The major glycoprotein of Sendai virus is dispensable for efficient virus particle budding

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A temperature-sensitive mutant of Sendai virus, ts271, when grown at restrictive temperature is known to produce virions lacking integral haemagglutinin–neuraminidase (HN). In this study, it is shown that the transmembrane–cytoplasmic tail of HN is not detected either. This apparent complete lack of HN does not affect budding efficiency.

Assembly of Sendai virus, a member of the Paramyxoviridae family, takes place at the cell plasma membrane. The new virion is produced after formation of a bud which pinches off the membrane, resulting in the release of a particle. Participating in this assembly process are (i) the viral glycoproteins, which are inserted in the plasma membrane with their ectodomain exposed at the cell surface, (ii) the matrix (M) protein, which is partly embedded in the lipid bilayer on the cytoplasmic side and organized as a leaflet structure and (iii) the nucleocapsid, composed of the viral RNA associated with NP, P and L proteins. According to the currently accepted model, which is based both on experimental data (for recent reviews see Peeples, 1991; Ray et al., 1991) as well as rational thinking, the M protein plays a central role in the organization of the budding complex in which the glycoproteins would be anchored in patches in the membrane through contacts between their transmembrane or cytoplasmic domains and the M protein leaflet. This M protein leaflet would in turn be recognized, from the cytoplasmic side, by the nucleocapsid. At present, none of these various contact points have been identified and therefore the real participants in this overall structure remain uncertain. For instance, although it is reasonable to assume that the nucleocapsid will make contact with the M protein through the NP protein, a contact between the M protein and the RNA cannot be excluded.

Another point yet to be determined is which components are the minimum needed for virus assembly. From the study of mixed (standard plus defective interfering particles) and persistent Sendai virus infections, reduction of virus particle budding has been correlated with an increase in the turnover of the M protein and haemagglutinin–neuraminidase (HN), suggesting that both of these proteins are required for efficient budding (Tuffereau & Roux, 1988; Roux et al., 1985; Roux & Waldvogel, 1982). On the other hand, a temperature-sensitive (ts) mutant, ts271, was shown to assemble into virus particles at the non-permissive temperature in the absence of detectable HN, thereby indicating that HN is dispensable (Portner et al., 1974, 1975; Markwell et al., 1985; Tuffereau et al., 1985). A vesicular stomatitis virus (VSV) mutant, ts045, has been shown to produce virions lacking the G protein at the non-permissive temperature (Schnitzer et al., 1979). These spikeless particles were shown, however, to contain the G protein membrane anchors, indicating that the G protein was indispensable for virus particle budding (Metsikkö & Simons, 1986). Therefore, it was relevant to determine whether a remnant portion of HN, i.e., its transmembrane–cytoplasmic portion, could be found in Sendai virus ts271 virions produced at the restrictive temperature.

The ts271 virion was first described as containing non-functional haemagglutinin and reduced neuraminidase activity when grown at non-permissive temperature (Portner et al., 1974, 1975); HN was not detected under restrictive conditions either by iodination of the infected cell surface proteins (Tuffereau et al., 1985), or in the virus particles after [35S]methionine labelling (Markwell et al., 1985). This latter point is shown in Fig. 1 and Table 1. Fig. 1 (a) shows a comparison of the [35S]methionine-labelled viral polypeptides found in wild-type (wt) or mutant (ts) virions grown under permissive (31 °C) or non-permissive (39 °C) conditions. At 31 °C the amount of HNwt incorporated into the virus particles was about twofold less than that of HNts271, when the amounts of HN were normalized to those of the other viral membrane protein, M (for clarity see HN and M in Fig. 1a, lanes 1 and 5, and Table 1, 31 °C lines). However, at
Fig. 1. HN protein content and budding efficiency of is271 virions. Samples of 10^7 LLCMK2 cells were infected with Sendai wt (lanes 1 to 4) or is271 (lanes 5 to 8) Sendai virus at an m.o.i. of 10 and incubated at 31 °C (lanes 1, 2, 5 and 6) or 39 °C (lanes 3, 4, 7 and 8). Medium was removed and replaced with 10 ml of fresh medium containing 30 μCi/ml [3S]methionine 20 h post-infection, and after 5 h of labelling, cell supernatants were collected and clarified. Virus particles were recovered by pelleting (500000 g, 4 °C) through 15% glycerol in 10 mM-Tris-HCl pH 7.5, 50 mM-NaCl, 1 mM-EDTA (TNE), resuspended in 80 μl PAGE sample buffer (Laemmli, 1970), electrophoresed on a 10% polyacrylamide gel and electroblotted onto nitrocellulose. The filter was reacted with Rab-HNmil (1:200) (Johnson et al., 1984) and then developed using alkaline phosphatase-conjugated goat anti-rabbit IgG and BCIP/NBT colour reagents (Promega). (a) Autoradiogram of the nitrocellulose sheet exposed to X-ray film. (b) A BCIP/NBT-stained blot. Lane 9, virus protein markers. Lanes 1, 3, 5 and 7, and 2, 4, 6 and 8 were loaded with a quarter (20 μl) and three-quarters (60 μl) of the virus particle preparation respectively.

39 °C, HN was barely detectable in the mutant virions, whereas its amount in the wt virus was not reduced relative to the other viral proteins and in particular to the M protein (Fig. 1a, lanes 4 and 8; Table 1, 39 °C lines). The data in Table 1 indicate that at 39 °C, the amount of HN_{is271} is reduced 15- to 20-fold compared with that of HN_{wt}, and 10-fold compared with that of HN_{is271} at 31 °C, when normalized to the amount of the M protein.

However, these results must reflect an overestimation of the amount of HN_{is271} incorporated into the virions at 39°C owing to a non-specific radioactive signal because HN was not detected by specific anti-HN antibodies in the Western blot of the samples (Fig. 1b, lanes 7 and 8). Therefore, within the limits of sensitivity of the Western blot, the mutant virions grown at non-permissive temperature appeared to be free of HN. Furthermore, this massive reduction in the incorporation of HN into virions appeared to occur with no significant impairment of virus budding because the production of wt and ts271 virus particles at 39 °C was identical (Fig. 1a, compare lanes 3 and 4 with lanes 7 and 8).

Previous data, which confirm the conclusions reached in previous reports (Portner et al., 1974, 1975; Tuffereau et al., 1985; Markwell et al., 1985), could not exclude the presence in the virions of the transmembrane–cytoplasmic portion of HN. HN_{is271} is efficiently synthesized at the non-permissive temperature, but is degraded before reaching the cell surface (Tuffereau et al., 1985); therefore HN degradation could leave the transmembrane–cytoplasmic portion of HN in place. To investigate this possibility, an antiserum was raised against the N-terminal 20 amino acids (Rab-HN_{tail}) (Blumberg et al., 1985) by three subcutaneous injections into a rabbit of the peptide coupled to keyhole limpet haemocyanin. As expected, this antiserum does not react with the HN ectodomain expressed at the cell surface (Fig. 2, lanes 1 and 2); an antiserum raised against the whole viral particle (Rab-vir; Fig. 2, lanes 3 and 4) does react with the ectodomain. Under these conditions, Rab-vir mainly recognizes the two glycoproteins because they are the only ones expressed at the cell surface. In contrast, after cell disruption, which produces conditions in which Rab-

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### Table 1. Relative amounts of HN and M protein in virus particles produced at 31 °C and 39 °C

<table>
<thead>
<tr>
<th>Virus</th>
<th>Temperature (°C)</th>
<th>Amount tested†</th>
<th>HN</th>
<th>M</th>
<th>HN/M</th>
</tr>
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<tr>
<td>wt</td>
<td>31</td>
<td>1 x</td>
<td>3895</td>
<td>2135</td>
<td>1.82</td>
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<tr>
<td>wt</td>
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<td>3 x</td>
<td>12135</td>
<td>7363</td>
<td>1.65</td>
</tr>
<tr>
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<td>39</td>
<td>1 x</td>
<td>1441</td>
<td>859</td>
<td>1.68</td>
</tr>
<tr>
<td>wt</td>
<td>39</td>
<td>3 x</td>
<td>5027</td>
<td>2360</td>
<td>2.13</td>
</tr>
<tr>
<td>ts271</td>
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<td>1 x</td>
<td>2625</td>
<td>3245</td>
<td>0.81</td>
</tr>
<tr>
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<td>3 x</td>
<td>6078</td>
<td>7676</td>
<td>0.79</td>
</tr>
<tr>
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<td>1 x</td>
<td>55</td>
<td>736</td>
<td>0.07</td>
</tr>
<tr>
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<td>39</td>
<td>3 x</td>
<td>235</td>
<td>2310</td>
<td>0.10</td>
</tr>
</tbody>
</table>

† The autoradiogram in Fig. 1(a) was scanned (Image Quant, Molecular Dynamics) and the amounts of HN and M protein were expressed as arbitrary units.

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† 1 x and 3 x indicates that the gel was initially loaded with 20 μl or 60 μl of the virus particle preparation.
then chased for 0 (four samples, odd-numbered lanes) or 70 min (four samples, even-numbered lanes). Cell samples were either directly incubated in situ (cell surface immune precipitation) with 1 ml of 10-fold diluted Rab-HNtail or Rab-vir (rabbit serum raised against the HN tail). Lanes 1 and 2, surface IP using Rab-HNtail; lanes 3 and 4, surface IP using Rab-vir; lanes 5 and 6, total cell IP using Rab-HNtail; lanes 7 and 8, total cell IP using Rab-vir. V, Viral protein markers.

Fig. 2. Rab-HNtail specifically binds to HN<sub>ts271</sub> only when its cytoplasmic tail is accessible. Eight samples of 10<sup>5</sup> BHK cells were infected with ts271. After 23 h of infection at 33 °C, the infected cells were pulse-labelled with [35S]methionine (150 μCi/ml) for 10 min and then chased for 0 (four samples, odd-numbered lanes) or 70 min (four samples, even-numbered lanes). Cell samples were either directly incubated in situ (cell surface immune precipitation) with 1 ml of 10-fold diluted Rab-HNtail or Rab-vir (rabbit serum raised against the whole purified virus; Mottet et al., 1986), or disrupted in Triton-SDS buffer before exposure to the antibodies (IP: total immune precipitation (IP)). Both types of IP were performed exactly as described (Roux & Waldvogel, 1983). Lanes 1 and 2, surface IP using Rab-HN<sub>ts271</sub>; lanes 3 and 4, surface IP using Rab-vir; lanes 5 and 6, total cell IP using Rab-HN<sub>ts271</sub>; lanes 7 and 8, total cell IP using Rab-vir. V, Viral protein markers.

The data presented here indicate that the HN glycoprotein, which contains the function essential for virion attachment, is not essential for virus particle formation; even a possible role for the transmembrane–cytoplasmic portion of the protein was excluded. Lack of HN participation in virion budding was supported, moreover, by the fact that the extensive reduction of HN incorporation into virus particles does not appear to impair budding efficiency. These conclusions contrast with the results reported for the VSV G protein, the counterpart of Sendai virus HN. The VSV G protein was judged to be essential for virus budding because its transmembrane–cytoplasmic tail was found in spikeless virions (Metsikkö & Simons, 1986).

In both Sendai virus and VSV, the M protein plays a central, strategic role in budding (Yoshida et al., 1976; Tuffereau & Roux, 1988; Knipe et al., 1977). However, the two viruses differ in that VSV contains only one glycoprotein, whereas Sendai virus contains HN and F<sub>0</sub>. If one assumes that the anchor portion of one glycoprotein is essential for budding, the G protein would not be dispensable in the case of VSV, whereas in the absence of HN, F<sub>0</sub> could fulfil this function in Sendai virus.

The fact that a Newcastle disease virus is mutant, with a mutation in the fusion protein, is equally capable of budding in the absence of F<sub>0</sub> (Matsumura et al., 1990) is relevant to this question, although whether these virions...
contain the $F_0$ transmembrane–cytoplasmic portion is not known. For Sendai virus, we initially considered $F_0$ as having an accessory function in virus budding, based mainly on the observation that its stability and function appeared to be unaffected in situations of restricted virus budding. In these situations, however, instability and/or malfunction of Sendai virus HN and the M protein were always observed (Tuffereau & Roux, 1988; Roux et al., 1984, 1985; Roux & Waldvogel, 1982; Yoshida et al., 1979). We therefore postulated that a preferential interaction between HN and the M protein (rather than between $F_0$ and the M protein) would be essential for efficient virus budding. The present data indicate that the M protein function (i.e. ability to organize a bud) does not depend on HN, and that HN, although it may normally interact with the M protein, is not an essential component of the budding process. Therefore, if a glycoprotein is needed in paramyxovirus budding, $F_0$ and not HN must be involved. The consistent mutations or deletions in the cytoplasmic tail of the measles virus F protein observed in persistent infections (subacute sclerosing panencephalitis), in which budding is restricted, appear to support this hypothesis (Cattaneo et al., 1989). Alternatively, it may turn out that for Sendai virus particle budding, both glycoproteins are dispensable. In contrast to VSV, where the shape of the virion is rigid and strictly controlled, i.e. the length of the bullet-shaped virion is proportional to the length of the nucleocapsid, SV virions are very pleomorphic in size.

Finally, it is evident that a minimal amount of HN or its transmembrane–cytoplasmic domain, in amounts that could not be detected in these experiments, cannot be excluded as being essential for budding. For comparison however, the number of G protein anchors in tsO45 particles corresponded to the full complement of G proteins (Metsikkö & Simons, 1986). Moreover, although complementation of tsO45 with wt G protein in transfection experiments rescued VSV particle infectivity, the increased availability of G proteins did not result in a global increase in virus particle budding (Whitt et al., 1989). These results indicate the requirement of a large initial amount of the essential glycoprotein (or glycoprotein anchor) for virus budding. Once this initial amount is reached, the efficiency of budding would not be affected by an excess of glycoproteins. It is evident that these fundamental questions concerning viral budding can only be fully addressed using a more genetically controlled system.

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References


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