Identification of an Additional Sendai Virus Non-structural Protein Encoded by the P/C mRNA

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SUMMARY

Peptide antiserum and monoclonal antibodies have detected a previously unrecognized small non-structural protein in Sendai virus-infected cells. This protein, designated X, appears to represent the C-terminal 95 amino acids of the P protein reading frame. The X protein appears to be almost as abundant as the P protein on a molar basis in vivo. No evidence of a precursor-product relationship between the X and P proteins could be found.

Sendai virus, a member of the parainfluenza virus genus of the paramyxovirus family, encodes its entire genetic information within a non-segmented 15 kb negative strand (−) RNA genome (Kingsbury, 1974; Kolakofsky et al., 1974). Upon infection, this genetic information is expressed via positive-sense mRNAs transcribed from the genome by the virion-associated polymerase. These transcripts are largely monocistronic, encoding the NP, M, F, HN and L proteins. The P mRNA, however, has been demonstrated to be polycistronic. In addition to encoding the 568 amino acid (aa) P protein, it also codes for a pair of non-structural proteins, C (204 aa) and C' (181 aa) (Curran et al., 1986; Dethlefsen & Kolakofsky, 1983; Giorgi et al., 1983) which represent a C-coterminal nested set and are derived from an overlapping open reading frame (ORF) (see Fig. 1). The C overlapping ORF has also been conserved in the closely related human parainfluenza virus 3 (Spriggs & Collins, 1986a) as well as the two morbilliviruses examined to date, measles virus and canine distemper virus (Barrett et al., 1985; Bellini et al., 1985). Curiously, in the case of the parainfluenza viruses simian virus 5, Newcastle disease virus and mumps virus, non-structural proteins are again encoded within the P mRNA, but here these non-structural proteins appear to share tryptic peptides with the P protein and are therefore thought to be encoded by the same ORF (Collins et al., 1982; Herrler & Compans, 1982; Paterson et al., 1984). Only in the case of respiratory syncytial virus, a member of the more distinctly related pneumovirus genus, is the P mRNA thought to be monocistronic (Collins et al., 1984).

Recently, Herman (1986) has found that the NS mRNA of the rhabdovirus vesicular stomatitis virus (VSV), which is thought to be the VSV equivalent of the parainfluenza virus P mRNA, also codes for a second small protein, representing the C-terminal 62 aa of the 265 aa long NS protein. Since the Sendai virus P protein and the VSV NS protein have several characteristics in common, and antiserum specific for the C-terminal 16 aa of the Sendai virus P protein is available, we examined whether the same also applies to Sendai virus.

Sendai virus (Harris strain)-infected BHK cells were labelled for 2 h at 18 h post-infection, when marked cytopathic effects are apparent, as well as a parallel uninfected culture. Under these conditions, host protein synthesis has largely been displaced by the viral protein synthesis and the virus structural proteins and the non-structural C protein can clearly be detected above the remaining cellular background (lanes 1 and 2, Fig. 2). When these extracts were immunoprecipitated with the P peptide antiserum, several smaller proteins in addition to the P protein were precipitated by the antiserum; the most prominent of these was the smallest, designated X. A protein migrating at this position could be discerned directly in infected cell
Fig. 1. A schematic representation of the ORFs of the Sendai virus P/C mRNA. Other AUGs in the P (+1) reading frame are marked above the line and those in the C (+2) reading frame are marked below the line. The figure is not to scale.

Fig. 2. Identification of the X protein in vivo and in vitro. Sendai virus-infected or mock-infected BHK cells were labelled with [35S]methionine 18 to 20 h post-infection. Samples of cytoplasmic extracts were either analysed directly (lane 1, mock-infected cell extract; lane 2, infected cell extract) or immunoprecipitated with a P peptide antiserum (Curran et al., 1986) raised to the precise C-terminal of the P protein (lane 3, mock-infected cells; lane 4, infected cells). Proteins were analysed on a 12.5% polyacrylamide gel and visualized by fluorography. The entire P-coding region was inserted in the plasmid SP 65 and the gene transcribed as a 5' capped RNA. The transcript was translated in a wheatgerm extract and the products were analysed on a 15% polyacrylamide gel (lane 5). A control was provided by performing the translation in the absence of exogenous RNA (lane 6).
extracts (lane 2) but it appeared to comigrate with at least one host cell protein (lane 1). None of the host proteins, however, were recognized by the peptide antiserum (lane 3).

We also considered the possibility that the X protein was not in fact of viral origin, but simply a host cell protein which associated with the P protein even under the RIPA buffer conditions used for the immunoprecipitation. The entire Sendai virus P gene was therefore cloned into the RNA expression vector SP 65, a capped transcript was made in vitro (Konarska et al., 1984) and then translated in a wheatgerm extract. As shown in lane 5, Fig. 2, a protein which migrated at the position of X was a prominent band among the in vitro translation products. The X protein synthesized in vitro was also recognized specifically by the P peptide antiserum (not shown). This suggests that the X protein found in vivo by immunoprecipitation was of viral origin, and the translation product of the P mRNA.

Lane 5 (Fig. 2) also shows that the major translation product of the P mRNA in vitro is the C protein, as is the case in vivo (Curran et al., 1986), although this effect appears exaggerated in this in vitro reaction. The C’ protein is also visible among the in vitro translation products whereas a separate C’ protein cannot be detected among the in vivo proteins (lane 2). Although it is possible that undetectable amounts of C’ protein were made during this viral infection, it is also possible that the two C proteins are comigrating in the gel, as has previously been shown to occur under some conditions. [The C’ protein is actually 26 aa shorter than the C protein and the reason for its aberrant slower migration than the C protein on some gels is unknown (Curran et al., 1986).] Finally, lane 5 (Fig. 2) shows that the protein designated Y, which is also found in vivo, appears to be yet another product of the P mRNA. This protein appears to be encoded by the C ORF since its migration varies among different virus strains in a pattern that mirrors that of the two C proteins (not shown). However, it cannot be immunoprecipitated by peptide antiserum specific to either the N or C termini of the C protein, and thus its origin remains obscure.

We have also investigated whether the X protein is a specific degradation product of the P protein both in vivo and in vitro. In vivo, labelling experiments in the presence of canavanine and pulse–chase experiments both failed to demonstrate a product–precursor relationship between X and P. In vitro, kinetic analysis of the reaction products showed that the ratio of X to P did not increase over time in the reaction. The X protein therefore does not appear to be a degradation product of the P protein either in vivo or in vitro. However, further experiments will be required to determine whether the X protein, like the C proteins, results from independent ribosomal initiation.

The [35S]methionine-labelled X protein was found to comigrate with the [3H]glucosamine-labelled F2 protein of Sendai virus during SDS–PAGE [the F2 protein does not contain any methionine residues (Blumberg et al., 1985)]. However, unlike the F2 protein, the X protein could not be labelled with glucosamine (not shown). On this basis, we estimated its mol. wt. as 10K to 12K. Because the protein is in the same reading frame as P and must be C-coterminal, it was predicted to initiate from one of two possible AUGs in the P reading frame, at nucleotide position 1505 to 1507 or 1523 to 1525 on the P/C mRNA (Giorgi et al., 1983), if indeed this protein was the result of an independent ribosomal initiation as has been suggested for the VSV 7K protein.

The X protein was found to be recognized by four separate P monoclonal antibodies. We have used one of these antibodies as well as the peptide antiserum to estimate the relative amounts of X and P proteins in infected cells by densitometric analyses of immunoprecipitations from five separate infections. No attempt was made to estimate the relative amounts of these proteins directly, since the extent of viral displacement of host protein synthesis was seldom complete, e.g. actin continues to be made at a significant rate (lane 2, Fig. 2). Both antisera gave similar results, and when expressed on a molar basis (Table 1) the X protein was found to be almost as abundant as the P protein and considerably more abundant than the L protein. However, it should be noted that if these specific antisera preferentially recognize the smaller protein, the ratio of X to P will be overestimated. Even with this qualification the X protein accumulates in Sendai virus-infected BHK cells to significant levels.

The Sendai virus P protein, like the VSV NS protein, is thought to function in concert with the L protein in transcribing the (−) genome template (NP-RNA). By analogy with VSV, the L
Table 1. Molar abundance of the X and L proteins relative to the P protein in Sendai virus-infected cells

<table>
<thead>
<tr>
<th>Protein</th>
<th>No. of methionines</th>
<th>Relative molar abundance*</th>
</tr>
</thead>
<tbody>
<tr>
<td>P</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>X1</td>
<td>3</td>
<td>0.4 ± 0.15</td>
</tr>
<tr>
<td>X2</td>
<td>2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>L</td>
<td>47</td>
<td>0.07 ± 0.02</td>
</tr>
</tbody>
</table>

* The figures, normalized to the relative methionine content of each protein, were determined by densitometric analyses of five different gels, representing different virus infections, labelled between 18 to 20 h post-infection (Giorgi et al., 1983; Shioda et al., 1986). The ratio of X to P was determined from immunoprecipitations using both monoclonal (three times) and peptide antisera (twice); that of L to P was determined directly. The two results for X (i.e. X1 and X2) reflect the two potential start sites for X protein synthesis based on its estimated mol. wt. (position 1505 to 1507 or 1523 to 1525 on the P/C mRNA). The initiating methionines were not included in the calculation since both the Sendai virus NP and M proteins, the only two virus proteins examined to date, have been shown to have this methionine removed in vivo (Blumberg et al., 1984a, b).

The X protein is thought to be the polymerase, whereas the role of the P (or NS) protein is less clear. When closely related strains of VSV and parainfluenza virus are examined for amino acid homology, the P and NS protein are found to be the mostly highly divergent of all the virus proteins, yet their greatest homology lies in the C-terminal portion of each protein (Gill & Banerjee, 1985; Spriggs & Collins, 1986b). This relative conservation of the C terminus of the parainfluenza P protein suggests that this region is required for function. If the Sendai virus P protein were to contain more than one functional domain, e.g. the VSV NS protein is found to bind to the genome template both directly and via the L protein (Gill et al., 1986), the generation of a separate C-terminal portion of the protein might offer a way of modulating the different functions of the P protein in vivo.

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REFERENCES


Short communication


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