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Temperature-dependent internalization of virus glycoproteins in cells infected with a mutant of Semliki Forest virus

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When the ts-1 mutant of Semliki Forest virus (SFV) was grown in chick embryo or BHK 21 cells at the restrictive temperature (39°C), its membrane glycoproteins were arrested in the endoplasmic reticulum, but started to migrate to the cell surface once the cultures were shifted to the permissive temperature (28°C). If the temperature of infected cells was raised back to 39°C, ts-1 glycoproteins disappeared from the cell surface as evidenced by loss of surface immunofluorescence and by radioimmunoassay based on the binding of ^{125}I-labelled protein A. This phenomenon was specific for ts-1 at 39°C as it was observed neither in cells infected with wild-type SFV at 39°C nor with ts-1 at 28°C. The disappearance of the ts-1 glycoproteins was due to internalization. The internalized proteins were digested, as shown by specific decrease of virus glycoproteins labelled with [^{35}S]methionine at 39°C before shift to 28°C, and by concomitant release of acid soluble ^{35}S-activity into the culture medium. Ts-1 infected cells were treated before shift back to 39°C with Fab' fragments, prepared from IgG against the viral membrane glycoproteins. After shift back to 39°C, the Fab' fragments disappeared from the cell surface. In the presence of chloroquine, they could be visualized in vesicular structures, using an anti-IgG-fluorescein isothiocyanate conjugate. The internalization of ts-1 glycoproteins was not inhibited by carbonylcyanide *p*-trifluoromethoxy phenylhydrazone, chloroquine, cytochalasin B, vinblastine, colcemid, or monensin.

Key words: virus/glycoproteins/internalization/radioimmunoassay

Introduction

Semliki Forest virus (SFV) is an enveloped RNA virus, which has three glycoproteins, E1 (mol. wt. 49 000), E2 (mol. wt. 52 000), and E3 (mol. wt. 10 000) on its surface. The nucleocapsid core consists of a 42S RNA genome and the capsid protein (mol. wt. 30 000) (for reviews, see Kääriäinen and Söderlund, 1978; Garoff *et al.*, 1982; Kääriäinen and Pesonen, 1982).

The structural proteins are synthesized as a polyprotein from a subgenomic 26S mRNA, which is identical to the 3' third of the 42S RNA. The complete nucleotide sequence of this part of the genome has been determined, allowing the deduction of the primary structure of the four structural proteins (Garoff *et al.*, 1980a, b). During the translation of the 26S RNA the amino-terminal capsid protein is cleaved off rapidly, and the ribosome then attaches rapidly to the rough endoplasmic reticulum (ER) membrane. The precursor protein p62 of E3 and E2 is translocated through the membrane, followed by the carboxy-terminal E1 protein (Garoff *et al.*, 1978). In normal infection p62 and E1 migrate as a dimer to the plasma membrane (Simons and Garoff, 1980; Ziemiecki *et al.*, 1980).

We have used temperature-sensitive mutants of SFV (Keränen and Kääriäinen, 1974) as models for intracellular transport of membrane proteins. The ts-1 mutant has a reversible defect in the transport of the virus glycoproteins (Saraste *et al.*, 1980). At the restrictive temperature (39°C) the glycoproteins remain in the ER (Kääriäinen *et al.*, 1980) and have exclusively high mannose-type glycans (Pesonen *et al.*, 1981). When ts-1 infected cultures are shifted to the permissive temperature (28°C), in the presence of cycloheximide, the viral glycoproteins migrate to the cell surface (Saraste *et al.*, 1980). During transport, parts of the high mannose chains are converted to complex glycans (Pesonen *et al.*, 1981). The transport of ts-1 glycoproteins to the Golgi complex has been established by immunofluorescence and immunoelectron microscopy (Kääriäinen *et al.*, 1980; Saraste, 1981). The transport from the ER is inhibited by carbonylcyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) (10 – 20 μM), an uncoupling agent of oxidative phosphorylation, whereas the carboxylic ionophore monensin (10 μM) allows the accumulation of ts-1 glycoproteins in the Golgi complex, but inhibits further transport to the cell surface (Kääriäinen *et al.*, 1980; Saraste, 1981).

Here we show that the ts-1 glycoproteins can also serve as a model for internalization of membrane proteins. The ts-1 glycoproteins, which had been transported to the cell surface, were specifically internalized and degraded at 39°C, but not at 28°C. This internalization process could not be inhibited by FCCP, monensin, the lysomotropic agent chloroquine, or drugs affecting the cytoskeleton network of the cell.

Results

Immunofluorescence of ts-1 infected cells

When ts-1 infected cells were incubated at 39°C for 5 or 9 h, no surface immunofluorescence could be seen after treatment of paraformaldehyde-fixed cells with anti-envelope serum followed by anti-IgG-fluorescein isothiocyanate (FITC) conjugate (Figure 1A). When the cultures were transferred to 28°C, in the presence of cycloheximide (100 μg/ml), and incubated for 2 h, the arrested glycoproteins migrated to the cell surface (Figure 1C), as reported previously (Saraste *et al.*, 1980). Intracellular staining revealed an intensive fluorescence of the juxtanuclear reticular structure (Figure 1D), which indicated that the virus glycoproteins were present in the Golgi complex (Kääriäinen *et al.*, 1980). Similar staining was not seen in cells maintained at 39°C for 5 – 9 h (Figure 1B). When the cells were incubated for 5 h at 39°C, transferred for 2 h to 28°C in the presence of cycloheximide, and shifted back to 39°C for 2 h, the surface immunofluorescence disappeared (Figure 1E). Cells which were not shifted back to 39°C, but incubated at 28°C for 4 h, displayed an intensive surface fluorescence (Figure 1F). These results suggest that ts-1 glycoproteins first appear at the cell surface, after the infected cells have been transferred to 28°C, but disappear when shifted back to the restrictive temperature.

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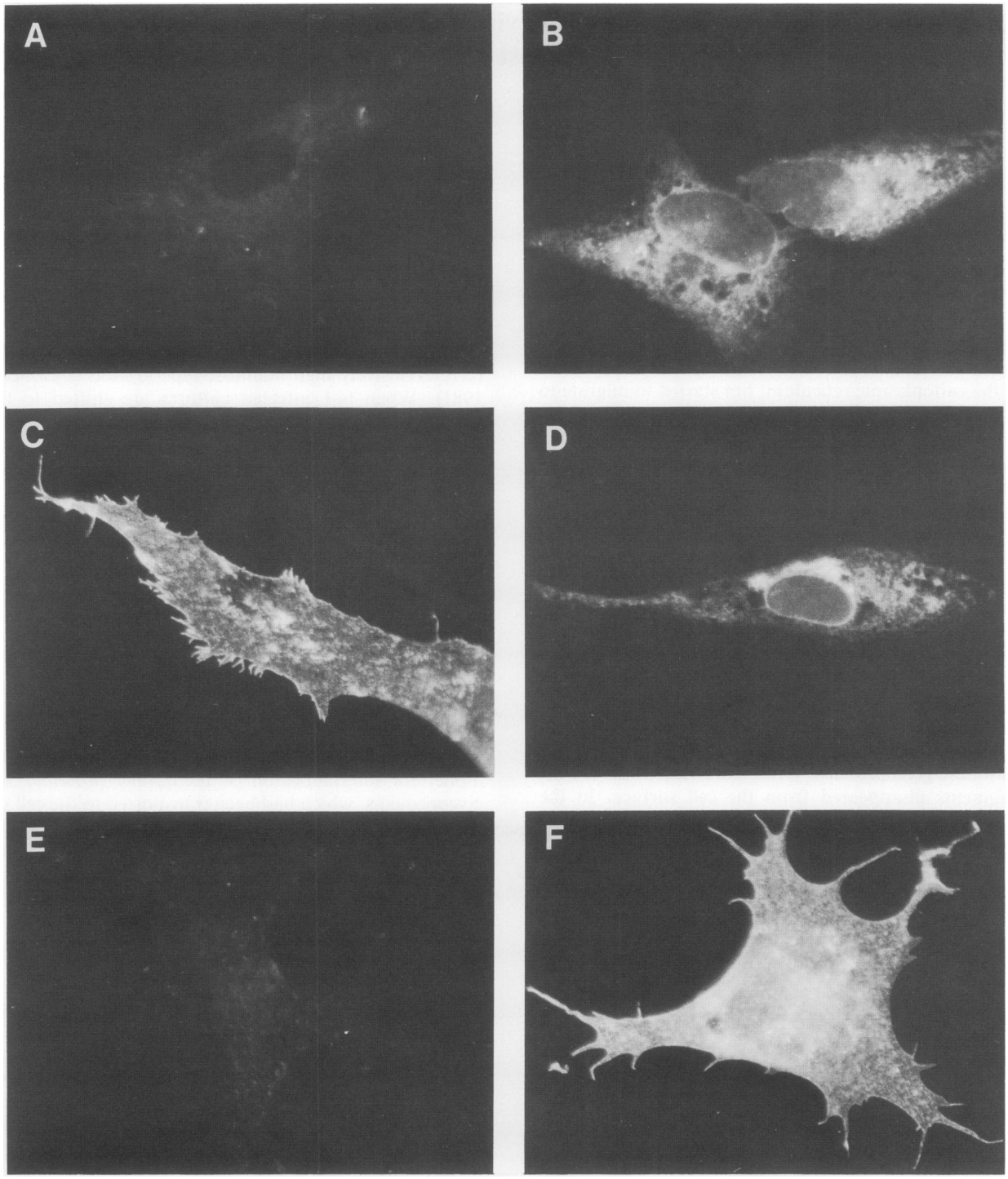

Fig. 1. Immunofluorescence of virus glycoproteins in ts-1 infected chick embryo fibroblasts. The cells were fixed with paraformaldehyde before treatment with antiserum and anti-IgG-FITC conjugate. For intracellular staining the cells were permeabilized with Triton X-100. **(A)** Surface staining of infected cells maintained for 5 h at 39°C. **(B)** Intracellular immunofluorescence of infected cells incubated for 9 h at 39°C. **(C)** Surface immunofluorescence of infected cells incubated for 5 h at 39°C followed by a 2-h incubation at 28°C. **(D)** Intracellular immunofluorescence of infected cells treated as in **C**. **(E)** Surface staining of infected cells shifted back to 39°C for 2 h after incubation first at 39°C (5 h) and then at 28°C (2 h). **(F)** Surface immunofluorescence of infected cells maintained for 4 h at 28°C after an initial incubation of 5 h at 39°C.

Kinetics of disappearance of ts-1 glycoproteins at 39°C

At different times after shift back to 39°C, the ts-1 infected and paraformaldehyde-fixed cells were treated with anti-envelope serum followed by ^{125}I-labelled protein A from *Staphylococcus aureus*. This quantitation of virus glycoproteins revealed that most of them disappeared from the cell surface within 60 min. Cells incubated continuously at 28°C showed little, if any, decrease in the binding of [^{125}I]protein A (Figure 2A). The same was true for cells infected with wild-type SFV grown first for 5 h at 39°C, then for 2 h at 28°C, followed by shift back to 39°C. The disappearance of ts-1 glycoproteins from the cell surface could not be inhibited by any of the drugs tested (Table I).

The ts-1 infected cells shifted from 39°C to 28°C were

Table I. Effect of drugs on the internalization of ts-1 glycoproteins.

Experimental conditions	Drug[c]	Concentration	[^{125}I]protein A binding on the cell surface (%)[d]
Control[a]	None		100
Shift back to 39°C[b]	None		0
Shift back to 39°C[b]	Colcemid	1 μg/ml	2
Shift back to 39°C[b]	Colcemid	10 μg/ml	10
Shift back to 39°C[b]	Cytochalasin B	2 μg/ml	2
Shift back to 39°C[b]	Vinblastine	10 μg/ml	0.3
Shift back to 39°C[b]	Monensin	10 μM	15
Shift back to 39°C[b]	FCCP	20 μM	0.1

[a]Ts-1 infected chicken embryo fibroblasts were maintained for 5 h at 39°C. followed by incubation for 2 h at 28°C in the presence of cycloheximide.
[b]Cells treated as in [a] were shifted back to 39°C for 2 h.
[c]The drugs were added to the culture medium at the moment of shift back to 39°C.
[d]The radioactivity bound to the cell surface after incubation for 5 h at 39°C has been subtracted as background and the value obtained after 2 h shift to 28°C was defined as 100%.

treated with Fab' fragments prepared from anti-envelope IgG for 100–120 min. The amount of Fab' at the cell surface was measured after transfer of the cultures back to 39°C. Then the cells were incubated with anti-IgG followed by [^{125}I]protein A. The kinetics of disappearance of Fab' fragments were similar to those of virus glycoproteins (Figure 2B). The result was essentially the same when the incubation with Fab' fragments was carried out at 0°C instead of 28°C prior to shift back to 39°C.

The ts-1 glycoproteins in cells maintained continuously at 28°C for 5, 7, or 12 h followed by shift to 39°C disappeared from the cell surface with the same kinetics as shown in Figure 2A. This indicates that the synthesis of the ts-1 glycoproteins at the restrictive temperature was not essential for their rapid disappearance from the cell surface (data not shown).

Degradation of ts-1 glycoproteins at 39°C

Cells infected with ts-1 and maintained at 39°C were labelled with [^{35}S]methionine for 10 min at 5 h post-infection followed by a 10-min chase with unlabelled methionine and cycloheximide. The cultures were then shifted to 28°C, in the presence of cycloheximide, and after 2 h part of the cultures were shifted back to 39°C. The media and cells were harvested at different times. There was a clear increase in the acid-soluble [^{35}S]methionine activity in the media from cultures shifted back to 39°C as compared with those maintained at 28°C (Figure 3). Our attempts to obtain virus glycoproteins from the medium by immunoprecipitation gave negative results.

Analysis of intracellular proteins by polyacrylamide gel electrophoresis revealed that the amount of virus glycoproteins decreased after shift back to the restrictive temperature, but not if the cultures were maintained at 28°C for 4 h after shift to the permissive temperature (Figure 4). When the bands were quantitated for radioactivity, it could be estimated that 30–40% of the labelled virus glycoproteins were degraded during the incubation for 4 h at 39°C. We have previously calculated from the conversion of high mannose-type glycans to complex ones that maximally 50% of the ts-1 glycoproteins are transported to the plasma membrane (Pesonen *et al.*, 1981). Thus, the above results suggest strongly that the ts-1 glycoproteins are internalized and that a substantial part of the internalized proteins is degraded.

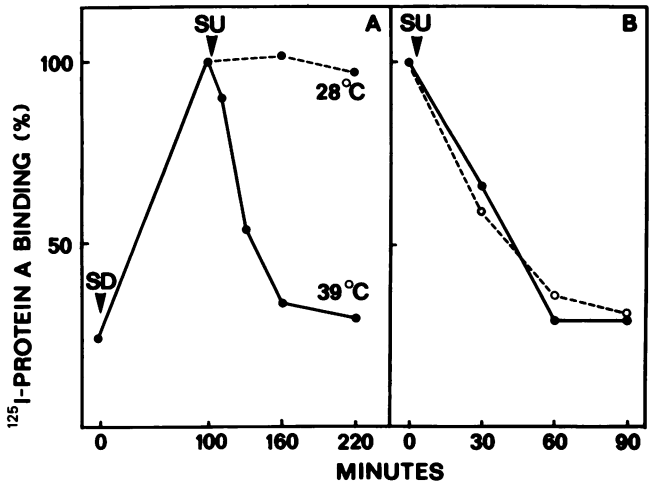

Fig. 2. Disappearance of ts-1 glycoproteins from the surface of infected cells shifted back to 39°C. Infected cultures were maintained for 5 h at 39°C followed by shift (SD) to 28°C in the presence of cycloheximide (100 μg/ml). **(A)** After 100 min at 28°C the cultures were shifted back to 39°C (SU) (——) or maintained at 28°C (----). At indicated times the cultures were fixed with 3% paraformaldehyde, treated with anti-envelope serum followed by [^{125}I]protein A. **(B)** The infected cultures maintained for 5 h at 39°C, followed by a 90-min incubation at 28°C, were exposed for 30 min to Fab' fragments prepared from anti-envelope serum before shift to 39°C. At indicated times the cultures were fixed and treated with anti-rabbit-IgG followed by [^{125}I]protein A (●). The control cultures which were not exposed to Fab' fragments were treated as in **A** (○).

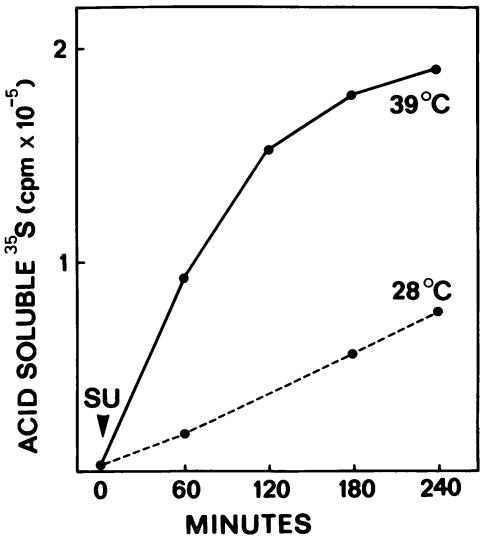

Fig. 3. Release of acid-soluble ^{35}S radioactivity from ts-1 infected cells. The cells were incubated for 5 h at 39°C and pulse-labelled for 10 min with [^{35}S]methionine (20 μCi/dish), followed by a chase for 10 min with a 20-fold excess of unlabelled methionine in the presence of cycloheximide (100 μg/ml). The cultures were shifted to 28°C for 100 min, after which part of them were shifted back to 39°C (SU). The culture media were harvested at indicated times, and the acid-soluble radioactivity was determined.

Internalization of Fab' fragments bound to ts-1 glycoproteins

Cells infected with ts-1 and incubated for 5 h at 39°C were shifted to 28°C for 2 h. The cultures were exposed to Fab' fragments, prepared from the IgG fraction of anti-envelope serum, for 30 min at 0°C to avoid fluid phase uptake. The unbound Fab' fragments were washed away and the cultures were shifted to 39°C or 28°C in the presence or absence of

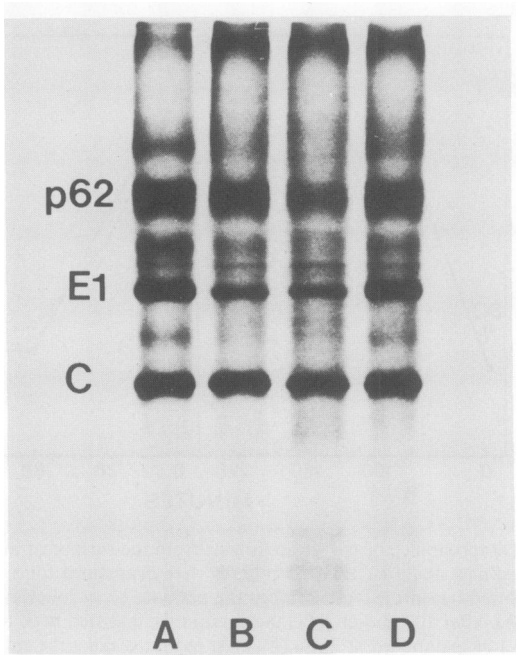

Fig. 4. Fluorogram of [^{35}S]methionine-labelled proteins from ts-1 infected cells. The cells were pulse-labelled and chased at 29°C as in Figure 3. Thereafter the cultures were incubated at 28°C for 100 min **(A)**, or for 340 min **(D)**, followed by a shift back to 39°C for 120 min **(B)**, or for 240 min **(C)**.

100 μM chloroquine. The cells were fixed with paraformaldehyde and part of the cultures were treated with Triton X-100 to permeabilize the cell membrane before staining with anti-IgG-FITC conjugate. Immediately after incubation, the Fab' fragments were found exclusively at the cell surface (Figure 5A), and no intracellular fluorescence could be seen in the permeabilized cells (Figure 5B). The surface fluorescence was retained in cultures incubated for 4 h at 28°C, whereas it disappeared almost completely from cells shifted back to 39°C, in accordance with the results in the [^{125}I]protein A binding assay (Figure 2B). Negligible intracellular fluorescence was seen in cells shifted back to 39°C for 2 h in the absence of chloroquine (Figure 5C), while in the presence of the drug a bright, presumably vesicular, fluorescence was observed (Figure 5D). In mock-infected cells neither surface nor intracellular fluorescence could be demonstrated, indicating that unspecific binding or internalization of Fab' fragments had not taken place.

Discussion

The ts-1 mutant of SFV has been useful in establishing the intracellular pathway of viral glycoproteins. At the restrictive temperature, the ts-1 glycoproteins remain in the ER, evidently because the cellular transport machinery fails to recognize them as transportable proteins, owing, perhaps, to a wrong

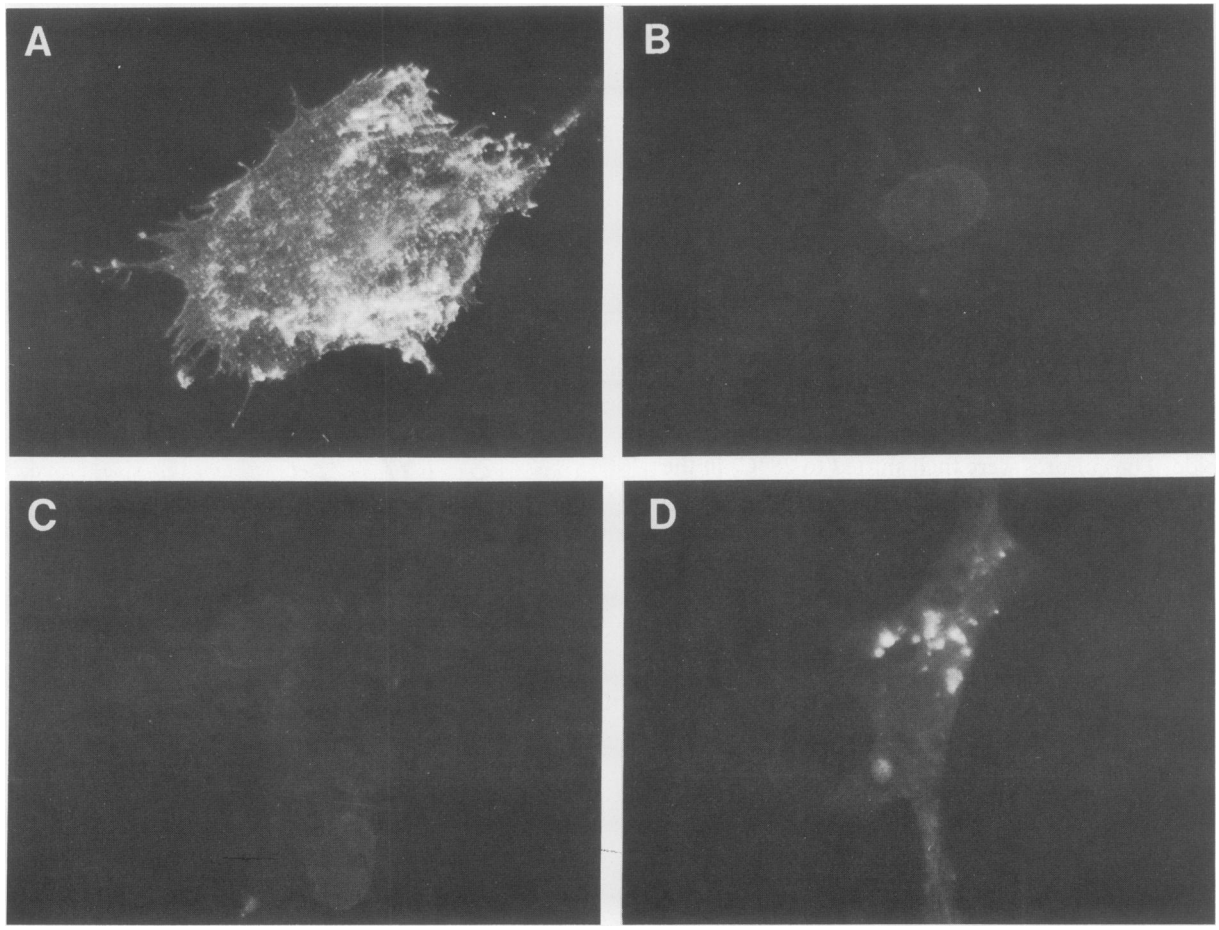

Fig. 5. Localization by immunofluorescence of Fab' fragments against envelope glycoproteins of SFV in ts-1 infected cells. The infected cells were maintained for 5 h at 39°C, shifted to 28°C for 2 h followed by exposure to Fab' fragments for 30 min at 0°C before shift back to 39°C, in the presence or absence of 100 μM chloroquine. Fixed cells were treated with anti-rabbit-IgG-FITC conjugate. For intracellular staining the cells were permeabilized with Triton X-100. **(A)** Surface and **(B)** intracellular staining of cells immediately after Fab' treatment. **(C)** Intracellular staining 2 h after shift back to 39°C. **(D)** Intracellular immunofluorescence 2 h after shift back to 39°C in the presence of chloroquine.

configuration or possibly to aggregation. This defect is reversible since, when the infected cultures are transferred to the permissive temperature, the viral glycoproteins are transported via the Golgi complex to the cell surface (Kääriäinen *et al.*, 1980; Pesonen *et al.*, 1981; Saraste *et al.*, 1980; Saraste, 1981). The transport from the ER to the Golgi complex is inhibited by FCCP, and from the Golgi complex to the cell surface by monensin and FCCP.

Here we have shown that ts-1 glycoproteins, synthesized either at 28°C or 39°C, disappear rapidly from the cell surface if they have been allowed to reach it. The disappearance is strictly temperature-dependent taking place only at 39°C, and specific for ts-1, since it was not observed in wild-type infected cells grown either at 28°C or 39°C. The glycoproteins are not shed to the medium but rather internalized and degraded as evidenced by the following results: (i) [^{35}S]methionine-labelled glycoproteins were degraded; (ii) acid-soluble radioactivity was released in substantial amounts from cells incubated at 39°C and in lesser amounts from cells incubated at 28°C; (iii) Fab′ fragments against viral glycoproteins disappeared from the cell surface with kinetics similar to those of the viral glycoproteins; and (iv) using anti-IgG-FITC conjugates, Fab′ fragments were found inside the cells, probably in vesicles, but only in the presence of chloroquine, which is known to inhibit lysosomal degradation of endocytosed proteins (Seglen *et al.*, 1979).

The internalization of ts-1 glycoproteins at 39°C resembles the fate of insulin and epidermal growth factor (EGF) receptors, once they have bound specific ligands. The receptor-ligand complex is internalized and apparently degraded in the lysosomes (Goldstein *et al.*, 1979; Haigler *et al.*, 1979; Kasuga *et al.*, 1981; King *et al.*, 1980; Schlessinger, 1980; Schlessinger *et al.*, 1980). The internalization of the receptor-ligand complex is preceded by clustering of the receptors (Schechter *et al.*, 1979; Schlessinger *et al.*, 1978; Zidovetzki *et al.*, 1981). In the case of ts-1, there is supposedly no specific ligand to react with the virus glycoproteins, and thus the internalization process must be elicited by the restrictive temperature. Addition of the monovalent ligand, Fab′ fragments, to the cells did not alter the kinetics of internalization at 39°C. It would be tempting to speculate that the internalization is preceded by clustering of ts-1 glycoproteins similar to the clustering of hormone receptors in the presence of their ligand.

Whatever mechanism elicits the internalization of ts-1 glycoproteins at 39°C, it may well be the same one which inhibits their transport from the ER immediately after synthesis. This would mean that the positive signal for internalization is negative for transport from the ER to the Golgi complex, i.e., different intracellular mechanisms operate in these two transport pathways. This is also supported by the inability of FCCP to inhibit the internalization process, whereas the drug efficiently inhibits the transport from the ER. The internalization of ts-1 glycoproteins is accompanied by their specific degradation, possibly in the lysosomes, as is the case with insulin and EGF receptor-ligand complexes. This would suggest that the internalization signal for both ts-1 glycoproteins and hormone-receptor complexes is also a signal for destruction. Thus, the ts-1 glycoproteins offer a model for investigations of the internalization of membrane glycoproteins, which have so far chiefly been made by following the fate of a specific ligand rather than of the receptor itself (Pastan and Willingham, 1981a, b).

Materials and methods

Virus and cells

The origin and cultivation of wild-type SFV and ts-1 mutant in secondary chick embryo fibroblast and BHK 21 cells have been described previously (Keränen and Kääriäinen, 1974; Saraste *et al.*, 1980).

Temperature shift experiments

Cells on 35 mm plastic dishes were infected with 50 PFU/cell of wild-type SFV or ts-1 in MEM supplemented with 0.2% bovine serum albumin and 20 mM HEPES, pH 7.0 or 7.2 (MEM-BSA) at 39°C. After 1 h adsorption of the virus, the cells were washed and incubated at 39°C for 5 h as before (Saraste *et al.*, 1980). Cycloheximide (100 μg/ml) was added when the cultures were shifted to the permissive temperature (28°C) for 2 h. Again a new prewarmed medium was added and the cultures were transferred to 39°C. Cultures incubated throughout at 39°C and cultures incubated for 4 h after shift to 28°C served as controls.

Labelling and analysis of viral proteins

Labelling with [^{35}S]methionine (20 μCi/dish) was carried out for 10 min at 39°C at 5 h post-infection in methionine-free MEM-BSA, followed by a chase with 20-fold excess of unlabelled methionine and cycloheximide (100 μg/ml) (Saraste *et al.*, 1980). Proteins were isolated and analyzed in 12% polyacrylamide slab gels as described earlier (Saraste *et al.*, 1980). After fluorography, the desired bands were cut from the dried gel and the radioactivity was determined as before (Hashimoto *et al.*, 1981). The acid soluble radioactivity was determined after precipitation with 10% TCA.

Fluorescence microscopy

Indirect immunofluorescence microscopy using antiserum against the 29S envelope protein complex of SFV and anti-rabbit-IgG-FITC conjugate (Wellcome) was performed as before (Kääriäinen *et al.*, 1980; Saraste *et al.*, 1980). The Fab′ fragments were prepared from anti-envelope serum according to Mage (1980). Mock-infected cells and cells infected with either ts-1 or wild-type SFV were exposed to Fab′ fragments (650 μg/ml) in MEM-BSA for 30 min at 0°C followed by three washes at the same temperature. The cultures were shifted in prewarmed MEM-BSA either to 28°C or 39°C. At desired times the cultures were washed and the cells were fixed with 3% paraformaldehyde as before (Saraste *et al.*, 1980). Half of the cultures were permeabilized by 0.05% Triton X-100 (Laurila *et al.*, 1978), and all were treated with anti-rabbit-IgG conjugate.

[^{125}I]protein A binding assay

Infected or mock-infected cultures were treated either before or after fixation with Fab′ fragments (50 μg/ml) or anti-envelope serum, respectively. In the case of antiserum treatment [^{125}I]protein A was added directly. Cells treated with Fab′ fragments were incubated with anti-IgG for 30 min at 37°C, washed three times with Dulbecco's PBS containing 0.5% BSA, and labelled with [^{125}I]protein A as described previously (Kääriäinen *et al.*, 1980).

Acknowledgements

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References

Garoff,H., Frischauf,A.-M., Simons,K., Lehrach,H., and Delius,H. (1980a) *Nature,* **288**, 236-241.

Garoff,H., Frischauf,A.-M., Simons,K., Lehrach,H., and Delius,H. (1980b) *Proc. Natl. Acad. Sci. USA,* **77**, 6376-6380.

Garoff,H., Kondor-Koch,C., and Riedel,H. (1982) *Curr. Top. Microbiol. Immunol.*, in press.

Garoff,H., Simons,K., and Dobberstein,B. (1978) *J. Mol. Biol.,* **124**, 587-600.

Goldstein,J.L., Anderson,R.G.W., and Brown,M.S. (1979) *Nature,* **279**, 679-685.

Haigler,H.T., McKanna,J.A., and Cohen,S. (1979) *J. Cell Biol.,* **81**, 382-395.

Hashimoto,K., Erdei,S., Keränen,S., Saraste,J., and Kääriäinen,L. (1981) *J. Virol.,* **38**, 34-40.

Kääriäinen,L., Hashimoto,K., Saraste,J., Virtanen,I., and Penttinen,K. (1980) *J. Cell Biol.,* **87**, 783-791.

Kääriäinen,L., and Pesonen,M. (1982) in Horowitz,M.I. (ed.) *The Glycoconjugates,* Volume III, Academic Press, NY/London, in press.

Kääriäinen,L., and Söderlund,H. (1978) *Curr. Top. Microbiol. Immunol.,* **82**, 15-69.

Kasuga,M., Kahn,C.R., Hedo,J.A., van Obberghen,E., and Yamada,K.M. (1981) *Proc. Natl. Acad. Sci. USA,* **78**, 6917-6921.

Keränen,S., and Kääriäinen,L. (1974) *Acta Pathol. Microbiol. Scand. Sect. B.*, **82**, 810-820.
King,A.C., Hernaez-Davis,L., and Cuatrecasas,P. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 3283-3287.
Laurila,P., Wartiovaara,J., and Stenman,S. (1979) *J. Cell Biol.*, **80**, 118-127.
Mage,M.G. (1980) *Methods Enzymol.*, **70**, 142-150.
Pastan,I., and Willingham,M. (1981a) *Annu. Rev. Physiol.*, **43**, 239-250.
Pastan,I., and Willingham,M. (1981b) *Science (Wash.)*, **214**, 504-509.
Pesonen,M., Saraste,J., Hashimoto,K., and Kääriäinen,L. (1981), *Virology*, **109**, 165-173.
Saraste,J. (1981) Thesis, University of Helsinki, Helsinki.
Saraste,J., von Bonsdorff,C.-H., Hashimoto,K., Kääriäinen,L., and Keränen,S. (1980) *Virology*, **100**, 229-245.
Schechter,Y., Hernaez,L., Schlessinger,J., and Cuatrecasas,P. (1979) *Nature*, **278**, 835-838.
Schlessinger,J. (1980) *Trends Biochem. Sci.*, **5**, 210-214.
Schlessinger,J., van Obberghen,E., and Kahn,R. (1980) *Nature*, **286**, 729-731.
Schlessinger,J., Schechter,Y., Willingham,M.C., and Pastan,I. (1978) *Proc. Natl. Acad. Sci. USA*, **75**, 2569-2663.
Seglen,P.O., Grinde,B., and Solheim,A.E. (1979) *Eur. J. Biochem.*, **95**, 215-225.
Simons,K., and Garoff,H. (1980) *J. Gen. Virol.*, **50**, 1-21.
Zidovetski,R., Yarden,Y., Schlessinger,J., and Jovin,T.M. (1981) *Proc. Natl. Acad. Sci. USA*, **78**, 6981-6985.
Ziemiecki,A., Garoff,H., and Simons,K. (1980) *J. Gen. Virol.*, **50**, 111-123.