Location and Abundance of Poly (A) Sequences in Sendai Virus Messenger RNA Molecules

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SUMMARY

Adenine-rich sequences from 18S Sendai virus messenger RNA species were 99% adenylate, 3'-OH terminal, and were present in at least 50% of the RNA molecules. Intact virus messenger RNA molecules were resistant to exonucleolytic attack by polynucleotide phosphorylase, suggesting that their 3'-termini are masked.

Most messenger RNAs of eukaryotic cells and their viruses contain adenine-rich sequences (Weinberg, 1973). Sendai virus, a paramyxovirus, is no exception. It specifies messenger RNAs which are complementary in base sequences to virus particle RNA; most, if not all, of these RNAs sediment at about 18S (Kingsbury, 1973); they contain adenine-rich sequences which sediment at about 4S (Pridgen & Kingsbury, 1972). We now report more details about the structure of these adenine-rich sequences.

In all experiments, RNA was obtained from chick embryo lung (CEL) cells which were infected with 10 p.f.u. of low multiplicity passage Sendai virus per cell and incubated at 37 °C. At 48 h after infection, when virus production was at its peak, cells were treated for 1 h with 50 μg of actinomycin D per ml. A radioactive precursor of RNA was then added and incubation at 37 °C was extended in the presence of actinomycin D for 4 h. Cytoplasmic extracts were made by the method of Penman (1966) and RNA was isolated from them with SDS and phenol (Pridgen & Kingsbury, 1972). The extracts were centrifuged in sucrose velocity gradients and RNA sedimenting at 18S was selected. This RNA sedimented again as a single peak at 18S when it was boiled for 3 min in 0.01 M-tris HCl, pH 7.4, rapidly cooled, and recentrifuged.

All enzymes used were products of Worthington Biochemical Corporation, and were the purest available.

About 10% of [32P]-labelled 18S virus RNA was resistant to a mixture of bovine pancreatic and T1 ribonucleases. Infected cells were labelled with 250 μCi of carrier-free [32P]PO4− per ml of medium compounded without orthophosphate but containing 50 μg of actinomycin D/ml. The 18S RNA was digested for 30 min at 24 °C with 50 μg of pancreatic ribonuclease and 1 μg of T1 ribonuclease per ml of 0.3 M-NaCl, 0.001 M-EDTA, 0.005 M-tris-HCl (pH 7.4). The digest was passed through a Sephadex G50 column equilibrated with the same buffer containing 7 M-urea. Material which emerged in the void volume was hydrolysed with alkali and the ribonucleotides were separated and identified (East, 1968). The nucleotide composition of this ribonuclease-resistant material was 99% adenylate (Table 1), whereas the 18S RNA itself contained about equal amounts of [32P] in each of the four ribonucleotides (data not shown).

In view of its base composition, we will refer hereafter to the ribonuclease-resistant, adenine-rich fragment as the 'poly (A)' portion of Sendai virus messenger RNA. We now present evidence which shows that this poly (A) is located at the 3' terminal position in the Sendai virus messenger RNAs, in common with other virus and cellular messenger RNAs.
Table 1. Base composition of the ribonuclease-resistant portion of Sendai virus 18S messenger RNA

<table>
<thead>
<tr>
<th>Mol %*</th>
<th>Adenine</th>
<th>Uracil</th>
<th>Cytosine</th>
<th>Guanine</th>
</tr>
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<tbody>
<tr>
<td>99.3 (± 0.5)</td>
<td>0.1 (± 0.1)</td>
<td>0.3 (± 0.3)</td>
<td>0.3 (± 0.2)</td>
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* The numbers in parentheses are the standard deviations of the means of 10 determinations.

(Weinberg, 1973). The poly (A) obtained by combined T1 and pancreatic ribonuclease digestion of [3H]-adenine-labelled 18S RNA was treated with Micrococcus luteus polynucleotide phosphorylase under conditions where the phosphorylase acts as a 3'-OH terminal exonuclease (Sheldon et al. 1972b). At an enzyme concentration of 0.6 units/ml in 0.005 M-MgCl₂, 0.01 M-sodium phosphate, 0.1 M-tris-HCl (pH 8.5), the poly (A) was 90 % digested in 20 min and completely digested in 40 min at 37 °C. This experiment was controlled in several ways. The rate of enzymatic degradation did not increase after pre-treatment of the poly (A) with Escherichia coli alkaline phosphatase. The polynucleotide phosphorylase itself was free of contaminating phosphatase by two criteria: it was inactive against p-nitrophenyl phosphate (Garen & Levinthal, 1960) and it digested no more than 40 % of a commercial poly (A) preparation that had been incubated briefly with pancreatic ribonuclease at low ionic strength to generate fragments bearing both 3’-and 2’-terminal phosphate. Thus, it was established that most of the poly (A) segments from Sendai virus 18S messenger RNA terminated in native 3'-OH groups.

Attempts to digest intact Sendai virus 18S RNAs with polynucleotide phosphorylase were unsuccessful (Fig. 1). [3H]-adenine labelled RNA was incubated at 37 °C with 0.6 units of polynucleotide phosphorylase per ml, as described before. At intervals, samples were placed in ice-cold 0.0125 M-EDTA, 0.15 M-NaCl to stop the digestion, and either precipitated with 5 % trichloroacetic acid or ribonuclease-treated and then acid precipitated. It can be seen that neither intact 18S mRNA nor the poly (A) segment derived from it by ribonuclease treatment was affected by polynucleotide phosphorylase under these conditions. This was unexpected in view of the ease with which other messenger RNAs and our nuclease-released poly (A) were digested (Sheldon et al. 1972b; Williamson, Crossley & Humphries, 1974).

A simple explanation is that intramolecular secondary structure blocks the 3’-termini of the intact Sendai virus messenger RNAs. In an attempt to expose 3’-termini by denaturing the RNAs they were boiled in water and cooled rapidly before adding the phosphorylase. The result was the same; no enzymatic degradation occurred, indicating that the 3’-termini became blocked again. However, a more drastic treatment was effective. Another 18S mRNA preparation was treated with 0.3 M-KOH for 1 min at 23 °C and neutralized by passage through a small Dowex 50 (H⁺) column before treatment with phosphorylase (Fig. 1). About 16 % of the alkali-treated 18S mRNA was digested by the enzyme and the poly (A) portion was more rapidly and more extensively digested, confirming that it was in a 3' terminal position.

Longer or shorter alkali treatments reduced the amount of 18S RNA digested by the enzyme. Presumably, shorter treatments cleaved fewer molecules, releasing fewer free 3'-OH terminal fragments, whereas longer treatments reduced the lengths of these fragments. Although the evidence indicates that 3’-termini are blocked in intact Sendai virus
messengers under these conditions, the relevance of this finding to the in vivo conformations or functions of these RNAs is not clear.

In other experiments we measured the proportion of Sendai virus 18S messenger RNAs which contained poly (A) using polynucleotide binding methods. From 60 to 85% of the RNA was bound by poly (U) filters (Sheldon, Jurale, & Kates 1972a) in different experiments, whereas about 50% bound either to unmodified cellulose (Kitos, Saxon & Amos, 1972) or to poly (U)-Sepharose (Lindberg & Persson, 1972). The RNA had been labelled with 3H-adenine in the latter two cases, so that ribonuclease treatment could be used to estimate the poly (A) content of the bound and unbound material. The unbound RNA was less than 10% ribonuclease-resistant whereas the bound RNA was more than 20% resistant to ribonuclease after elution from the columns. Poly (A) segments are heterogeneous in length (Weinberg, 1973) and it has been shown that different polynucleotide binding procedures have different selectivities (Gorski et al. 1974). Thus, at least half of Sendai virus 18S RNA molecules contain poly (A) sequences long enough to bind to cellulose or to poly (U)-Sepharose. The remainder are relatively deficient in poly (A).

The RNA molecules deficient in poly (A) are not cell-specified messenger RNAs or slowly sedimenting virus-specific RNAs of the virus particle type. Actinomycin D eliminates the former (Blair & Robinson, 1968) and low-multiplicity passage of virus prevents the emergence of defective-interfering virus particles which generate the latter (Kingsbury & Portner, 1970).

Similar data have been obtained with vesicular stomatitis virus (Soria & Huang, 1973),
another 'negative-strand virus' (Baltimore, 1971). Here, too, a significant portion of RNA molecules which qualify as virus messenger RNA by virtue of sizes and base sequences are deficient in poly (A). Some insight into the function of poly (A) may be gained by determining whether these poly (A)-deficient RNAs can act as templates for virus proteins and whether they originate as such or are derived from poly (A)-rich congeners.

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REFERENCES


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