Synthesis of Sendai Virus Polypeptides by a Cell-free Extract from Wheat Germ

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(Accepted 27 May 1976)

SUMMARY

The ‘18S’ RNA population from Sendai virus-infected cells efficiently directed the synthesis of three virus-specific polypeptides P, NP and M, in wheat germ cell-free extracts. In agreement with previous results obtained with a reticulocyte extract, there was little or no production of virus glycopolypeptides. Analyses of tryptic peptides revealed close correspondence between the primary structures of NP and M made in vitro and the authentic virus polypeptides.

INTRODUCTION

The first group of viruses to reveal a negative-strand strategy of genetic information transfer (Baltimore, 1971; Mahy & Barry, 1975) was the paramyxoviruses (Kingsbury, 1966; Bratt & Robinson, 1967). Negative-strand viruses contain single-stranded RNA, but this is opposite in polarity (complementary) to virus messenger RNAs, which are generated by transcription in infected cells.

Some of the messenger RNA species of a model paramyxovirus, Sendai virus, were translated in a cell-free system from rabbit reticulocytes (Kingsbury, 1973). Immune precipitation of the in vitro products was used in that demonstration, to reduce interference by a high background of endogenous polypeptide synthesis. We have now translated Sendai virus messenger RNAs in wheat germ extracts, which provide much improved background levels, and we have further characterized the products.

METHODS

Messenger RNA. The extraction of total RNA from Sendai virus-infected chick embryo lung cells was described by Kingsbury (1973). Material sedimenting in sucrose gradients with 18S ribosomal RNA (from about 14S to 22S) was used in the present work. The RNA was washed repeatedly with ethanol, dried, dissolved in water, and stored at -60 °C.

RNA 4 (the 14S monocistronic coat protein message) from brome mosaic virus (BMV; Shih & Kaesberg, 1973) was employed as a control.

Cell-free translation. Extracts were made from wheat germ (General Mills) or with embryos freshly prepared from dried wheat seeds, and were incubated with or without added RNA as previously described (Davies & Kaesberg, 1973).
Table 1. In vitro amino acid incorporation directed by virus messenger RNAs

<table>
<thead>
<tr>
<th>Messenger</th>
<th>Leucine incorporation*</th>
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<tbody>
<tr>
<td></td>
<td>ct/min</td>
</tr>
<tr>
<td>Zero time</td>
<td>428</td>
</tr>
<tr>
<td>None</td>
<td>734</td>
</tr>
<tr>
<td>Sendai virus RNA†, 12 µg</td>
<td>5568</td>
</tr>
<tr>
<td>BMV RNA, 10 µg</td>
<td>32356</td>
</tr>
</tbody>
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* The incubation mixture (0.1 ml), contained 0.1 µCi of 14C-leucine (26 Ci/mol) and was incubated for 60 min at 31°C.
† Total 18S RNA from chick embryo lung cells infected with Sendai virus.

Polyacrylamide gel electrophoresis. The discontinuous system of Laemmli (1970) was used. Incubated reaction mixtures were treated with ribonuclease and EDTA, followed by urea, mercaptoethanol, and SDS (Davies & Kaesberg, 1973). Samples were diluted fivefold in electrophoresis sample buffer (Laemmli, 1970) and separated on 10% polyacrylamide slabs 0.75 mm thick and 10 cm long. A potential gradient of 6 V/cm was applied for 6 h. After electrophoresis, gels were fixed with 12.5% trichloroacetic acid, dried and placed on X-ray film. Autoradiograms were scanned with an Ortec Model 4310 recording densitometer.

Analysis of tryptic peptides. Radiolabelled polypeptide species were isolated from polyacrylamide gels, digested with trypsin, and chromatographed on Dowex-50 columns (Davies & Kaesberg, 1973).

RESULTS

Amino acid incorporation

Most of the transcript species made in Sendai virus infection sediment close to 18S ribosomal RNA (Roux & Kolakofsky, 1975). Material sedimenting in this range stimulated amino acid incorporation in wheat germ cell-free extracts (Table 1). The stimulation produced by 120 µg of RNA per ml was sevenfold above a control that received no RNA. If zero-time background is subtracted, the difference was 17-fold.

Sendai virus messenger RNA appears to be at least as efficient as the coat protein message of brome mosaic virus (BMV) in the wheat germ system. Sendai virus RNA induced the incorporation of about 14 pmol of leucine into protein per µg of RNA in an hour. The yield with BMV message was 109 pmol/µg of RNA. However, most of the Sendai virus infected cell 18S RNA is ribosomal. Assuming that the virus messenger content of the 18S RNA pool is about 10% by weight (Darnell, 1968), leucine incorporation stimulated by Sendai virus messenger RNA was about 135 pmol in an hour.

With Sendai virus RNA, leucine incorporation was most rapid for the first 20 min, decreasing markedly by 40 to 60 min after the start of the reaction (Fig. 1a). The response to Sendai virus RNA in a 10 min incubation was linear up to about 250 µg/ml. (Fig 1b). On the same assumption of a messenger RNA content of 10%, the system was saturated by about 25 µg of Sendai virus messenger/ml, an estimate that compares well with results obtained with BMV coat protein message (Shih & Kaesberg, 1973).

Electrophoresis of the products

The electrophoretic behaviour of the in vitro product is compared with Sendai virion polypeptides in Fig. 2. As with the reticulocyte system, the major product migrated like the principal nucleocapsid polypeptide, NP. There were significant amounts of material
Fig. 1. (a) Time course of leucine incorporation by a wheat germ extract which received 120 μg of Sendai virus infected cell 18S RNA ml. (b) Leucine incorporation as a function of Sendai virus infected cell 18S RNA concentration in a 10 min incubation. Zero time background ct/min were subtracted from all data.
migrating like the lesser nucleocapsid polypeptide, P, and the non-glycosylated virus envelope polypeptide, M. In addition, there was a small peak in the position of polypeptide F, embraced by other minor peaks that may have counterparts in virions (Fig. 2). However, no products with apparent mol. wt. greater than that of P (about 75000; Stone, Kingsbury & Darlington, 1972) were seen, and there was scant material in the positions of envelope glycopolypeptides HN, the haemagglutinin-neuraminidase, or F₀, the precursor of the small 'fusion' polypeptide, F (Scheid & Choppin, 1974). The small amount of radioactivity incorporated by endogenous reactions was distributed diffusely in the gel (Davies, Kaesberg & Diener, 1974).

There was a large portion of low mol. wt. product that migrated close to the dye front (Fig. 2). It probably represents prematurely terminated polypeptide chains.

**Tryptic peptides**

Putative NP and M from electrophoretic separations of reaction products were extracted from polyacrylamide gels and digested with trypsin. Their peptide profiles were distinctive, and each corresponded closely to the peptide profile of the authentic polypeptide from virions (Fig. 3). Some minor differences, indicated by arrows in the figure, may be due to contamination by endogenous wheat polypeptides, lack of complete removal of the initiation-terminal methionine *in vitro*, or other modifications occurring in the extract, such as proteolytic enzyme action.
**Sendai virus polypeptide synthesis**

**Fig. 3.** Ion exchange chromatography of tryptic hydrolysates of Sendai virus polypeptides NP and M. Solid lines: authentic virion polypeptides; broken lines: *in vitro* products.

**DISCUSSION**

This work confirms the messenger function of the slowly-sedimenting class of Sendai virus transcripts. This '18S' class of messenger RNA species specified polypeptides ranging in mol. wt. from about 40,000 (M) to about 75,000 (P), a span that includes all the known virion polypeptides but one, a minor and very large polypeptide that may be specified by a 33S transcript (Lamb, 1975; Roux & Kolakofsky, 1975; Zaides et al. 1975).

Improved separation of tryptic peptides confirmed that NP can be made *in vitro* and made possible a convincing demonstration that M was also synthesized. M had not been clearly identified among the products of the reticulocyte extract (Kingsbury, 1973), perhaps because it was not effectively precipitated by the antibodies used to recover virus-specific products.

It seems that translation of the glycopolypeptides HN and F₀ was inefficient or altogether absent in wheat germ extracts. Non-glycosylated forms of these polypeptides might have been made, and these might migrate slightly faster in SDS gels than the glycosylated polypeptides from virions (Knipe, Rose & Lodish, 1975; Both, Moyer & Banerjee, 1975). But none of the unidentified polypeptide species shown in Fig. 2b in the region between P and M was made in amounts comparable to the amounts of HN and F₀ (or the cleavage product, F) that occur in virions or in infected cells (Lamb, Mahy & Choppin, 1976). Deficient *in vitro* synthesis of virus glycopolypeptides has been noted in several other studies (Morrison et al. 1974; Wengler, Beato & Hackemack, 1974; Etkind & Krug, 1975). Special requirements for translation of the relevant messenger RNAs are suggested, but recent work indicates that inefficient extraction of membrane bound glycopolypeptide message may be partially responsible (Knipe et al. 1975; Morrison & Lodish, 1975). Monocistronic messenger RNAs coding for the Sendai virus glycopolypeptides are expected to have sedimented in the 18S region, if they were extracted, since the sizes...
of polypeptides HN and F₀ are within the limits of the messages that were translated (Fig. 2).

As previously indicated for the reticulocyte extract (Kingsbury, 1973), and now demonstrated in the wheat germ system, the non-glycosylated Sendai virus polypeptides are made in vitro in proportions that are not equimolar and that resemble their in vivo proportions. This indicates differences in abundances or in translation efficiencies of the different messengers. We are currently separating the mRNAs of Sendai virus on polyacrylamide gels. Translation of the individual mRNA species in vitro should illuminate this question.

We gratefully acknowledge the help and laboratory facilities provided by Dr Paul Kaesberg, and thank Ms Claudia Benike for technical assistance.

This work was supported in part (J.W.D.) by research grants AI-08909 and AI-01466 from the National Institute of Allergy and Infectious Diseases (U.S. Public Health Service), and by special grant 759 from the American Cancer Society. The work of A.P. and D.W.K. was supported by research grants AI-05343 and AI-11949 from the National Institute of Allergy and Infectious Diseases and by ALSAC.

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*(Received 30 March 1976)*