double infection). For infection with SFV ts1 alone the cells were treated and pre-incubated similarly but without UUK infection (mock-infection) followed by ts1 infection as above (single infection). At 2.5 h after ts1 infection the culture medium was replaced by methionine-free MEM. One hour later 100 μCi per dish of [35S]methionine (1420 Ci/mmol, Amersham) in 250 μl was added for 10 min. After the pulse the cells were washed with pre-warmed medium containing a 100-fold excess of unlabeled methionine and transferred to 28°C for chases of different time periods.

At indicated times the cells were washed with ice-cold phosphate-buffered saline (PBS) and lysed in NET-buffer [1% Nonidet P-40, 0.005 M EDTA, 0.05 M Tris–HCl (pH 8.0), 0.4 M NaCl, 100 IU of aprotinin (TrasyloI, Bayer AG, Leverkusen, FRG) per ml] (Gahmberg et al., 1986).

SFV E1 protein was immunoprecipitated with a specific rabbit antisem (Hashimoto et al., 1981; Viäanen, 1982) in 1 ml of NET-buffer as described previously (Kuismanen et al., 1984). Part of the immunoprecipitates was treated with endoglycosidase H (Seikagaku, Tokyo, Japan), 0.02 U/ml for 24 h at 37°C in 0.125 M citrate buffer, pH 5.0 (Kuismanen et al., 1984; Tarentino et al., 1974).
Surface labeling with the periodate-NaBH₄ technique was carried out as described previously (Gahmberg and Andersson, 1977) and the lactoperoxidase-catalyzed iodination according to Hubbard and Cohn (1972). The labelled cells were treated with NET-buffer and E1 was immunoprecipitated as above.

The labeled proteins were analyzed in 10% SDS polyacrylamide gels according to Laemmli (1970). Fluorography was carried out according to Bonner and Laskey (1974). For quantification of radioactivity the bands were excised, solubilized in NCS tissue solubilizer (Amersham) and the radioactivity determined in a LKB-Wallac 1211 Rackbeta counter in toluene-Permablend cocktail. The band intensities were determined by scanning in an.autoscanner (Helena Laboratories, TX).

Radioimmunoassay
The amount of SFV glycoproteins at the surface of double- and single-infected cells was determined after fixation with 3% paraformaldehyde using rabbit anti-SVF envelope protein antiserum (Saraste et al., 1980) essentially as described previously (Kääriäinen et al., 1980), except that commercial [125I]protein A (40 mCi/mI) (Amersham) was used.

Acknowledgements
We thank Annikki Kallio and Kaija Kettunen for technical assistance, Kirsti Tuominen and Marjut Puranen for typing the manuscript, Carl G. Gahmberg (Department of Biochemistry, University of Helsinki) for advice and for providing the reagents for the surface-labeling experiments, and Johan Peränen for helpful discussions. This work was supported by the Sigrid Juselius Foundation.

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Received on 18 July 1986; revised on 1 September 1986