JOURNAL OF VIROLOGY, Sept. 1972, p. 555-559
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Vol. 10, No. 3
Printed in U.S.A.

NOTES

Transfer Ribonucleic Acid Nucleotidyltransferase and Transfer Ribonucleic Acid in Sendai Virions

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Received for publication 15 May 1972

Sendai virions contain both transfer ribonucleic acid (tRNA) nucleotidyltransferase and its substrate, tRNA missing its CCA-OH end.

Ribonucleic acid (RNA)-dependent RNA polymerase activity has recently been found in Newcastle disease virus and Sendai virus (7, 11, 13). This communication reports that Sendai virions also contain a transfer RNA (tRNA) nucleotidyltransferase activity which represents the predominant incorporation of cytidine nucleotide under the assay conditions used to detect polymerase activity, and that at least some of the 4*S* RNA found in Sendai virions (1) can serve as a substrate for this reaction.

Sendai virus, Harris strain (obtained from the laboratory of R. D. Earry, Cambridge) was grown in 9-day embryonated chicken eggs. The allantoic fluid was harvested 72 hr after infection and centrifuged for 5 min at 10,000 × *g* to remove debris, and the virus was then pelleted by layering the allantoic fluid over a 15-ml cushion of 20% glycerol in TNE [50 mM tris-(hydroxymethyl)aminomethane (Tris)-chloride (*p*H 7.5), 25 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA)] and centrifuging for 90 min at 36,000 rev/min in an International IEC A-170 rotor. The virus pellet was resuspended in TNE with the aid of a Dounce homogenizer, centrifuged for 5 min at 10,000 × *g* to remove insoluble material, and then layered on top of a preformed gradient of potassium tartrate (10 to 40%, w/w) in TNE and centrifuged for 2 hr in a Spinco SW27 rotor at 26,000 rev/min. The virus was collected by puncturing the tube just below the visible virus band, diluted with two volumes of TNE, and rebanded in a potassium tartrate gradient as above. The virus was then diluted with an equal volume of TNE, pelleted by centrifugation for 90 min at 40,000 rev/min in a Spinco 50 rotor, resuspended in TNE, and stored at −70 C. Virus prepared in this fashion had no detectable phosphatase activity as described by Mizutani and Temin for Rous sarcoma virus (10), in that adenosine-5′-monophosphate was not hydrolyzed to adenosine.

When Sendai virions were assayed for endogenous polymerase activity, it was noted that cytidine triphosphate (CTP) incorporation was significantly higher than that of either guanosine triphosphate (GTP) or uridine triphosphate (UTP) (Table 1). Moreover, nearest neighbor analysis of the reaction products gave startlingly different results depending on which α-^{32}P-ribonucleoside triphosphate was used. When labeled GTP or UTP was used, nearest neighbors were approximately equal, but when labeled CTP was used it was found that C was incorporated next to C greater than 60%, next to A approximately 25%, and seldom next to G and U (Table 1). Since most tRNA species that have been sequenced end in . . . ACCA-OH (8), the relatively higher incorporation and unusual nearest neighbor analysis when labeled CTP was used could be explained if, in addition to a polymerase activity, Sendai virions also contained both a tRNA nucleotidyltransferase activity and tRNA missing its CCA-OH end, the substrate for this enzyme.

To test this possibility, RNA was extracted from Sendai virions, and the 4*S* component was isolated by sucrose gradient centrifugation and added to the polymerase reaction mixture containing radioactive CTP. The results (Table 1 and Fig. 1) demonstrate that Sendai 4*S* RNA stimulated the incorporation of CTP in a linear fashion up to at least sevenfold, and that this activity, like that of the polymerase activity reported (7, 11, 13), is dependent upon the presence of a nonionic detergent, Nonidet P-40 (NP40).

The nature of the reaction product labeled with CTP was examined. Reaction products were syn-

TABLE 1. *Incorporation and nearest neighbor analysis using α-^{32}P-ribonucleoside triphosphates*[a]

α-^{32}P-ribonucleoside triphosphate used	Additions	Total incorporation		3'-Monophosphate label							
				C		A		G		U	
		Counts/min	Picomoles	Counts/min	%	Counts/min	%	Counts/min	%	Counts/min	%
GTP		4,612	0.42	211	10.1	639	30.8	570	27.4	654	31.5
UTP		3,840	0.37	556	27.2	443	21.7	607	29.6	438	21.4
CTP		5,941	2.54	2,381	62.5	908	23.8	340	8.9	183	4.8
CTP	4S RNA	44,806	19.2	18,235	61.2	8,909	29.6	1,699	5.6	1,292	4.3
CTP[b]	4S RNA			5,718	64.7	2,234	25.3	361	4.1	501	5.7

[a] Reaction mixtures contained in a total volume of 50 μliters: Tris-chloride (*p*H 7.5), 75 mM; $MgCl_2$, 10 mM; NaCl, 12.5 mM; dithiothreitol, 3 mM; NP40, 0.4%; three nonradioactive 5'-ribonucleoside triphosphates, 0.4 mM; one α-^{32}P-5'-ribonucleoside triphosphate, 0.02 mM (for specific activities, see table); Sendai virion, 85 μg of protein; and, where indicated, 6 μg of Sendai 4S RNA. Tubes were incubated for 2 hr at 29 C; 200 μliters of stopping mixture (1 × standard saline citrate plus 10% $Na_2P_2O_4$) was added, followed by cold trichloroacetic acid to a final concentration of 6%, and the precipitates were then filtered on Whatman GF/C filters and washed with 6% trichloroacetic acid. After counting by liquid scintillation, the filters were removed from the vials, washed with toluene, dried, and heated for 90 min at 100 C in 10% piperidine in sealed tubes. The filters were then removed, the hydrolysate was twice reduced to dryness, marker 3'-ribonucleoside monophosphates were added, and the samples were subjected to electrophoresis on Whatman 3MM paper in pyridine-acetate, *p*H 3.5, for 5,000 volt-hours. Spots were located under ultraviolet light, cut out, and counted by liquid scintillation.
[b] See legend to Fig. 2.

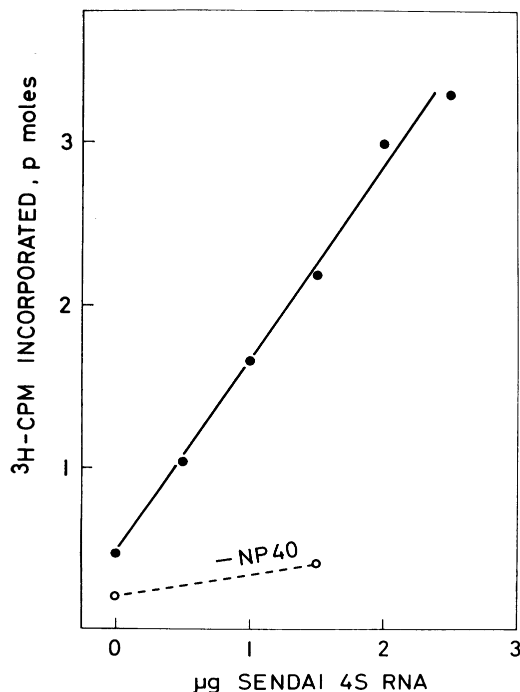

FIG. 1. *Effect of Sendai 4S RNA on the incorporation of ^{3}H-CTP. Reaction mixtures were as described in the legend to Table 1, except that reactions were carried out in a total volume of 25 μliters, 45 μg of protein of Sendai virus and 0.02 mM ^{3}H-CTP (2,140 counts per min per pmole) were used, and where indicated, NP40 was omitted.*

thesized with and without added Sendai 4S RNA and analyzed by electrophoresis on polyacrylamide gels. The results (Fig. 2) show that, whether or not the reaction had been stimulated by Sendai 4S RNA, virtually all of the reaction product comigrated with $tRNA_{Metf}$ (*E. coli*) added as marker. Because of the sharp 4S peak obtained on polyacrylamide gels and the nearest neighbor analysis when radioactive CTP was used to follow incorporation, we conclude that Sendai virions contain a tRNA nucleotidyltransferase activity and that some of the Sendai 4S RNA is tRNA whose CCA-OH end is missing.

To determine whether the Sendai tRNA whose CCA-OH end was missing represented a unique species, the α-^{32}P-CTP-labeled product synthesized in the presence of Sendai rS RNA (see Fig. 2) was digested with ribonuclease T_1 both in the presence and absence of bacterial alkaline phosphatase (BAP) and fingerprinted by the method of Sanger et al. (12). Figure 3 shows the fingerprint of the T_1 ribonuclease digest without added BAP. The solid black spots represented in the left-hand panel of Fig. 3 were found in identical positions in the fingerprint of the digest by T_1 ribonuclease and BAP (fingerprint not shown) and therefore represent oligonucleotides without 3'-phosphate groups. Analogously, hatched spots represent oligonucleotides that changed their position when BAP was included in the digestion and therefore do contain 3'-phosphates. As shown in Fig. 3, there are 16 detectable oligonucleotides whose position is un-

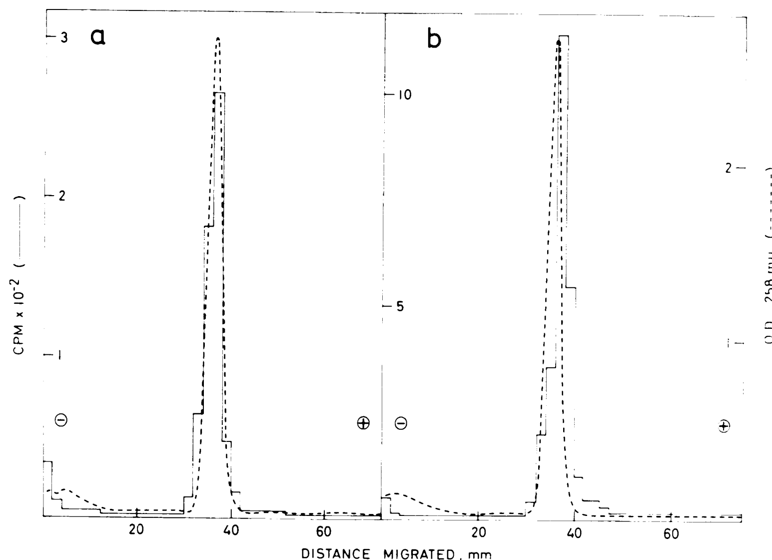

FIG. 2. *Polyacrylamide gel electrophoresis of α-^{32}P-CTP-labeled products. Fourfold reaction mixtures, with (b) and without (a) added Sendai 4S RNA, were incubated as described in Table 1, but were then digested with Pronase (0.5 mg/ml) in the presence of 0.1% sodium dodecyl sulfate, extracted with phenol, and passed through a 2-ml column of Sephadex G-50 in 20 mM Tris-chloride (pH 7.5) to remove unincorporated triphosphates. The excluded peak was located by Čerenkov counting and pooled, and the RNA was precipitated with two volumes of ethanol. The RNA was then collected by centrifugation and dissolved in distilled water. Samples of the isolated products were mixed with 0.4 optical density unit of $tRNA_{Met\,f}$ (E. coli) and subjected to electrophoresis on 0.6 by 7.5 cm polyacrylamide gels (7.5%, 3% bisacrylamide) in 50 mM Tris-chloride (pH 7.5), 10 mM KCl, and 10 mM $MgCl_2$, until the bromophenol blue marker had just run off the end of the gel. The gels were first scanned at 258 nm to locate the position of the marker $tRNA_{Met\,f}$ and then cut into 2-mm slices, and radioactivity was determined in a liquid scintillation spectrometer by Čerenkov counting.*

changed after BAP treatment and therefore represent 3′ ends, and these oligonucleotides comprise the major share of the reaction product. We conclude that Sendai tRNA which is missing its CCA-OH end does not represent a unique species. However, since there is considerable variation in the yield of oligonucleotides without 3′-phosphate groups, it is unlikely that all tRNA species missing their 3′ ends are equally represented.

RNA found in Sendai virions has been shown to consist of a large RNA, 50 to 57*S* in size (1, 2, 9), but the presence or absence of a small or "4*S*" component is unclear. Barry and Buckrinskaya (1), using virus grown in eggs for 72 hr and labeled with ^{32}P, found this small RNA. However, Blair and Robinson (2) and Kingsbury et al. (9), using virus grown in chicken embryo cell cultures and labeled with ^{3}H-uridine 11 hr and 24 hr after infection, respectively, did not find this component. We have consistently found this small RNA, comprising 40 to 60% of the total viral RNA, by using egg-grown virus, both unlabeled and labeled with ^{32}P, or virus grown in chicken embryo fibroblasts for 24 hr and labeled with ^{3}H-uridine immediately after infection. If this small component represented host RNA which was included in the virion during viral assembly and release, then the failure to detect this small RNA in the virion when the viral RNA was labeled late in infection might be due to the possible shut-off of the synthesis of this small RNA in the host.

We have further fractionated this "4*S*" component obtained by sucrose gradient centrifugation by means of gel filtration on Sephadex G-100 into excluded and included fractions (data not shown). The included fraction, which comprises approximately half of the Sendai "4*S*" RNA, coelectrophoreses on polyacrylamide gels as a sharp peak with *E. coli* $tRNA_{Met\,f}$, whereas the excluded fraction is heterogeneous and larger in size (5 to 12*S*) by the same criterion (data not shown). As reported in Table 1, 16.7 additional pmoles of CTP were incorporated into trichloroacetic acid-precipitable material in response to 6 μg of unfractionated Sendai "4*S*" RNA. Assuming, therefore, that approximately half of the Sendai "4*S*" RNA is tRNA, this would correspond to 16.7 pmoles of CTP incorporated per 120 pmoles of tRNA; i.e., only a small fraction of the tRNA present in Sendai

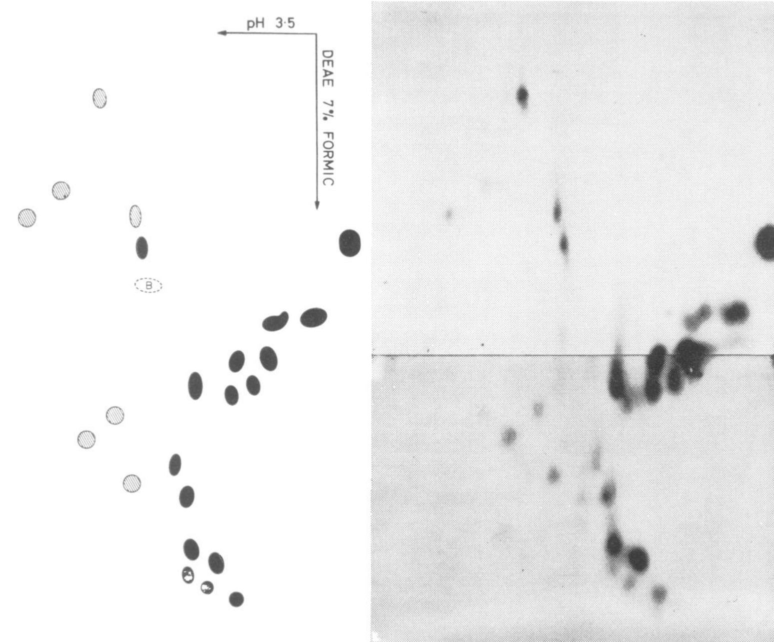

FIG. 3. *Fingerprint of the α-^{32}P-CTP-labeled product. A sample of the reaction product prepared as described in the legend to Fig. 2, containing 15,000 counts/min, was mixed with 15 μg of carrier QB RNA, digested with 1 μg of ribonuclease T_1 in 10 mM Tris-chloride (pH 7.5) and 1 mM EDTA for 30 min at 37 C, and then fingerprinted by the method of Sanger et al. (12). The right-hand panel shows a photograph of the autoradiograph, the left-hand panel a drawn representation of the autoradiograph (for explanation see text).*

virus has served as a substrate for the tRNA nucleotidyltransferase.

It is indeed difficult to speculate on what role, if any, the tRNA nucleotidyltransferase might play in the replication of Sendai virus. Mizutani and Temin (10) have recently demonstrated the presence of hexokinase and lactate dehydrogenase (cellular enzymes found in high concentrations in the cytoplasm and not generally believed to be involved in viral replication) in another budding virus, the Schmidt-Ruppin strain of Rous sarcoma virus. However, they failed to detect the presence of a third such enzyme, glucose-6-phosphate dehydrogenase. Similarly, Erikson and Erikson (6) have shown that avian myoblastosis virus (AMV), also a budding virus, contains some aminoacyl synthetases but not others, and several groups (3, 5, 14) have shown that AMV contains some tRNA species but not others. It seems possible that the apparent selectivity with which some enzymes are included in budding viruses may simply reflect their affinity towards substrates that are packaged in the virion. In the case of Sendai virus, we estimate that there are 100 to 150 molecules of tRNA per 57*S*, or 6.7 × 10^6 molecular weight RNA (1; and *see above*). Since it is known that tRNA nucleotidyltransferase does have an affinity for tRNA (4), it seems likely that this enzyme is included in Sendai virions as an enzyme-substrate complex. However, the question of why Sendai virus contains these components of the translation system remains unanswered.

I thank Pierre-François Spahr for generous advice, encouragement and support, Ray Gesteland for helpful discussions, and Andrée Bruschi for excellent technical assistance. α-^{32}P-ribonucleoside triphosphates were the kind gift of Martin Billeter (University of Zürich).

This work was supported by a research grant no. 3.595.71 from the Fonds National Suisse de la Recherche Scientifique.

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