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Polyadenylic Acid Sequences in Rhinovirus RNA Species from Infected Human Diploid Cells

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Polyadenylic acid sequences were shown to be present in rhinovirus virion RNA. Virus-specified RNA from human embryo lung cells infected with rhinovirus also contained polyadenylic acid but did not contain any polyuridylic acid sequences.

The virion RNA of picornaviruses is a single molecule of infectious single-stranded (SS) RNA, which is presumed to act as a messenger for its own RNA transcriptase. Tracts of homopolynucleotides have been found in the virion RNAs of a variety of picornaviruses. In the genomes of cardioviruses and foot-and-mouth disease viruses polycytidylic acid sequences have been found (4, 17). Polyadenylic acid (poly[A]) segments have been observed in poliovirus virion RNA and in certain of the poliovirus-specified RNA species (1, 19, 20). In addition, the virion RNA of mengovirus (12) and rhinovirus (14) have been shown to be polyadenylated. The mRNAs of a number of members of other virus families have also been shown to be polyadenylated (1, 6, 8).

Polyuridylic acid (poly[U]) is absent from the genome RNA of several groups of viruses (11), suggesting the nontranscriptional adenylation of the messengers of these viruses. However, poly(U) has been found in poliovirus-specified replicative RNA structures (18, 20), and this raises the possibility of polyadenylation by transcription.

This paper confirms the observation of Nair and Owens (14), with a different method of oligo (dT)-cellulose chromatography, that rhinovirus virion RNA contains a segment of poly(A) and also shows that the virus-specified RNA in diploid human embryo lung (HEL) cells infected with rhinovirus type 2 is also polyadenylated. In this report none of the rhinovirus-specified RNA species were found to contain poly(U) sequences.

Rhinovirus type 2 was grown, titrated, and purified as previously described (9). Labeling of the virus was achieved by growth in low-phosphate medium containing [^{32}P]orthophosphate. Purified labeled virion RNA was passed through an oligo (dT)-cellulose column under the conditions used by Aviv and Leder (2), which allowed RNAs to be bound and recovered in 100% yields. Polyadenylated RNA species bound to the column in a high-salt buffer (0.4 M NaCl, 0.01 M Tris, pH 7.6, 1 mM EDTA, 0.1% sodium dodecyl sulfate [SDS]) and were eluted from the column in a low-salt buffer (0.01 M Tris, pH 7.6, 1 mM EDTA, 0.1% SDS). An average of 70% of the RNA bound to oligo (dT)-cellulose in the high-salt buffer (a mean of several determinations). On a second passage through the column, the nonbinding material bound to about 70%, implying that all the rhinovirus virion RNA was polyadenylated. This result is in agreement with previous results of Nair and Owens (14), who used a filter binding technique.

Digestion of labeled virion RNA with pancreatic ribonuclease A (5 μg/ml) and T_1 ribonuclease (30 U/ml) for 30 min at 37 C, which does not destroy poly(A) tracts, was done in order to obtain the poly(A) portion of the RNA molecule. An oligo (dT)-cellulose column was used to recover the nondegraded material. The fraction of the RNA digest that bound to the column was about 1.2%. As the molecular weight of the rhinovirus type 2 virion RNA is between 2.4×10^6 and 2.8×10^6 (9, 13), the segment of poly(A) in the virion RNA is about 90 nucleotides in length. An insufficient quantity of this material was available for an accurate estimation of the size of the poly(A) sequence of the virion RNA with polyacrylamide gel electrophoresis.

Monolayers of HEL cells were used as a source of rhinovirus RNA species synthesized during replication. Cells were infected with rhinovirus type 2 at an input multiplicity of 10 PFU per cell for 1 h at 33 C before washing and further incubation at 33 C. Time after infection was measured from the time of addition of virus. Actinomycin D (1 μg/ml) was used to inhibit cellular RNA synthesis and was added 4 h before labeling with [^{3}H]uridine. Radioactive

viral RNA was isolated and analyzed by polyacrylamide gel electrophoresis as described by Koliais and Dimmock (9).

Three types of virus-specified RNA have been identified using polyacrylamide gel electrophoresis, namely the replicative intermediate (RI), replicative form (RF), and SS RNA (9). The RI either just entered the gel or migrated a short distance into the gel as a heterogeneous peak. RF migrated in the gel close to the DNA marker and SS RNA had a mobility identical to that of virion RNA (Fig. 1a). The DNA was nucleic acid from the infected HEL cells which copurified with RNA.

To determine whether or not these rhinovirus-induced RNA species contained poly(A) sequences, [^{3}H]uridine-labeled RNA extracted from infected HEL cells was passed through an oligo (dT)-cellulose column. The electrophoretic mobility on polyacrylamide gels of the bound material was then compared with that of the total RNA (Fig. 1). The two profiles are similar, with the three types of virus-specified RNA appearing in both the oligo (dT)-cellulose-bound material and the total RNA. Therefore, all the types of rhinovirus-specified RNA molecules contain poly(A) sequences. The DNA and rRNA of the HEL cells did not bind to the oligo (dT)-cellulose columns.

Further experiments were done to determine if the SS RNA bound to oligo (dT)-cellulose to the same extent as the RI and RF forms. SS RNA was separated from RI and RF by fractionating the RNA species on CF11 cellulose columns (7) in the presence of 0.1% SDS. A second cycle of fractionation on cellulose columns produced complete separation of SS RNA species from multistranded RNA as judged by analysis on polyacrylamide gels.

Attempts were made to separate rhinovirus RI and RF species using techniques that have been successfuly applied to poliovirus (3, 15). However, the RI and RF species could not be cleanly separated using such methods. Possibly, the rhinovirus RI and RF are structurally more similar to each other than those of poliovirus.

Table 1 shows the binding to oligo (dT)-cellulose obtained with the rhinovirus-specified RNA species as compared to the binding of poly(A), poly(U), and HEL rRNA. The HEL rRNA would not be expected to contain any poly(A) sequences. The mixed RI plus RF species bound to a greater extent to oligo (dT)-cellulose than SS RNA, possibly because

TABLE 1. *The binding of various polyribonucleotides to oligo(dT)-cellulose columns*

Polyribonucleotide	Total counts/min	Counts/min binding to oligo(dT)-cellulose	Binding (%)
Poly(A)	40,643	40,603	100
Rhinovirus RI/RF[a]	11,770	8,046	68
Rhinovirus SS RNA	54,183	32,418	59
HEL rRNA	25,914	628	2
Poly(U)	35,336	120	0

[a] The rhinovirus RI/RF was separated from SS RNA by CF11 cellulose chromatography as described in the text.

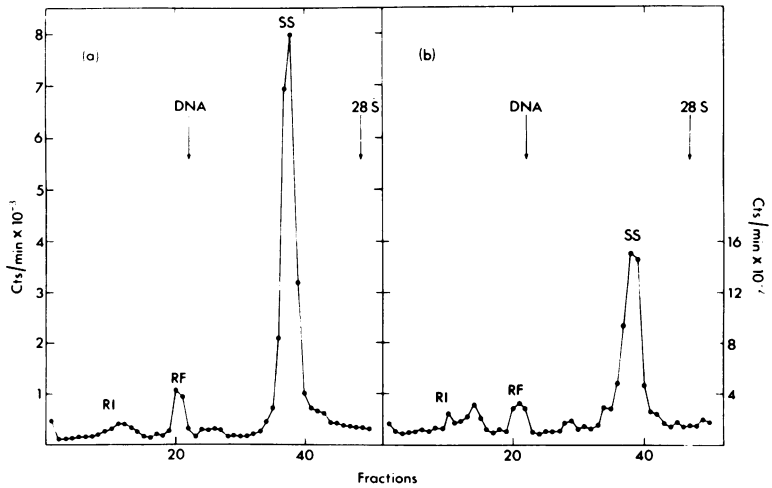

FIG. 1. *Electrophoresis on 2.4% polyacrylamide gels of RNA extracted from rhinovirus-infected HEL cells labeled with [^{3}H]uridine from 6 to 8 h after infection. The arrows are taken from the E_{260} trace, indicating the position of the "marker" RNA and DNA. (a) Total RNA: (b) oligo (dT)-cellulose-bound RNA with added marker HEL ribosomal RNA and DNA.*

TABLE 2. *Binding of various polyribonucleotides to poly(A)-Sepharose columns*

Polyribonucleotide	Total counts/min	Counts/min binding to poly(A)-Sepharose	Binding (%)
Poly(U)	32,129	32,031	100
Rhinovirus RI/RF[a]	10,446	328	3
Rhinovirus SS RNA	46,117	554	1
HEL rRNA	23,418	281	1
Poly(A)	44,196	102	0

[a] The rhinovirus RI/RF was separated from SS RNA by CF11 cellulose chromatography as described in the text.

the SS RNA on passage through the oligo (dT)-cellulose column takes up a conformation that partially masks the poly(A) sequence and prevents the RNA molecule from binding to oligo (dT)-cellulose. The polyacrylamide gel profiles (Fig. 1) show that RI and RF both bind to oligo (dT)-cellulose.

The presence of poly(A) sequences in the rhinovirus-specified RNA was also investigated by binding the RNA species to nitrocellulose filters under conditions used by Nair and Owens (14), which favor the binding of polyribonucleotides containing poly(A) (10). This method was generally found to be less satisfactory than using oligo (dT)-cellulose chromatography as less of the RNA bound to nitrocellulose filters than oligo (dT)-cellulose columns. Nevertheless, the rhinovirus SS RNA and replicative RNA species bound to nitrocellulose filters in approximately the same proportions as had been found with oligo (dT)-cellulose chromatography. These results suggest that poly(A) sequences are present in all three RNA species found in HEL cells infected with rhinovirus.

Poly(U) sequences have been found in the negative strands of poliovirus RF and RI (18, 20). As this result has interesting implications for the way in which RNA replication takes place, poly(A)-Sepharose chromatography (20) was used to probe for the presence of poly(U) sequences in rhinovirus-specified RNA. The binding of the rhinovirus-specified RNA species, poly(A), poly(U), and HEL rRNA to poly(A)-Sepharose is shown in Table 2. The HEL rRNA would not be expected to contain any poly(U) sequences. The rhinovirus-specified RNA species did not bind to the column, indicating that these species do not contain poly(U) sequences.

This report shows that not only is poly(A) found in rhinovirus virion RNA, but also on three virus-specified RNA species in HEL cells infected with rhinovirus. None of these RNA species contain poly(U), which suggests that poly(A) in rhinovirus RNA is synthesized nontranscriptively.

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