Characterization of the Low-Molecular-Weight RNAs Associated with the 70S RNA of Rous Sarcoma Virus

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Two low-molecular-weight RNAs are associated with the 70S RNA complex of Rous sarcoma virus: a previously described 4S RNA and a newly identified 5S RNA. The 4S RNA constitutes 3 to 4% of the 70S RNA complex or the equivalent of 12 to 20 molecules per 70S RNA. It exhibits a number of structural properties characteristic of transfer RNA as revealed by two-dimensional electrophoresis of oligonucleotides obtained from a T1 ribonuclease digest of the 4S RNA species. The 5S RNA is approximately 120 nucleotides in length, constitutes 1% of the 70S RNA complex or the equivalent of 3 to 4 molecules per molecule of 70S RNA, and is identical in nucleotide composition and structure to 5S RNA from uninfected chicken embryo fibroblasts. Melting studies indicate that the 5S RNA is released from the 70S RNA complex at the same temperature required to dissociate 70S RNA into its constituent 35S subunits. In contrast, greater than 80% of the 4S RNA is released from 70S RNA prior to its conversion into subunits. The possible biological significance of these 70S-associated RNAs is discussed.

Virions of Rous sarcoma virus (RSV) contain two major size classes of RNA: 60 to 70S RNA, presumed to be the viral genome, and a low-molecular-weight population consisting of a number of discrete RNA species. In addition, RSV 70S RNA can be denatured into a number of subunits of relatively large mass ranging from approximately 10^6 to 3×10^6 daltons (9), and into several low-molecular-weight RNAs referred to as 70S-associated RNAs (8, 12, 14). The 70S-associated RNAs include a previously identified 4S RNA (8, 12) and a 5S RNA to be described in this communication. Recent studies have suggested that the 70S-associated RNAs may function in either of two capacities: (i) as a primer involved in the initiation of DNA synthesis by the oncornavirus RNA-directed DNA polymerase when 70S RNA is utilized as a template (8, 25, 27, Bishop et al., Second Annual Steenbock Symposium, in press, and Faras et al., submitted for publication), and (ii) as a linker responsible for the maintenance of the subunit nature of the 70S RNA complex (20; Canaani et al., Proc. Nat. Acad. Sci. U.S.A., in press).

We have initiated studies on the 70S-

associated RNAs of RSV in order to elucidate the structure of these RNA species. In this communication we report the presence of an additional low-molecular-weight RNA contained within virions of RSV. This 5S RNA species is found both free in the virion and associated with the 70S RNA genome, and it is identical in structure to the 5S RNA obtained from uninfected chicken embryo fibroblasts. In addition, we report that the RSV 70S-associated 4S RNA and free 4S RNA exhibit a number of structural similarities to chicken fibroblast tRNA.

MATERIALS AND METHODS

Reagents. The sources and preparation of most of the pertinent materials used in this study have been described in previous communications (5, 6). RNase A (pancreatic RNase) was from Worthington Biochemical Corp., and T1 RNase was from Sankyo Company, Ltd., Tokyo, Japan.

Purification of RSV. The propagation and purification of the Schmidt-Ruppin strain (subgroup A) of Rous sarcoma virus have been described previously (5).

Preparation of nucleic acid samples. All procedures for purification of nucleic acids were carried out as previously described (4, 5). RSV RNA was extracted from purified virus with sodium dodecyl

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sulfate (SDS)-phenol at room temperature. Ninety percent of the viral RNA extracted by either SDS-phenol or chloroform-phenol bound to polydeoxythymidylic acid columns (2, 16). This suggests that there is no preferential loss of polyadenylic acid from viral RNA during phenol extraction (18, 19, 22). Cellular RNA was prepared from chicken embryo fibroblasts by phenol extraction at 60 C in 0.05 M sodium acetate and 0.01 M EDTA, pH 5.0.

Electrophoresis in polyacrylamide gels. We have previously described procedures for electrophoresis in ethylene diacrylate cross-linked gels of polyacrylamide (5). Conditions of electrophoresis are given for individual experiments.

Two-dimensional electrophoresis. The procedure for two-dimensional electrophoresis was essentially that described by Sanger et al. (24). RNA was hydrolyzed with T1 RNase at an enzyme-to-RNA ratio of 1:20 in 0.02 M Tris-hydrochloride (pH 7.4) and 0.002 M EDTA in a volume of 5 μ liters for 30 min at 37 C. The hydrolysate was applied to a cellulose acetate strip (30 mm by 550 mm; Schleicher and Schuell Co.) and separated by high-voltage electrophoresis in pyridine acetate (pH 3.5) at 100 V/cm for approximately 2 h. The oligonucleotides were transferred to DEAE paper (Whatman DE 81) and subjected to two-dimensional electrophoresis in 7% formic acid at 17 V/cm for approximately 16 h. The positions of oligonucleotides in the two-dimensional fingerprint were located by radioautography. For further analysis, oligonucleotides were eluted with triethylamine carbonate and characterized by electrophoresis on DEAE paper in pyridine acetate (pH 3.5) after digestion with pancreatic RNase (0.1 mg/ml) in 0.01 M Tris-hydrochloride, pH 7.5, and 0.001 M EDTA at 37 C for 30 min (1).

Nucleotide composition. RNA samples which had been evaporated to dryness on a polyethylene sheet were digested with 5 μ liters of 0.3 N NaOH at 37 C for 16 h. Mononucleotides were separated by electrophoresis on Whatman 3 MM paper in pyridine acetate, pH 3.5. Appropriate optical density markers were added where necessary, the pyridine was removed from the paper in a NH_4 atmosphere, and the marker was visualized under a short-wave UV lamp.

RESULTS

Identification of 5S RNA in virions of RSV.

Analysis of the free low-molecular-weight RNAs of RSV by electrophoresis in gels of 10% polyacrylamide is illustrated in Fig. 1a. In addition to the previously described 4S and 7S RNA molecules (5, 6, 23), RNA with the same electrophoretic mobility as that of host cell 5S RNA can be demonstrated (Fig. 1a). This 5S RNA comprises 2% of the total RNA associated with virions of RSV. In contrast, 4S and 7S RNA represent 20 and 5% of the total RSV RNA, respectively (Table 1). The 5S RNA is not eliminated by pretreatment of virus with RNase (50 μ g/ml for 1 h at 37 C) prior to extraction, suggesting that it is contained within the virion particle rather

than merely adsorbed to the outer envelope of the virus. Furthermore, the yield of 5S RNA from purified RSV is independent of the time interval (1 h or 12 h) between harvests of RSV from cells, suggesting that it does not arise from degradation of another RNA species (3).

Denaturation of avian myeloblastosis virus (AMV) 70S RNA dissociates it into its constituent 35S subunits and releases a 4S RNA species (12, 13). Denaturation of the 70S RNA from RSV releases both 4S and 5S RNAs (Fig. 1b). The high-molecular-weight subunit constituents of RSV 70S RNA do not penetrate gels of this pore size and thus accumulate at the origin

FIG. 1. Electrophoresis of RSV low-molecular-weight RNAs and 70S-associated RNAs in 10% polyacrylamide gels. a, Analysis of the free low-molecular-weight RNA species. The RNA of RSV was extracted with phenol and fractionated on sucrose gradients as previously described (5, 6). The 70S region and 4 to 10S region of the gradient were pooled separately and ethanol precipitated. The low-molecular-weight RNAs were then subjected to electrophoresis in gels of 10% polyacrylamide at 5 mA per gel for 4 h. b, Analysis of 70S-associated RNAs. The 70S RNA, purified as described in Fig. 1a, was denatured by treatment with 95% dimethylsulfoxide in 0.02 M Tris-hydrochloride, pH 7.4, and 0.01 M EDTA for 30 min at 37 C and then ethanol precipitated. The RNA was resuspended in 0.02 M Tris-hydrochloride, pH 7.4, and 0.01 M EDTA, heated at 80 C for 1 min to disrupt any aggregation which may have occurred during ethanol precipitation of the dimethylsulfoxide-treated RNA (17), and finally subjected to electrophoresis as in Fig. 1a. Arrows indicate the positions of differentially labeled RSV 7S RNA, and 5.5S, 5S, and 4S chicken embryo fibroblast RNAs run in the same gel.

TABLE 1. RNAs of *Rous sarcoma virus*^a

RNA species	Amount ^b (%)	Moles per virion ^c
70S	70	1
Aggregate of:		
20 to 35S subunits	(95)	3-4
5S	(1)	3-4
4S	(4)	12-20
28S ribosomal RNA	2	<1
18S ribosomal RNA	1	<1
7S	5	7
5S	2	7
4S	20	125

^a The table represents a summary of the RNAs contained in virions of RSV. Several of these RNAs have been described in previous communications (5, 6).

^b Expressed as the relative percentage of the total radioactivity. Cells were labeled with $^{32}\text{PO}_4^{2-}$ for 12 h before virus harvests to insure steady-state labeling of RNA in all experiments.

^c Computed by using the following molecular weight values: 70S, 10×10^6 ; 28S, 1.6×10^6 ; 18S, 0.7×10^6 ; 7S, 10^5 ; 5S, 4.2×10^4 ; and 4S, 2.5×10^4 .

(Fig. 1b). The 70S-associated 4S RNA represents 3 to 4% of the total 70S RNA complex. Assuming a molecular weight of 3×10^6 for 35S RNA and a molecular weight of 2.5×10^4 for 4S RNA, approximately 4 to 5 molecules of 4S RNA are present per molecule of 35S (Table 1). These proportions conform to those obtained by Erikson and Erikson for the 4S RNA associated with the 70S RNA complex of AMV (12). The 5S RNA molecule represents 0.75 to 1.0% of the total 70S RNA or the equivalent of one molecule per 35S RNA species, assuming a molecular weight of approximately 4.2×10^4 for 5S RNA. The 7S RNA appears to be absent from the RSV 70S RNA complex (Fig. 1b).

Thermal transition of 70S-associated RNAs. Experiments were performed to explore the nature of the association of 4S and 5S RNA to the 70S RNA complex. RSV 70S RNA was heated at various temperatures and analyzed on disk acrylamide gels composed of 6.5 cm of 10% polyacrylamide overlaid with 3.5 cm of 2.25% polyacrylamide. Composite gels of this nature resolve both high- and low-molecular-weight RNAs and therefore allow simultaneous analysis of both 70S RNA and 70S-associated RNAs (12). We have used this procedure to obtain T_m values for both the conversion of RSV 70S RNA into its constituent subunits and the release of the 70S-associated RNAs from the 70S RNA complex (Fig. 2). With increasing temperature, RSV 70S RNA dissociates into its constituent high-molecular-weight subunits. By virtue of

their inability to penetrate the 10% polyacrylamide region of the gel, the 35S subunit species accumulate at the interface between the 2.25% and 10% gels and can readily be differentiated from the 70S RNA complex. The release of 4S and 5S RNA from 70S RNA is monitored by the appearance of these species in the 10% gel (12) and is quantified by summing the radioactivity in appropriate regions of the gels (Fig. 2). Nearly all (80%) of the 70S-associated 4S RNA is released from the 70S complex before the conversion of 70S RNA to its 35S RNA subunits (Fig. 3). The release of 5S RNA occurs concomitantly with the dissociation of 70S RNA into its constituent subunits. These studies permit the assignment of T_m values, i.e., the temperature at which half of a particular species of RNA is released from the 70S RNA complex or dissociated into subunits (Fig. 3). For example, the 70S-associated 4S RNA has a T_m which is substantially lower (42°C) than that of 5S RNA (51°C); 5S RNA has a T_m identical to that of 70S RNA. The broad melting profile (Fig. 3) of the 70S-associated 4S RNA suggests that differences may exist within the 4S RNA species with regard to the extent that they are associated with the 70S RNA complex. The temperature range at which the 70S-associated RNAs are released from RSV 70S RNA suggests that they are hydrogen bonded, rather than nonspecifically aggregated, to the 70S RNA complex. The T_m 's for both the transition of 70S RNA to 35S RNA subunits and the release of 4S RNA from the 70S RNA complex are lower than those previously reported (8). However, these differences probably reflect the nature of the ionic conditions in which 70S RNA was heated.

Nucleotide compositions. The nucleotide compositions of RSV 4S and 5S RNA and of the low-molecular-weight RNAs from chicken embryo fibroblasts are shown in Table 2. Both 70S-associated 5S RNA and free 5S RNA are identical in base composition to 5S RNA obtained from chicken embryo cells. The 70S-associated 4S RNA and free 4S RNA are similar in base composition to host cell 4S RNA and the 70S-associated 4S RNA of AMV (12). The base ratios of both 70S-associated RNA species differ considerably from that of RSV 70S RNA, reinforcing the conclusion that the 4S and 5S RNAs are not random degradation products of viral 70S RNA.

Separation and analysis of the T1 RNase oligonucleotides from RSV 4S and 5S RNAs by two-dimensional electrophoresis. A more detailed characterization of the structure of RSV 4S and 5S RNA was obtained by two-dimensional electrophoresis of oligonucleotides

generated by T1 RNase digestion of these RNA species (24). Two-dimensional fingerprints of T1 oligonucleotides from 70S-associated 5S RNA, 5S RNA obtained from the free low-molecular-weight RNA species, and 5S RNA purified from chicken embryo fibroblasts, are compared in Fig. 4. The number, relative position, and yield of oligonucleotides obtained from the 5S RNAs of RSV and of the host cell are identical. Furthermore, subsequent analysis of each oligonucleotide indicates that those occurring in the same position on all three of the 5S RNA fingerprints are identical in composition (Table 3). The fingerprints of RSV 5S RNA and chick cell 5S RNA are similar to that reported for 5S RNA obtained from KB cells (15).

The 70S-associated 4S RNA and the 4S RNA obtained from the free low-molecular-weight class of RSV RNA were subjected to similar analyses, and the resultant fingerprints of the T1 oligonucleotides were compared (Fig. 5). The fingerprints of both 70S-associated 4S RNA and free 4S RNA (Fig. 5a, b) are quite similar, particularly in the region of the larger T1 oligonucleotides. A comparison of fingerprints of equal intensity indicate that the 4S RNAs of RSV contain fewer oligonucleotides than does that of chicken embryo fibroblast 4S RNA, suggesting that fewer species of 4S RNA are contained in virions of RSV than in the host cell. Both 70S-associated and free 4S RNAs contain pGp and a prominent spot in the region of T ψ CG (24) representing approximately 3 to

FIG. 2. Electrophoresis of heat-treated 70S RNA in composite gels of 2.25% and 10% polyacrylamide. 32 P-labeled RSV 70S RNA was dissolved in 0.02 M Tris-hydrochloride, pH 7.4, and 0.01 M EDTA, heated at the indicated temperature for 2 min, chilled, and layered directly onto the gel. The composite gels consisted of 6.5 cm of 10% polyacrylamide overlaid with 3.5 cm of 2.25% polyacrylamide and were run for 4 h at 5 mA per gel. Symbols: Two separate scales are present on the ordinate. The scale represented by the solid line corresponds to 70S RNA and its constituent 35S subunits. The scale represented by the broken line corresponds to 70S-associated 4S and 5S RNA. The 35S subunits of 70S RNA are represented by the shaded area for purposes of computing the data illustrated in Fig. 3. Arrows indicate the positions of poliovirus 35S RNA and of cellular 5S RNA and 4S RNA markers.

4% of the total radioactivity. The presence of these particular oligonucleotides suggests that

FIG. 3. Thermal transitions of the release of 70S-associated 4S and 5S RNAs. The percentage of 35S, 5S, and 4S RNA released from the RSV 70S RNA complex after heating RSV 70S RNA at various temperatures was computed from the data in Fig. 2. The fraction of each RNA species released by heating was determined by summing the radioactivity present under the shaded areas and the 4S and 5S RNA peaks and computing the percentage of the total radioactivity represented by each. One hundred percent of the 4S and 5S RNAs were considered released from the 70S RNA complex when no additional radioactivity could be detected in the 4S and 5S regions of the gel upon further heating. Symbols: ●, thermal transition of release of 4S RNA; ○, thermal transition of the conversion of 70S to 35S subunits; △, thermal transition of release of 5S RNA.

RSV 4S RNA is similar to tRNA, a finding that conforms to results obtained from the 4S RNA of AMV (12). Included in Fig. 5 are fingerprints of two additional low-molecular-weight RNA species: host cell 5.5S RNA (originally referred to as 7S RNA [21]), which is hydrogen-bonded to 28S ribosomal RNA, and RSV 7S RNA (6) which has recently been identified in several RNA tumor viruses and in uninfected chicken embryo fibroblasts (Erikson et al., submitted for publication). A comparison of the oligonucleotides produced by RNase T1 digestion of 7S RNA from RSV, murine sarcoma murine leukemia virus, and AMV reveal striking similarities in structure, to the extent that these RNA molecules differ by at most two oligonucleotides (Erikson et al., submitted for publication). The fingerprints of RSV 7S RNA and host cell 28S-associated 5.5S RNA differ appreciably from 4S RNA and the newly identified 5S RNA contained within virions of RSV (Fig. 5d, e).

DISCUSSION

The present data describe a number of structural features of the low-molecular-weight RNAs contained within virions of RSV. Of particular interest is the identification and characterization of an additional low-molecular-weight RNA associated with RSV. This RNA species is found both free in the virion and associated with the 70S RNA genome. It is approximately 120 nucleotides in length (Table 3), comigrates with host cell 5S RNA on polyacrylamide gels, and is identical in base composition and structure to the 5S RNA associated with the large ribosomal subunit in eukaryotic cells. Although virtually nothing is known about

TABLE 2. Nucleotide compositions of RSV RNAs^a

RNA	Cytidine	Adenosine	Guanosine	Uridine	Guanosine + cytidine/ adenosine + uridine	Guanosine + cytidine (%)
70S	24.6 ± 0.15	22.8 ± 0.35	26.7 ± 0.35	25.9 ± 0.49	1.053	51.3
5S	27.7 ± 0.03	19.1 ± 0.06	30.9 ± 0.14	22.4 ± 0.10	1.414	58.6
5S (70S-associated)	27.5 ± 0.12	18.9 ± 0.18	30.6 ± 0.10	22.2 ± 0.11	1.414	58.1
5S (cell)	28.1 ± 0.19	18.7 ± 0.18	30.6 ± 0.28	22.7 ± 0.22	1.417	58.7
4S	27.7 ± 0.07	19.3 ± 0.24	32.7 ± 0.25	20.8 ± 0.42	1.507	60.4
4S (70S-associated)	27.9 ± 0.15	18.2 ± 0.21	33.1 ± 0.31	21.7 ± 0.27	1.528	61.0
4S (cell)	28.4 ± 0.30	18.7 ± 0.15	31.6 ± 0.32	20.9 ± 0.09	1.515	60.0
7S	28.5 ± 0.18	19.5 ± 0.02	35.1 ± 0.19	16.5 ± 0.05	1.604	62
5.5S (cell)	28.1 ± 0.29	20.9 ± 0.20	27.9 ± 0.32	23.0 ± 0.35	1.275	56.0

^a The low-molecular-weight RNAs were purified as described in Fig. 4. The results are expressed as mole percentages of nucleotide ± standard error of the mean. A minimum of 10 separate determinations was used for each RNA species. Nucleotide compositions of RSV 70S RNA were taken from previously published results (6).

FIG. 4. Radioautographs of two-dimensional fractionations of RNase T1 digests of 5S RNA from RSV and chicken embryo fibroblasts. ^{32}P -labeled RNA was extracted from RSV and chicken embryo fibroblasts, subjected to preliminary fractionation by rate zonal sedimentation, and further purified by electrophoresis in gels of 10% polyacrylamide for 4 h at 5 mA per gel (5, 6). Elution of RNA from the gels was accomplished by incubating the individual gel slices with 1 ml of a solution of 0.02 M Tris-hydrochloride, pH 7.4, 0.01 M EDTA, and 0.2% SDS for 24 h at 4 C. The RNA was concentrated by ethanol precipitation, and the oligonucleotides produced by T1 RNase digestion were separated by two-dimensional electrophoresis (24). The number, relative electrophoretic mobilities, and tentative nucleotide compositions of the major oligonucleotides in these radioautographs are identical. The fingerprint of cellular 5S RNA contains several additional oligonucleotides which are represented on the diagram (d) by letters A through G. These oligonucleotides are present in less than 0.2 mole per molecule and probably represent a minor contaminant of host cell 5S RNA with another RNA. a, 5S RNA from RSV 70S RNA; b, 5S RNA from the free low-molecular-weight fraction of RSV RNA; c, 5S RNA from chicken embryo fibroblasts; d, diagrammatical representation of 5S RNA. The spots labeled A through G and represented by the broken line correspond to oligonucleotides present in host cell 5S RNA in yields of less than 0.2 mole per molecule.

the function of 5S RNA in eukaryotic cells, evidence is accumulating which suggests that 5S RNA is required for protein synthesis in prokaryotic systems (10). The biological significance of RSV 5S RNA relative to the processes of RSV infection, replication, and transformation is not presently known. It is conceivable that the 5S RNA is incorporated into virions at random during the process of virus maturation and therefore does not constitute a functionally significant virion molecule. However, the reproducibility of yields of 5S RNA from various preparations of RSV (A. J. Faras, unpublished data), and its association with the RSV 70S RNA genome argues against this interpretation. Although the 70S-associated 5S RNA is released from the 70S RNA complex concurrently with the conversion of 70S RNA into its constituent subunits, we do not know whether it functions as a linker in the subunit structure of the RSV genome. Attempts to reconstruct 70S RNA from its constituent 35S subunits and 70S-associated RNAs have been unsuccessful to date (P. Dues-

berg and R. Erikson, personal communications).

The 4S RNA contained in virions of AMV can be aminoacylated and is therefore functionally similar to tRNA (7, 11, 26). We, and others, have demonstrated that the 4S RNAs of RSV have a number of structural properties characteristic of tRNA (12). Their role, if any, in the propagation of RNA tumor viruses remains obscure. A population of 4S RNAs, with structural features similar to those of tRNA, is also associated with the RSV 70S RNA genome (12, and present findings). A number of recent reports suggest a possible role for the 70S-associated 4S RNA. Studies on RNA-directed DNA synthesis by the oncornavirus DNA polymerase indicate that DNA synthesis is initiated on a small polyribonucleotide associated with the 70S RNA template (8, 25, 27). Studies from our laboratory suggest that the primer molecule required for initiation of DNA synthesis is 4S RNA (Bishop et al., in press; Faras et al., submitted for publication). Partial se-

FIG. 5. Radioautographs of two-dimensional fractionation of a ribonuclease T1 digest of chicken embryo fibroblast 4S, 5.5S RNA, RSV 4S, and 7S RNA. The RNAs were purified as described in Fig. 4. The oligonucleotides marked 1 and 2 are in the positions of pGp and T ψ CG, respectively (24). a, 4S RNA from the RSV 70S RNA; b, 4S RNA from the free low-molecular-weight fraction of RSV RNA; c, 4S RNA from chicken embryo fibroblasts; d, 5.5S RNA from chicken embryo fibroblasts; e, 7S RNA from the 4 to 7S fraction of RSV RNA.

TABLE 3. *Yields and compositions of oligonucleotides from RSV 5S RNA*

Oligonucleotide number	Composition ^a	Relative yield ^b
1	G	17.1
2	CG	3.0
3	CCG	0.8
4	AG	1.5
5	[A, C]G	1.2
6	[A, C ₂]G	0.9
7	AAG	1.0
8	UG	1.8
9	UCG	0.8
9	CUG	1.3
10	UAG	1.0
11	AUG	0.8
12	[U, C ₂]G	1.3
13	[(AC), C ₂ U]G	0.8
14	[(AAC), U]G	1.3
14	[U, C]AAG	1.3
15	[(AAU), (AC), C]G	0.8
16	UUAG	0.9
17 ^c	[U ₂ , C]X OH	0.9
18	[(AU), U, C ₂]G	1.2
19	[A, U ₂ , C ₃]G	0.8
20	[A ₂ , U ₂ , C ₄]G	0.8
21	[A, U ₃]G	0.9

^a Oligonucleotides were located on fingerprints by radioautography and eluted from the paper, and their composition was determined after pancreatic RNase digestion as described in Material and Methods and by comparison with the position of oligonucleotides of known sequence (24). Oligonucleotides without brackets or within parentheses indicate known sequence. The order of oligonucleotides within brackets is unknown. A, adenosine; C, cytidine; G, guanosine; U, uridine; X, unknown ribonucleoside.

^b Obtained by first determining the average count per minute per phosphate and then using this average to compute yields on the basis of the total radioactivity present in each oligonucleotide and the deduced length of each oligonucleotide.

^c Probably at the 3' terminus of 5S RNA, because it is the only oligonucleotide that lacks guanosine. We would not have detected the 5' terminus under these conditions of analysis.

quence studies of the purified 4S RNA primer reveal structural similarities to the 4S RNA found associated with the RSV 70S RNA complex (Faras et al., manuscript in preparation). Canaani et al. (in press) have demonstrated that the 70S-associated 4S RNA becomes associated with RSV 70S RNA as the 70S complex is formed from 35S subunit species during virus maturation. Differences in template efficiencies with the RSV DNA polymerase can be demonstrated between 30 to 40S RNA found in immature virus and 70S RNA found in mature virus. Correspondingly, differences can be detected in

the relative amount of 4S RNA associated with template RNA obtained from immature and mature virus. The possibility that the 70S-associated 4S RNA functions as a linker responsible for the maintenance of the subunit structure of the 70S RNA complex was also proposed (20, Canaani et al., in press).

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