Three Variations in the Cell Surface Expression of the Haemagglutinin–
Neuraminidase Glycoprotein of Sendai Virus

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SUMMARY

The fate of the haemagglutinin–neuraminidase glycoprotein (HN) of Sendai virus in three types of infection was studied by measuring its sensitivity to endoglycosidase H and its rate of appearance and turnover at the cell surface. HN behaved differently in the three types of infection. When highly expressed at the surface, as in a lytic standard virus infection, HN accumulated at the surface in a stable form (half-life of disappearance from the surface > 10 h). When moderately expressed, as in a non-lytic standard virus plus defective interfering virus infection, HN reached the membrane normally, but turned over rapidly (half-life about 2 h) and was re-internalized. When poorly expressed, as in long-term persistent infection, HN did not reach the cell surface and appeared to be degraded before reaching it. In contrast to HN, the other viral glycoprotein, F0, exhibited a similar turnover rate at the cell surface in the three situations. However, when compared to surface expression in standard virus-infected cells under standardized conditions, F0 surface expression in persistently infected cells was reduced. This reduction correlates with a decreased maturation rate in these cells.

INTRODUCTION

Sendai virus, a member of the Paramyxoviridae family, can participate in vitro in two distinct types of infection involving the majority of the infected cells. It can destroy the infected cells within days or it may allow complete survival of the infected culture, and establish a persistent infection in which all the cells are infected. This drastic modulation in the outcome of the infection is triggered by homologous defective interfering (DI) particles (Roux & Holland, 1979). Homologous F- particles are known to modulate the infection of many other RNA viruses (Holland et al., 1980), but Sendai virus DI particles are unique in that they provoke an abrupt change in the fate of the infection involving the majority of the infected cells (all or none effect). For this reason the study of the initial stages of an infection leading to cell survival was feasible, and led to the conclusion that the only difference between standard (St) virus infection causing cell death and standard plus DI (SD or mixed) virus infection allowing cell survival and establishment of persistent infection (pi), was a difference in viral glycoprotein expression at the surface of the infected cells (Roux & Waldvogel, 1981, 1983; Roux et al., 1984).

Sendai virus codes for six structural proteins, two of which are glycosylated and form the spike structures on the envelope: HN and F0 (for review, see Choppin & Compans, 1975). HN has an apparent molecular weight of about 72 000 and is the site of two viral activities: haemagglutinin and neuraminidase. F0, with an apparent molecular weight of 65 000, is responsible for the fusion of the viral envelope with the cell membrane envelope. F0 has to be activated by a specific protease for the virus to be infectious. When activated, F0 is resolved by polyacrylamide gel electrophoresis under reducing condition into two polypeptides, F1 (mol. wt. 52 000) and F2 (mol. wt. 12 000) (Homma & Ohuchi, 1973; Scheid & Choppin, 1974). Both HN and F0 contain only N-linked oligosaccharides, F0 containing three oligosaccharide chains of the complex type, and HN presumably containing two oligosaccharides of type A (nomenclature of Johnson &
During infection, the two glycoproteins, once synthesized on membrane-bound polysomes, are glycosylated and inserted into the cell plasma membrane from which the newly formed virus particles bud. Our previous data demonstrated that DI particles could mainly modulate the expression of one of the two viral glycoproteins at the surface of the infected cells late in infection. Indeed, HN protein was shown to be less efficiently expressed at the surface of SD virus-infected cells than at the surface of St virus-infected cells 40 to 44 h post-infection. Furthermore, HN expression was almost totally abolished at the surface of persistently infected cells. This restriction in expression was demonstrated by iodination of the infected cells followed by immunoprecipitation of the viral protein expressed at the cell surface (Roux & Waldvogel, 1983) or by binding of anti-HN or anti-F₀ monoclonal antibodies to the cell surface (Roux et al., 1984). In correlation with these variations in surface expression, variation in the stability of the protein was found: in persistently infected cells, HN (HN_{pi}) was less stable than in SD virus-infected cells (HN_{SD}), which in turn was found less stable than HN in St virus-infected cells (HN_{St}).

This direct correlation between HN instability and poor expression led us to follow more closely in the present work the fate of HN in the three types of infected cells: first, from its synthesis via its glycosylation pathway to its insertion into the plasma membrane; second, after its insertion into the plasma membrane. A parallel study was carried out with F₀, whose expression and stability were shown not to vary significantly in the three types of infection (Roux & Waldvogel, 1983; Roux et al., 1984).

METHODS

Cells, virus and infections. BHK-21 cells (American Type Culture Collection) were used routinely. BHK-21 cells persistently infected with Sendai virus (BHK_{pi}) have been described before (Roux & Holland, 1979). Sendai virus Harris strain and DI H-HP4 stocks (Kolakofsky, 1976) were prepared and St or St plus DI (SD) virus infections were performed as described before (Roux & Holland, 1979).

[{}^{35}S]Methionine pulse-chase labelling of infected cells. Thirty min prior to labelling, infected cells were incubated with methionine-free medium. Label (300 μCi/ml of [{}^{35}S]methionine) was then added for 15 or 30 min (see Results), and at the end of the labelling time cells were either harvested or further incubated in normal MEM containing 100 mM-methionine and 2% foetal bovine serum (FBS) for various periods of time (see Results).

[{}^{125}I]-labelling and chase of iodinated cells. Surface iodination of cells was performed according to Hubbard & Cohn (1972), exactly as described before (Roux & Waldvogel, 1983). After iodination, cells were either collected or further incubated with MEM containing 2% FBS and 10 mM-NaI for various periods of time (see Results). At the end of each incubation period, cells and supernatants were collected.

Purification and analysis of iodinated virus particles. Supernatants of iodinated cells were clarified (20000 g for 10 min, 4 °C) and virus particles were pelleted (200000 g, 1 h, 4 °C) through a cushion consisting of 25% glycerol in TNE (50 mM-NaCl, 10 mM-Tris-HCl pH 7.5, 2 mM-EDTA). They were resuspended in 50 μl of polyacrylamide gel electrophoresis sample buffer (Laemmli, 1970) and electrophoresed on a 12.5% gel. The dried gel was exposed on Kodak X-Omat XS-5 film and quantitative computation of the viral proteins was performed as described below.

Immunoprecipitations. Two techniques of immunoprecipitation were used, a cell surface immunoprecipitation and a total cell immunoprecipitation. Cell surface immunoprecipitation was designed to score only the protein exposed at the surface of the infected cells, while total cell immunoprecipitation was designed to score all the cell-associated proteins. These two methods have been described in detail earlier (Roux & Waldvogel, 1983). In brief, they differ in that intact cells are incubated at 4 °C with the antibody in the cell surface immunoprecipitation, but disruption of the cells is performed so that the cellular extract can react with the antibody in the total cell immunoprecipitation.

Quantitative computation of the viral proteins. The following precautions were taken in the computation of the viral proteins. First, as described previously (Roux & Waldvogel, 1983), antibodies were titrated to ensure their slight excess in the immunoprecipitations reactions. Second, quantification of [{}^{35}S]-labelled protein was carried out by scanning autoradiograms of different exposure times to ensure direct proportionality between darkening of the film and amount of protein. The area under the scanned peak was then integrated. Third, quantifications of [{}^{125}I]-labelled proteins were performed by excising the protein band from the dried gel after superimposition of the autoradiogram, and direct counting of the radioactivity in a gamma counter. Unless otherwise specified, a band next to that of interest was cut to estimate the background in each lane.

Digestion of viral proteins by endo-β-N-acetylglucosaminidase-H. Viral proteins were precipitated by total cell
immunoprecipitation as described above except that, at the end, the immunoprecipitates were resuspended in 1% SDS, 0.1 M-Tris–HCl pH 7.5, 0.5% 2-mercaptoethanol and boiled for 5 min. A sample of the immunoprecipitated protein was precipitated by 8 vol. ice-cold acetone, resuspended in 50 mM-sodium citrate containing 2 mM-phenylmethylsulphonyl fluoride (PMSF). Half the sample was mixed with endo-β-N-acetylgalactosaminidase-H (endo-H) from Streptomyces griseus (Sigma) at 100 mU/ml and incubated overnight at 37 °C, while the other half was mock-treated. The proteins were finally acetone-precipitated, resuspended in electrophoresis sample buffer and electrophoresed on a 10% polyacrylamide gel.

Monoclonal antibodies. Monoclonal antibodies to HN (S-16) and to F0 (M-16) were prepared and characterized as described before (Portner, 1981 and unpublished). They were shown to be specific for HN or F0 and to bind to these proteins at the surface of infected cells (Roux et al., 1984).

RESULTS

HNst, HNsd, Fosst and Foso en route to the plasma membrane

To follow the fate of HN and F0 en route to the plasma membrane, we first [35S]methionine pulse-labelled the infected cells, recovered the proteins by total cell immunoprecipitation after increasing intervals of chase and tested their sensitivity to cleavage by endo-H. Endo-H is known to cleave the N-linked high-mannose oligosaccharides (GlcNAc2, Man9, Glc3) attached to the protein as long as they have not been processed to complex oligosaccharides (Tai et al., 1979). Oligosaccharides that are intermediates in processing exhibit intermediate sensitivities to the enzyme (Tarentino & Maley, 1974; Tarentino et al., 1974; Robbins et al., 1977). Simple oligosaccharides are known to be added en bloc to the protein in the rough endoplasmic reticulum (RER) and then, as the protein moves from the RER to the Golgi apparatus, sugar residues are modified stepwise until they acquire the final composition that they exhibit on the mature protein (Hubbard & Ivatt, 1981). Sensitivity of the protein to endo-H thus reflects its RER location, while acquisition of resistance reflects its departure from RER to the Golgi apparatus and processing of the glycoprotein by maturation of the oligosaccharide moiety.

Fig. 1 compares the endo-H sensitivity of HNst and HNsd recovered at different times after their synthesis. About the same percentages of HNst and HNsd were sensitive to endo-H at the different times of analysis, and 2 h after their synthesis, only about 10% of both proteins was still sensitive. Fig. 2 shows the same analysis done with the F0 protein. Here, endo-H digestion allowed the generation of four distinct molecules. Three of these molecules (presumably reflecting the three possible intermediates of glycosylation containing three, two or one high-mannose oligosaccharides and respectively zero, one or two complex sugar residues, plus the molecule fully resistant to endo-H containing the three complex-type sugars) gradually disappeared in similar proportion for Fosst and Foso with increasing time of chase. Again, 2 h after their synthesis Fosst and Foso acquired almost complete resistance to endo-H. Therefore, these results indicated that both glycoproteins HN and F0 were transported to the Golgi apparatus and glycosylated at a similar rate in both types of infected cells.

Appearance of newly made viral glycoproteins at the surface of standard and mixed virus-infected cells

The next step was to follow the rate of appearance of HN and F0 at the surface of both types of infected cells. This was done by monitoring the amount of [35S]labelled proteins capable of binding at the cell surface to HN or F0 monoclonal antibodies added at different times after a pulse of [35S]methionine (cell surface immunoprecipitation). This method of immunoprecipitation was shown previously to precipitate specifically the viral proteins exposed at the cell surface (Roux & Waldvogel, 1983). Fig. 3(a) shows an example of such an experiment and Fig. 3(b) and (c) show a computation of the results obtained in a different experiment done in duplicate. In these experiments, since no effort has been made to compare the recovery of each protein, the rate of surface appearance of each protein has to be considered separately. Therefore, only the shape of the curves in (b) and (c), and not the absolute amounts of proteins, are significant. That is why the proteins are expressed as ‘relative amounts’. Both HNst and HNsd appeared at the cell surface after an initial lag of a few minutes (no protein seen at the surface at time point 0), but thereafter HNst accumulated continuously at the surface, while HNsd accumulation soon
Fig. 1. Endo-H sensitivity of HN$_{st}$ and HN$_{sd}$. Forty h post-infection, (a) St or (b) SD virus-infected cells (one 10 cm diam. Petri dish per sample) were pulse-labelled with $[^{35}S]$methionine for 15 min and chased for 0, 40 or 120 min. HN was recovered by total cell immunoprecipitation using 10 $\mu$l of S-16 antibody and 60 $\mu$l of a 50% suspension of Protein A-Sepharose. HN immunoprecipitated from cells harvested at 0, 40 or 120 min of chase were acetone-precipitated and resuspended in 80 $\mu$l of sodium citrate. Half of each sample was treated with endo-H (+), while the other half was mock-treated (−) before analysis by gel electrophoresis. Exposure of the gel was (a) 0 min lanes, 3 days; 40 and 120 min lanes, 24 h; (b) 3 days. V, viral protein marker.

Fig. 2. Endo-H sensitivity of F$_{os}$ and F$_{osd}$. As in Fig. 1, except that M-16 antibody was used. Exposure of the gel was 24 h.
Variation in Sendai virus HN expression

Fig. 3. Appearance of HN and F₀ at the surface of infected cells. Forty h post-infection, samples of 3 × 10⁶ cells were pulse-labelled with [³⁵S]methionine for 15 min and chased for the indicated periods of time (min). At the end of the chase period, the cells were incubated with 100 μl of a 20-fold dilution of S-16 or M-16 antibodies (cell surface immunoprecipitation). Forty μl of a 50% suspension of Protein A–Sepharose were used. Immunoprecipitates were analysed by polyacrylamide gel electrophoresis. (a) Example of autoradiograms obtained in such experiments. SURF tracks: immunoprecipitates obtained by cell surface immunoprecipitation. TOT tracks: immunoprecipitates obtained from companion samples recovered by total cell immunoprecipitation after 60 min of chase, using the same conditions of antibody and Protein A–Sepharose. V, viral protein markers. (b, c) Computation of the amounts of (a) HN (HNˢᵗ, ○; HNˢᵈ, □) and (b) F₀ (F₀ˢᵗ, ●; F₀ˢᵈ, □) recovered in a duplicate experiment similar to that in (a). The means of the two measures are shown along with their variations.

(a) Variation in Sendai virus HN expression

(b) Time of chase (min)

(c) Time of chase (min)

levelled off: from 10 to 60 min, HNˢᵗ increased at the surface by a factor of 8 to 10, whereas HNˢᵈ at the most doubled during the same interval (Fig. 3b). In contrast to HN, F₀ was observed at the membrane of both types of infected cells without any lag and the rates of surface accumulation of F₀ˢᵗ and F₀ˢᵈ were not significantly different. F₀ˢᵗ increased by a factor of 3
between 0 and 60 minutes of chase, while $F_{SD}$ increased by a factor of 2 during the same interval, but the surface accumulation of both proteins levelled off similarly by 15 to 20 minutes of chase (Fig. 3c). In both St- and SD-infected cells, the recovery of $^{35}$S-HN and $^{35}$S-$F_0$ by total cell immunoprecipitation (Fig. 3a, TOT lanes) demonstrated that the surface immunoprecipitation quantitatively precipitated the proteins, since in both cases, 60 to 70% of the total $^{35}$S-labelled proteins precipitable after 60 min of chase were actually recovered at the cell membrane. Similar results were obtained when a rabbit antiserum raised against the whole virus rather than monoclonal antibodies against HN and $F_0$ were used (data not shown). In conclusion, the rates of maturation of the viral glycoproteins were found to be similar in both types of infection, whereas the rate of accumulation of HN$_{St}$ and HN$_{SD}$ at the cell surface differed significantly.

**Fate of HN and $F_0$ exposed at the surface of standard and mixed virus-infected cells**

At least two possible explanations could be raised to account for the differences in HN$_{St}$ and HN$_{SD}$ surface accumulation. First, HN$_{SD}$, which matured normally from the RER to the Golgi apparatus, could be blocked from the Golgi apparatus onwards, in its transport to the plasma membrane. Second, the accumulation of HN$_{SD}$ could be counterbalanced by a faster turnover at the membrane. We tested this latter possibility by iodinating the infected cells with lactoperoxidase and following the fate of the iodinated proteins. Fig. 4(a) shows the amount of $^{125}$I-HN recovered by surface immunoprecipitation soon after the iodination reaction or after further incubation of the iodinated cells at 37 °C in MEM. The results obtained in the experiment presented in Fig. 4(a), as well as from other similar experiments, are plotted in Fig. 4(b). They demonstrate very clearly a different turnover of the HN$_{SD}$ and HN$_{St}$ exposed at the cell surface: whereas HN$_{SD}$ almost disappeared from the cell surface within 4 h, 80% of HN$_{St}$ was still present at the membrane 10 h after iodination. Moreover, it seems that the HN$_{St}$ which had not turned over within 2 h was bound never to leave the membrane. In contrast to HN, $F_{St}$ and $F_{SD}$ exhibited a quite similar turnover at the cell membrane, showing a half-life of about 4 to 6 h (Fig. 5a and b).

To trace the fate of the $^{125}$I-labelled proteins after they leave the membrane, virus particles were purified from the supernatants after the various chase periods and their protein composition was determined by polyacrylamide gel electrophoresis. In parallel, one-fifth of the infected cells which had produced the virus particles were similarly analysed, so that the efficiency of production of viral proteins in particles could be computed. Total cell material rather than proteins obtained from immune precipitation was preferred for this computation to match the direct method of analysis that viral particle purification represented. Fig. 6(a) shows the gel analysis of the cellular extracts and of the viral particles, and Fig. 6(b) and (c) present the computation obtained by excising and counting the protein bands of the gel. Of the 25% of HN$_{St}$ leaving the cell surface during the 4 h of chase, about 50% was recovered in virus particles. In contrast, of the 65% of HN$_{SD}$ leaving the membrane during the same interval, only about 10% was recovered in virus particles. Thus, disappearance of HN$_{SD}$ from the cell surface did not result from its incorporation into virus particles. Furthermore, attempts to recover by TCA or immune precipitation possible 'free' glycoproteins shed into the medium were unsuccessful (data not shown). Consequently, HN$_{SD}$ was likely to have been re-internalized. In an attempt to demonstrate re-internalization, iodinated HN$_{SD}$ was recovered by total cell immunoprecipitation to see whether it could be shown to turn over less rapidly, since re-internalized HN would be detected by this method. No difference in HN$_{SD}$ turnover could be detected in this way, indicating that, when re-internalized, HN$_{SD}$ was degraded very rapidly (not shown). Similarly, attempts to detect HN degradation products were not conclusive.

Computation of $^{125}$I-$F$ protein was complicated by the low amount of $^{125}$I label incorporated into the protein (iodination *in situ* is known to label $F_0$ poorly; Roux & Waldvogel, 1983). Despite this fact, Fig. 6(b) and (c) show that $^{125}$I-$F_{St}$ accumulated to some extent in viral particles during the chase, while $^{125}$I-$F_{SD}$ did not, suggesting that F$_{SD}$ was also re-internalized rather than incorporated into virus particles when leaving the cell surface.
Variation in Sendai virus HN expression

Fig. 4. Turnover of $^{125}$I-HN at the surface of St and SD virus-infected cells. Forty h post-infection, samples of St and SD virus-infected cells ($6 \times 10^6$) were iodinated. After iodination, cells were either harvested (0 h) or chased for 2 or 4 h (10 h). Cells were then resuspended in 1 ml of phosphate-buffered saline. (a) Two hundred $\mu$1 aliquots were incubated with 2 $\mu$L of S-16 antibody (cell surface immunoprecipitation). After cell disruption, the immunoprecipitates were recovered with 60 $\mu$L of a 50% suspension of Protein A–Sepharose and analysed by polyacrylamide gel electrophoresis. (b) $^{125}$I-HN protein bands were cut from the dried gel shown in (a) or similar, counted in a gamma counter and the fractions of $^{125}$I-HN (HN$_{St}$, ●; HN$_{SD}$, ○) found at the surface plotted as a function of chase intervals. Exposure of the gel (a) was St part, 15 h; SD part, 48 h.

Fate of HN and F$_0$ in persistently infected cells (HN$_{pi}$, F$_{0pi}$)

Iodination of persistently infected BHK cells (Roux & Waldvogel, 1983) as well as binding of anti-HN monoclonal antibodies at the cell surface (Roux et al., 1984) showed that HN$_{pi}$ surface expression was almost abolished, although HN$_{pi}$ was synthesized normally relative to the other viral proteins (Roux & Waldvogel, 1982). This lack of surface expression correlated with a very
Fig. 5. Turnover of $^{125}$I-F at the surface of St and SD virus-infected cells. As in legend to Fig. 4, except that M-16 monoclonal antibody was used. (b) $F_{St}$ (●) = $F_{6St}$ + $F_{1St}$; $F_{SD}$ (○) = $F_{6SD}$ + $F_{1SD}$.

The rapid degradation of the $^{35}$S-labelled protein, whose half-life was found to be less than 2 h (Roux et al., 1984). The question remained whether lack of expression was due to very rapid turnover of the protein once it had reached the surface of the cell, or whether HN$_{pi}$ was degraded before it could reach the surface. To answer this question, a comparison was made with HN expression at the surface of St virus-infected cells after standardization to the amount of intracellular viral nucleocapsids, as described by Roux & Waldvogel (1983). Fig. 7(a) shows that in such standardized conditions, HN$_{pi}$ expression was dramatically decreased relative to that of HN$_{St}$ as estimated immediately after iodination (especially since threefold less material was loaded in the HN$_{St}$ track). Because of this poor expression, the fate of $^{125}$I-HN$_{pi}$ was difficult to follow. Yet,
Fig. 6. Computation of the fates of HN and F iodinated at the surface of infected cells. Samples of St or SD virus-infected cells were iodinated and chased as in legend to Fig. 4. When collected, cells were washed with phosphate-buffered saline, concentrated, resuspended in 250 μl of polyacrylamide gel electrophoresis sample buffer, sonicated and boiled for 3 min. Virus particles produced in the supernatants during the chase intervals were pelleted and resuspended similarly in 50 μl sample buffer. (a) Fifty μl samples of either cell extracts or virus particles were electrophoresed on a polyacrylamide gel. The St (b) and SD (c) protein bands, localized on the dried gel, were excised and counted in a gamma counter. The amount of radioactivity in either HN or F protein bands is plotted as a function of chase intervals. HNc (○) and Fc (○), proteins derived from cells; HNv (△) and Fv (△), proteins derived from virus particles. F = F0 + F1. No background was subtracted.
Fig. 7. Turnover of $^{125}$I-HN and $^{125}$I-F at the surface of persistently infected cells. St virus or persistently infected cells (about 3 years old) were iodinated, chased for the times (h) shown and processed as for Fig. 4 for recovery of HN and F by cell surface immunoprecipitation. Parallel samples were used to establish the amount of intracellular nucleocapsid in both populations of cells as described previously (Roux & Waldvogel, 1982), so that the amount of $^{125}$I-protein loaded onto the gels could be adjusted to the amount of intracellular viral protein. Note that the standardized material loaded on St lanes was threefold less than on Pi lanes.

Fig. 7(b) similarly compares the amount of F$_0$ expressed at the surface of St virus and persistently infected cells, and presents a follow-up of $^{125}$I-F$_0$ turnover. Again, these observations were difficult to make, mainly because of poor efficiency of F$_0$ iodination relative to HN iodination as reported before (Roux & Waldvogel, 1983; e.g. compare in this figure the iodination of HN$_{st}$ and F$_{os}$). Nevertheless, comparison under standardized conditions allowed the estimation of the reduction of F$_{op}$ surface expression relative to that of F$_{os}$, and clearly demonstrated that the difference in expression between F$_{op}$ and F$_{os}$ was much less than that observed between HN$_{pi}$ and HN$_{st}$. This correlates with our previous observation that F$_0$ was expressed at the surface of persistently infected cells under conditions where HN was not (Roux et al., 1984). The follow-up of the fate of $^{125}$I-F$_{op}$ after iodination indicated a half-life of about 6 h at the cell surface (data not shown), which was similar to those of F$_{os}$ and F$_{osd}$ (see above).

Next, we followed the fates of HN$_{pi}$ and F$_{op}$ en route to the plasma membrane by estimating their sensitivity to endo-H during $[^{35}]$methionine pulse-chase labelling experiments. Due to poor viral protein synthesis in the persistently infected cells, the cells were pulse-labelled for 30 min, and the amounts of cells used to recover enough protein to perform the analysis were doubled. As a consequence, the immunoprecipitates obtained contained a higher protein background (Fig. 8 and 9). In this background, the major band migrating above the viral protein marker M was presumably actin. Despite this complication, the viral glycoproteins were clearly identified.

Fig. 8 shows that, during the 2 h of chase, HN$_{pi}$ resistance to endo-H was not as complete as that of HN$_{st}$, suggesting that HN$_{pi}$ was slowed down on its way to the Golgi apparatus. Since the half-life of newly synthesized HN$_{pi}$ was shown to be about 2 h (Roux & Waldvogel, 1983), it is
Variation in Sendai virus HN expression

Fig. 8. Endo-H sensitivity of $H_{N_p}$. As described in legend to Fig. 1, except that the infected cells (St virus and 3-year-old BHKpi) were labelled for 30 min and chased for the times (min) indicated in this figure. Also, two 10 cm diam. Petri dishes instead of one were used for the isolation of $H_{N_p}$. Exposure of the gel was 8 days.

Fig. 9. Endo-H sensitivity of $F_{o_p}$. As described in legend to Fig. 8, except that M-16 antibody was used.
reasonable to assume that a large proportion of HN_{pi} was degraded while still partially sensitive to endo-H. Therefore, the above experiments suggested that HN_{pi} was degraded before it could reach the cell plasma membrane.

The follow-up of the 35S-Fopi maturation rate, estimated by endo-H sensitivity is presented in Fig. 9. Unexpectedly, instead of the three intermediates created by endo-H digestion of F_{0st} at 0 min of chase (see also Fig. 2 and related text), only two intermediates were generated with F_{opi} (three protein bands instead of four, shown by open symbols). Moreover, in conditions where F_{0st} was fully resistant to the enzyme (the band marked by a closed symbol is presumably irrelevant since it is present in the mock-treated sample as well), a considerable amount of F_{opi} was still sensitive. After 2 h of chase, the upper band of F_{opi} was only slightly enriched compared to that seen after 0 min of chase. Although we have no clear explanation for such a modified pattern of endo-H sensitivity, it indicates nevertheless that F_{opi} was considerably slowed down on its way to the Golgi apparatus and thus presumably to the cell surface, since movement from RER to the Golgi apparatus seems to be the limiting step in the rate at which glycoproteins reach the cell surface (Strous & Lodish, 1980; Morrison & Ward, 1984).

DISCUSSION

A comparison of the fate of HN in St virus-, SD virus-, and persistently infected cells showed that quantitative differences in the cell surface expression of this protein (as reported previously: Roux & Waldvogel, 1983; Roux et al., 1984) correlated with differences in its mode of expression. When normally expressed at the surface, as in St virus infection, HN accumulated on the cell surface in a stable form. Reduction of this expression, as in SD virus-infected cells, corresponded to a faster turnover of the protein after its insertion into the plasma membrane. Finally, absence of expression, as in persistently infected cells, was a consequence of the absence of insertion into the plasma membrane. In these latter cells, HN matured more slowly and was only slightly degraded before it could reach the cell surface. In contrast to HN, F_0 was expressed at the surface of the three types of infected cells (Roux & Waldvogel, 1983; Roux et al., 1984), and exhibited a similar surface turnover in the three situations. In persistently infected cells, however, F_0 surface expression was reduced and, like HN, F_0 matured more slowly. Thus, the different behaviours of HN and F_0 in the three types of infection account for the differences in cell surface expression reported here and previously.

It is not clear why maturation of both HN and F_0 was slowed down in persistently infected cells. These cells have a normal metabolism, since their growth rate is comparable to that of infected cells. It could be argued that, in persistently infected cells, viral proteins have to await their turn in the maturation pathway when the cellular functions are not as exclusively reserved for viral metabolism as in acutely infected cells. However, this is unlikely since Sendai virus does not induce a shut-off of cellular metabolism. In fact, in mixed virus infection, the cells survive with no apparent pathology, yet HN and F_0 mature at the same rate as in standard virus-infected cells. A more likely possibility is that mutations which accumulate in the viral proteins during persistent infections (Rowlands et al., 1980) modify the intrinsic properties of the glycoproteins such that they utilize a different pathway for movement from the RER to the Golgi apparatus. Such mutations could also be responsible for the loss of one intermediate in glycosylation of F_0 by suppressing one glycosylation site. Although it remains unexplained, this delay in viral glycoprotein maturation in persistently infected cells certainly contributes to the pattern of HN and F_0 surface expression in these cells: it prevents a very unstable HN protein from reaching the cell surface, and it reduces the surface expression of F_0, which exhibits a normal turnover rate once it has reached the surface.

The differences in HN turnover at the cell surface could merely be a consequence of the fate of the infected cells. One could indeed argue that dying cells are no longer capable of recycling their membrane proteins. Were this explanation correct, one would expect a similar behaviour for HN and F_0. However, F_0 turnover at the membrane, as well as its surface expression, was not significantly affected by the type of infection. For this reason, we would rather suggest that HN_{pi} accumulation at the cell surface is part of its normal behaviour during a lytic infection, as is the
slow turnover of F0. A faster HN\textsubscript{SD} turnover thus becomes a specific modification of this normal behaviour triggered by DI particles.

The potential importance of this change in HN surface expression for cell survival and establishment of persistent infection, and the possible mechanism responsible for this change, based on the role played by the viral M protein in anchoring HN in the plasma membrane, have been discussed previously (Roux \textit{et al.}, 1984). Since then, we have made little progress due to our unsuccessful attempts to locate M in the infected cells. We know however, from recent studies of a temperature-sensitive mutant (ts 271; Portner \textit{et al.}, 1975) with a ts lesion in HN, that M is normally incorporated into virus particles in the absence of HN (unpublished results). This fact, added to previous results showing that whenever M was reduced in virus particles or absent in infected cells, HN was also reduced in virus particles or efficiently degraded in infected cells (Roux \textit{et al.}, 1984) argues for a major role of M in regulating HN function and turnover, and not the reverse.

This modulation of HN expression, apart from the importance it may have for establishment and maintenance of persistent infections \textit{in vivo} by Sendai virus, is an example of how cell surface expression of a particular viral antigen can be specifically adjusted. The interest of such an adjustment has to be related to a viral infection in the whole animal. Infected cells which are destined to survive viral infection and are then potentially capable of sustaining viral infection in a latent form in the organism, have to escape immune surveillance. Restriction of viral antigen expression at the cell surface has been shown to be appropriate in this respect (Fujinami & Oldstone, 1980). Modulation of expression of viral antigens by specific antibodies has been proposed to achieve this goal (Joseph & Oldstone, 1975; Barret & Koschel, 1983). Alternative mechanisms are faster turnover at the cell surface in the absence of antibodies, degradation of a protein before it reaches the plasma membrane and slowing down of the maturation rate of a protein. It is noteworthy that these mechanisms correlate with the presence of DI genomes in infected cells. Verification of these mechanisms in the whole animal is not yet feasible. What is observed, however, in the whole animal is gradual and selective disappearance of viral antigens in the brain after infection with Sendai virus, measles virus or lymphocytic choriomeningitis virus (Kristensson \textit{et al.}, 1983; Johnson \textit{et al.}, 1981; Oldstone & Buchmeier, 1982).

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REFERENCES


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