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Adenylate-Rich Sequences in Sendai Virus Transcripts from Infected Cells

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Virus-specific complementary ribonucleic acid (RNA) from cells infected with Sendai virus was isolated by a procedure involving hybridization with virion RNA and isopycnic centrifugation of the RNA hybrids. The complementary RNA contained adenylate-rich sequences which sedimented at about 4*S*.

Adenylate-rich sequences have recently been described in numerous ribonucleic acid (RNA) species with putative or verified messenger function. These include cellular polyribosomal RNA (6), transcripts from deoxyribonucleic acid virus genomes (12, 17), and virion RNA from viruses whose genomes are single-stranded RNA (1, 9, 11, 14). Some RNA viruses with single-stranded genomes, the rhabdoviruses and the paramyxoviruses, are thought to use single-stranded complementary RNA transcripts of their genomes as message (2). Vesicular stomatitis virus, a rhabdovirus, may have adenylate-rich sequences in complementary RNA species from infected cells (15). In this study, we report that a paramyxovirus, Sendai virus, also has adenylate-rich sequences in genome transcripts. We show that these adenylate-rich sequences are covalently bound to RNA molecules which are virus-specific and complementary to virion RNA by virtue of their ability to hybridize with virion RNA.

A cloned stock of Sendai virus free of incomplete virions (13, 19) was added to chicken embryo lung (CEL) cells at 10 to 20 plaque-forming units per cell. After 48 hr of incubation at 37 C, cells were treated with 50 μg of actinomycin D per ml, and then were labeled in the presence of the drug for 1 hr with 50 μCi of adenine-*8*-^3H per ml (17 Ci/mmole) or for 1.5 hr with uridine-*5*-^3H (20 Ci/mmole) (both precursors from Schwarz BioResearch).

As shown in Fig. 1, total RNA, isolated from ^{3}H-uridine-infected cells, sedimented in sucrose gradients as previously described (3, 18). There were prominent peaks at 18*S* and 50*S*, representing complementary RNA and genome-like RNA (3), respectively (Fig. 1A). Ribonuclease-resistant material, thought to represent complexes involved in viral RNA synthesis (18), was found at 26*S* and in more rapidly sedimenting species (Fig. 1B), although the prominent 50*S* peak seen here has not been a usual finding. Noteworthy was the sensitivity to ribonuclease of the ^{3}H-uridine complementary RNA sedimenting at 18*S*, confirming the absence of significant double-helical structure in this RNA (3, 5). In contrast, when ^{3}H-adenine was the precursor (Fig. 1C), there was a prominent nuclease-resistant peak at 18*S*, representing 5% of the radioactivity in this experiment (Fig. 1D), and up to 10% in other experiments. Nuclease-resistant and nuclease-sensitive ^{3}H-adenine RNA sedimenting slower than 18*S* were also seen (Fig. 1, C and D), but the other RNA species labeled with either precursor were similar.

Nuclease-resistant RNA sedimenting more slowly than 18*S* is probably not virus-specific. Total RNA extracted from uninfected cells, labeled as above with ^{3}H-adenine in the presence of actinomycin D, had about as many nuclease-resistant counts as infected cells in this material. Some of the ^{3}H-adenine RNA from uninfected cells sedimented into the 18*S* region as well. However, when cytoplasmic RNA was examined, only 10 counts/min were recovered from uninfected cells for each 50,000 counts/min in virus-specific RNA. Thus, actinomycin D-resistant labeling of uninfected cells was confined to the nucleus, and cytoplasmic extracts of infected cells should provide solely virus-specific RNA species. However, to insure virus specificity, hybridization with Sendai viron RNA was used preparatively in the following work.

^{3}H-adenine virus-induced cytoplasmic RNA sedimenting slower than 24*S* was boiled for 3 min to dissociate putative hydrogen-bonded nuclease-resistant fragments and was recentrifuged on sucrose gradients. Although boiling did not generate any slowly sedimenting radio-

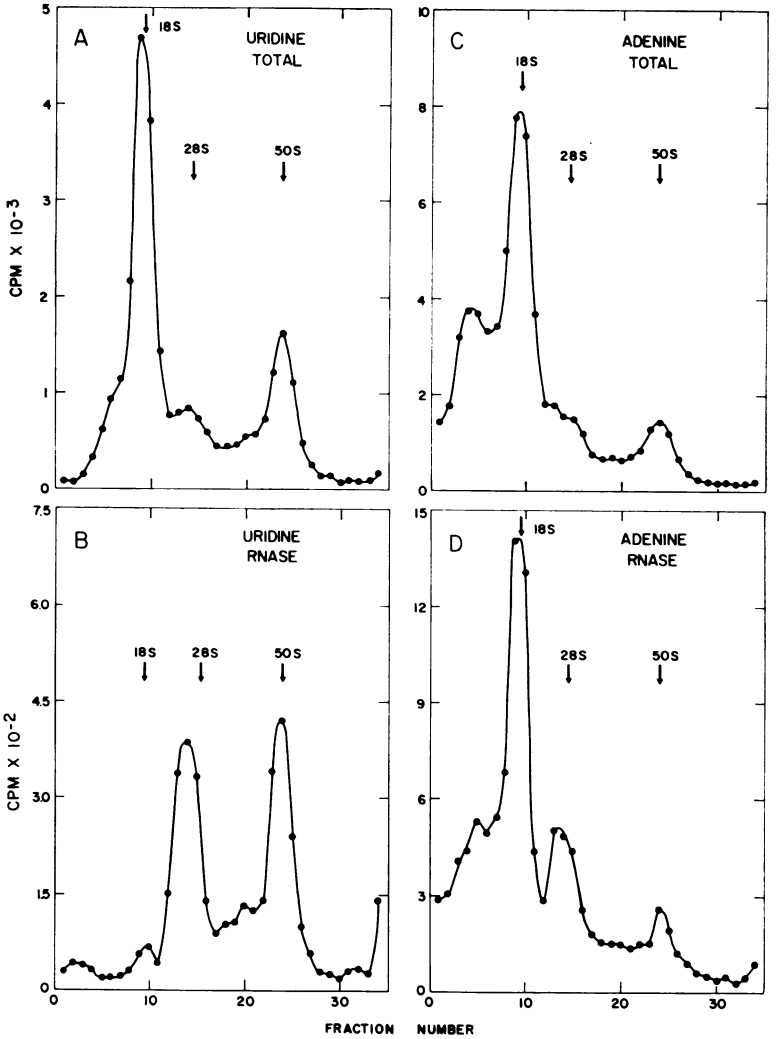

FIG. 1. *Sedimentation of Sendai virus-specific RNA in sucrose gradients. RNA labeled with uridine-5-^{3}H or adenine-8-^{3}H was extracted at 60 C with 0.5% sodium dodecyl sulfate (SDS) and phenol and centrifuged in 35-ml linear 15 to 30% (w/w) sucrose gradients in 0.005 M Tris-hydrochloride, 0.001 M ethylenediaminetetraacetic acid (EDTA), 0.1 M NaCl, 0.5% SDS (pH 7.4) at 18,000 rev/min, 20 C for 16 hr in a Spinco SW27 rotor. One fifth of each 1-ml gradient fraction was precipitated with 5% trichloroacetic acid and counted. The remainder of each gradient fraction was precipitated with ethanol and treated with pancreatic ribonuclease (50 μg/ml) and T1 ribonuclease (1 μg/ml) for 30 min at 24 C in 0.005 M Tris-hydrochloride, 0.001 M EDTA, 0.3 M NaCl (pH 7.4) before acid precipitation.*

activity, only RNA sedimenting faster than 8S was selected at this step. This RNA was annealed with 50S virion RNA, digested with pancreatic ribonuclease, and centrifuged isopycnically in KI (7), as described in the legend of Fig. 2. We found KI superior to Cs_2SO_4 for isopycnic centrifugation, since KI did not precipitate RNA (7). As shown in Fig. 2, RNA hybrids banded at 1.58 g/cm^3, well separated from virus-specific complementary RNA which banded symmetrically at 1.60 g/cm^3. Synthetic ^{14}C-polyadenylate banded sharply at 1.62 g/cm^3 (Fig. 2A, arrow). Thus, the procedure specifically isolated viral complementary RNA in the form of hybrids with virion RNA and precluded contamination by adenylate-rich fragments.

The six peak fractions of hybrids from KI gradients were pooled; the RNA was separated from KI by ethanol precipitation, dissolved in water, boiled 3 min to dissociate the hybrids, and treated with pancreatic and T1 ribonucleases in 0.3 M NaCl. As shown in Fig. 2B, nuclease-

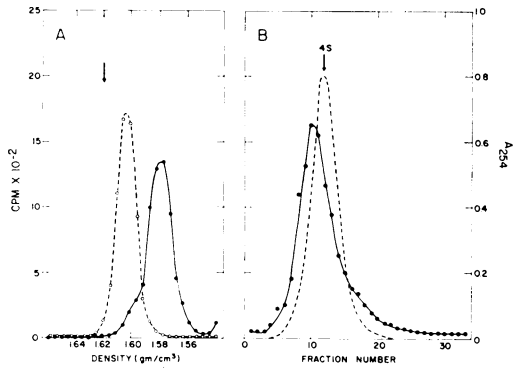

FIG. 2. *A, Isopycnic centrifugation of ^{3}H-adenine-labeled Sendai virus-specific RNA species in KI. Single-stranded complementary RNA sedimenting at less than 24S from infected cells was taken from a sucrose gradient like those shown in Fig. 1, except the RNA was isolated with SDS-phenol from a cytoplasmic extract made as described by Penman (16). The RNA was precipitated with ethanol in the presence of 200 μg of yeast RNA. The precipitate was dissolved in water, boiled for 3 min, and centrifuged in a sucrose gradient as before. RNA sedimenting faster than 8S was collected by ethanol precipitation, dissolved in 0.3 M NaCl, 0.03 M Na citrate (pH 7.0) and annealed with 20 μg of unlabeled Sendai virion RNA per milliliter at 80 C for 1 hr. After cooling to 24 C, pancreatic ribonuclease was added to a final concentration of 0.1 μg/ml; 10 min later the mixture was made 0.5% in SDS and precipitated with ethanol. The precipitate was dissolved directly in 3 ml of 5 M KI, 0.015 M Na citrate, 0.01 M Na bisulfite, 0.01 M K_2HPO_4 (pH 7.0) and was centrifuged for 64 hr at 36,000 rev/min, 20 C in a Spinco SW39 rotor. Densities were determined with a refractometer, and a portion of each fraction was processed for counting. Symbols in A: (●) annealed RNA, (○) unannealed Sendai virus complementary RNA run in a separate KI gradient. The arrow indicates the position of ^{14}C-polyadenylate from Miles Laboratories. B, Sedimentation of adenylate-rich segments from Sendai virus complementary RNA. The six peak fractions of annealed RNA from an isopycnic gradient like that shown in panel A were combined, collected by ethanol precipitation, dissolved in water, boiled for 3 min, and treated with a mixture of pancreatic and T1 ribonucleases in buffer containing 0.3 M NaCl as described in the legend of Fig. 1. The mixture was then made 0.5% in SDS and centrifuged at 26,000 rev/min, 20 C, for 40 hr in a 15 to 30% sucrose gradient. In a parallel gradient, yeast transfer RNA (Miles Laboratories) was centrifuged as a sedimentation marker. Symbols in B: (●) ribonuclease-resistant radioactivity, (---) A_{254}.*

resistant material, representing about 10% of the radioactivity taken for hybridization, sedimented at about 4S, close to marker transfer RNA.

These results identify adenylate-rich sequences in Sendai virus 18S complementary RNA, by methods which avoid contamination by cell RNA or by adenylate-rich fragments which are not linked covalently to the complementary RNA molecules. The sedimentation properties of the adenylate-rich sequences indicate a size comparable to adenylate-rich sequences found in other viral and cellular RNA species thought to have messenger function (1, 6, 8, 9, 12, 14).

The present state of knowledge about adenylate-rich sequences does not permit us to identify an RNA as message simply by its possession of such sequences. However, this finding should be weighed with other evidence for messenger function of paramyxovirus complementary RNA species which are smaller than viral genomes—their association with virus-specific polyribosomes (4, 5) and their generation by virion-associated transcriptase (2, 19). Moreover, it is likely that adenylate-rich sequences are absent from paramyxovirion RNA (9), arguing against a messenger function for paramyxovirus genomes.

With regard to the origin of polyadenylate in Sendai virus transcripts, it does not seem to be templated by virion RNA, since uridylate-rich sequences are not found in Sendai virion RNA (D. Gillespie, *personal communication*). Since Sendai virus replicates in the cytoplasm, adenylate-rich sequences could not be involved in transporting viral messenger RNA out of the nucleus. Rather, an involvement in transcription or translation (1) is indicated.

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