JOURNAL OF VIROLOGY, May 1974, p. 1126-1133
Vol. 13, No. 5
Printed in U.S.A.
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Transcription of DNA from the 70S RNA of Rous Sarcoma Virus

I. Identification of a Specific 4S RNA Which Serves as Primer

J. E. DAHLBERG, R. C. SAWYER, J. M. TAYLOR, A. J. FARAS,[1] W. E. LEVINSON, H. M. GOODMAN, AND J. M. BISHOP

Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706, and Departments of Microbiology and of Biochemistry and Biophysics, University of California, San Francisco, California 94143

Received for publication 23 January 1974

The enzymatic transcription of DNA from the 70S RNA of Rous sarcoma virus (RSV) is initiated on the 3′ terminus of a molecule of 4S RNA which is hydrogen bonded to the viral genome. We labeled this primer with radioactive deoxynucleotides, and demonstrated that its release from 70S RNA by thermal denaturation was accompanied by a reduction in the template activity of the viral RNA. Two-dimensional electrophoresis in polyacrylamide gels separated the 4S RNAs associated with the 70S RNA of RSV into approximately eight fractions, each of which appeared to contain a discrete species of tRNA. The RNA in one of these fractions served as the principal primer for initiation of DNA synthesis by both detergent-disrupted virions of RSV and purified RNA-directed DNA polymerase with RSV 70S RNA as template.

The natural template-primer for the DNA polymerase of RNA tumor viruses is the viral 70S RNA (1, 18, 27, 30), a complex containing three to four high-molecular-weight subunits (about 35S; 7, 11) and several low-molecular-weight RNAs (4, 10, 12). The latter RNAs include a 5S RNA (70Sa-5S RNA; ref. 12), and 4S RNAs (70Sa-4S RNA) which have structural (10, 12, 25) and functional (24) properties of tRNA. Transcription in vitro of the genome of avian RNA tumor viruses initiates by covalent attachment of DNA to the 3′ terminus of a low-molecular-weight RNA (17, 29, 31). This primer may be a special fraction of the total 70Sa-4S RNA (4, 14). We have developed techniques to identify and purify primer molecules associated with the genome of Rous sarcoma virus (RSV) (2, 14, 26), and now present further evidence that these primers are part of a limited fraction of the total 70Sa-4S RNA. The accompanying manuscript describes structural features which indicate that the principal 4S primer on the RSV genome is probably a single species of tRNA (13).

MATERIALS AND METHODS

Reagents. Unlabeled deoxynucleoside triphosphates were purchased from Calbiochem. [^{3}H]dATP (15 Ci/mmol) was purchased from Schwarz BioResearch. [α-^{32}P]dATP (150 Ci/mmol) was synthesized according to our modification of the procedure of Symons (27; personal communication). Uracil arabinoside triphosphate (araUTP) was a gift from D. Shannahoff and S. Hendler. Carrier-free [^{32}P]orthophosphate was obtained from International Chemical and Nuclear Corp. Nonidet-P40 was a gift from Shell Chemical Co.

Purification of Rous sarcoma virus and its DNA polymerase. The propagation and purification of the Schmidt-Ruppin strain (subgroup A) of RSV, the purification of RNA-directed DNA polymerase, and the conditions for enzymatic synthesis of DNA have all been described (3, 15). Standard reaction mixtures for purified polymerase contained 0.2 U of enzyme per ml (15), template RNA (2 μg/ml, unless otherwise indicated), 0.1 M Tris-hydrochloride (pH 8.1), 0.01 M $MgCl_2$, 0.01% (vol/vol) Nonidet-P40, 2% (vol/vol) β-mercaptoethanol, and deoxynucleoside triphosphates (10^{-5} M). Reaction mixtures with purified virus were identical, except that purified enzyme and template were replaced by virus (200 to 400 μg of protein/ml). All reactions were carried out at 37 C.

Preparation of viral RNA. RNA was extracted from purified RSV with sodium dodecyl sulfate-phenol and fractionated by rate zonal centrifugation as described previously (3). [^{32}P]70S RNA was prepared from virus propagated as follows. Confluent monolayers of chick fibroblasts transformed by and producing RSV were washed twice with PO_4-free medium 199 containing calf serum (5%, vol/vol) and dimethylsulfoxide (1%, vol/vol) and then exposed to fresh medium of the same composition for 2 to 3 h, followed by medium containing carrier-free [^{32}P]or-

[1] Present address: Department of Microbiology, University of Michigan, Ann Arbor, Mich. 48104.

thophosphate at 100 μCi/ml. The medium containing ^{32}P was harvested and replaced with fresh medium containing ^{32}P every 12 h for 3 to 5 days, and stored at −70 C until further processing. RNA prepared from virus labeled in this manner had a specific activity of about 10^6 dpm/μg at the time of extraction 1 week after the virus was harvested.

Preparation of 70S complexes between template and tagged primer RNA. Purified DNA polymerase of RSV, 0.2 U/ml (15) and RSV 70*S* RNA (2 μg/ml) were incubated in a standard polymerase reaction mixture (generally 1 to 2 ml) which contained dGTP, dCTP, araUTP, and either [^{3}H]dATP or [α-^{32}P]dATP. All nucleoside triphosphates, including the analogue, were used at 10^{-5} M. After incubation at 37 C for 1 h, the reaction mixture was treated with sodium dodecyl sulfate (0.5% wt/vol) and then purified by rate zonal centrifugation in gradients of 15 to 30% sucrose containing 0.1 M NaCl-0.001 M EDTA-0.02 M Tris-hydrochloride, pH 7.4 (18). The complex between template and radioactively tagged primer cosedimented with RSV 70*S* RNA (14, 18) and was recovered from the gradients by ethanol precipitation.

Denaturation of nucleic acids. Thermal denaturation was carried out with the nucleic acids dissolved in 0.01 M EDTA-0.02 M Tris-hydrochloride (pH 7.4). On occasion, the nucleic acids were treated with dimethylsulfoxide (85%, vt/vol) (12, 21) in order to completely dissociate RSV 70*S* RNA into its 4*S*, 5*S*, and 20-35*S* constituents (4, 10, 12).

Fractionation of RNA by rate zonal centrifugation. The centrifugation of RNA through density gradients of sucrose containing 0.1 M NaCl-0.001 M EDTA-0.02 M Tris-hydrochloride (pH 7.4) was carried out as described previously (3). RSV 70*S* RNA was isolated from gradients centrifuged in a Spinco SW41 rotor for 3 h at 40,000 rpm, 4 C. The RNAs derived from RSV 70*S* RNA by thermal denaturation were fractionated by centrifugation in either an SW41 rotor (15 to 30% sucrose, 40,000 rpm, 6 h, 4 C) or an SW27 rotor (5 to 20% sucrose, 25,000 rpm, 16 h, 4 C). Two populations of RNA were obtained: high-molecular-weight subunits (2.5×10^6 to 3×10^6 daltons) as originally described by Duesberg (7) and Erikson (11), low- and molecular-weight RNAs, including 4*S* (10) and 5*S* (12) RNAs, which are not resolved by this procedure. The RNAs were recovered from the gradients by ethanol precipitation.

Electrophoresis of RNA in gels of polyacrylamide. We have previously described our procedures for electrophoresis in cylindrical gels of polyacrylamide (3) and in one- and two-dimensional slab gels (20, 26). The first dimension of the two-dimensional procedure was in 10% polyacrylamide (9.5% [wt/vol] acrylamide, 0.5% [wt/vol] *N,N'*-methylene bis-acrylamide) for 3 h at 400 V and 15 C. A second dimension of electrophoresis and some single-dimensional analyses were carried out in 19% polyacrylamide-1% *N,N'*-methylene bis-acrylamide for 14 h at 400 V and 15 C. The electrophoresis buffer was 0.045 M Tris-0.045 M boric acid-0.0014 M EDTA at pH 8.3. [^{32}P]RNAs were located in the slab gels by autoradiography, appropriate regions of the gels were cut out, and Cerenkov radiation was measured in a scintillation spectrometer. When necessary, RNA was eluted from the gel into 0.3 M NaCl and recovered by ethanol precipitation.

Analysis of oligodeoxynucleotides by paper electrophoresis. [^{32}P]oligodeoxynucleotides were released from covalent linkage with primer RNA by hydrolysis with NaOH (0.2 N, 37 C, 18 h) and separated by electrophoresis at pH 3.5 on Whatman 540 paper (5,000 V, 1 h). The separated materials were then eluted and analyzed further by electrophoresis at pH 3.5 on Whatman DE 81 paper. Identification of the final products will be described elsewhere (J. M. Taylor et al., manuscript in preparation).

RESULTS

Tagging of primer RNA with radioactive oligodeoxynucleotides. Transcription of RSV 70*S* RNA by the RNA-directed DNA polymerase of the virus initiates with the sequence pdApdApdT ... (2, 14; Taylor et al., manuscript in preparation). Further transcription beyond the initial dT can be arrested by carrying out DNA synthesis with a reaction mixture in which TTP is replaced by either dideoxythymidine triphosphate or araUTP (14). (Only dideoxythymidine triphosphate was used in the previous study, but we have unpublished evidence that araUTP is equally effective.) The products of DNA synthesis arrested with araUTP are the sequences pdA, pdApdA, and pdApdAparaU, all covalently linked to the 3'-terminal adenosine of 4*S* RNA primer (14; Taylor et al., manuscript in preparation). When synthesized with radioactive dATP, these aborted DNA chains constitute convenient "tags" which serve to identify otherwise unlabeled primer RNA without appreciably affecting its physical properties (14).

Selective release of primer from the 70S RNA of RSV. Denaturation of RSV 70*S* RNA dissociates low-molecular-weight primer from template with a consequent large reduction in template activity for RNA-directed DNA polymerase (9, 15). We examined the release of tagged primer from RSV 70*S* RNA by thermal denaturation and compared this release with both the dissociation of total 70*S*a-4*S* RNA and the loss of template activity. Release of tagged primer was monitored by electrophoresis in composite gels of polyacrylamide (Fig. 1). Two discrete stages were apparent. (i) The primer remained attached to template at a temperature (65 C) which completely dissociated 70*S* RNA into its high-molecular-weight subunits; at this point, tagged primer and subunits migrated to the interface of the composite gel (Fig. 1b). (ii) At a higher temperature (80 C), tagged

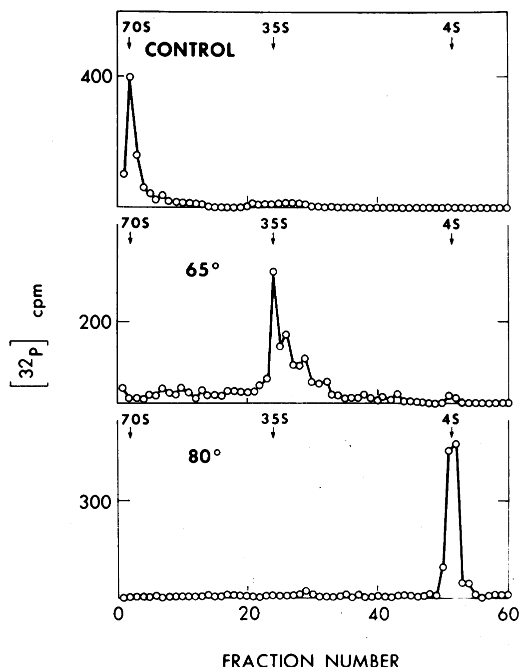

FIG. 1. *Release of primer from RSV 70S RNA. A complex between 70S RNA and primer tagged with [32P]dAMP was prepared as described under Materials and Methods. Samples (minimum of 2,000 counts/min) of this material were heated in 0.01 M EDTA-0.02 M Tris-hydrochloride, pH 7.4, at various temperatures for 2 min, quickly cooled in ice water, and analyzed by electrophoresis in composite gels of 2.25 and 10% polyacrylamide (6 mA/gel, 2.5 h, room temperature). The figure illustrates results with an unheated control and with samples heated at 65 and 80 C. The results at other temperatures are summarized in Fig. 2. Arrows indicate the locations of 70S RNA, high-molecular-weight subunits (35S), and 4S RNAs when denatured [32P]70S RNA devoid of nascent DNA was subjected to the same procedure. Recovery of radioactivity from the gels always exceeded 85%.*

primer was released from the subunits and migrated as 4S RNA (Fig. 1c). We have shown previously that the electrophoretic mobility of the released primer is attributable solely to the size of the RNA (14). Further documentation of this conclusion is presented below (Fig. 4).

Release of tagged primer and loss of template activity occurred over an identical range of temperatures (T_m = 69 C in 0.01 M EDTA-0.02 M Tris-hydrochloride, pH 7.4), whereas the bulk of 70Sa-4S RNA was released over a lower and much broader range of temperatures (Fig. 2). We conclude that the primer is a limited fraction of the total 70Sa-4S RNA, and that release of primer is both concomitant with and probably the cause of the loss of template activity. These data and conclusions are analogous to those of Canaani and Duesberg (4), except that we have directly identified the primer by virtue of its attached radioactive nascent DNA.

Fractionation of 70Sa-4S RNAs by two-dimensional electrophoresis in polyacrylamide gels. Two-dimensional electrophoresis in gels of polyacrylamide separates 4S RNAs of RSV into multiple fractions which represent discrete species of tRNA (26). We have used this technique to determine the nature of the 4S RNAs removed from RSV 70S RNA at different temperatures and to further substantiate our conclusion that a select portion of 70Sa-4S RNA serves as primer for DNA synthesis.

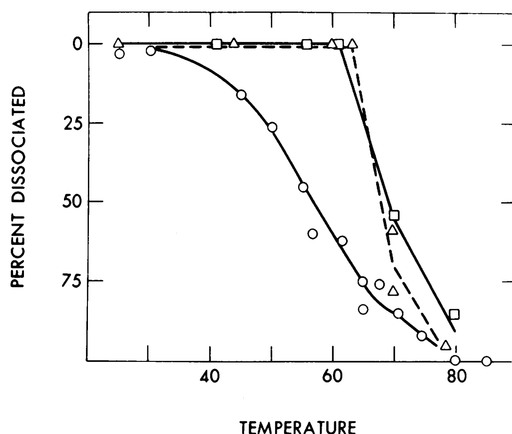

FIG. 2. *Selective release of primer from the 70S RNA of RSV. Denaturation of the complex between template and primer tagged with [32P]dAMP was analyzed as described for Fig. 1. In addition, samples of uniformly labeled [32P]70S RNA were heated at various temperatures as described for Fig. 1. Equal amounts of RNA from each sample were then tested as template for the purified DNA polymerase of RSV, and the remainder was analyzed by electrophoresis in composite gels of polyacrylamide identical to those illustrated in Fig. 1. The radioactivity migrating as 4S RNA was summed, normalized for the amount of material applied to the gel, and compared with the normalized amount of radioactivity recovered in the same region of the gel when 70S RNA was completely denatured by either boiling or treatment with dimethylsulfoxide (20). The data obtained from the experiment illustrated in Fig. 1 were subjected to the same computations and are plotted here. The template activities of various heated samples were compared with the activity of an unheated sample (20,000 counts/min of [3H]TMP incorporated into DNA in 1 h). The percentage decrease in template activity as a function of temperature is plotted on the same scale as the dissociation of the RNAs. Symbols: ○, 4S RNA; □, tagged primer; △, template activity.*

The bulk of 70Sa-4S RNA can be removed from high-molecular-weight subunits at a temperature (about 63 C) below the range of temperatures (65 to 80 C) required to disrupt the template-primer complex (Fig. 2). We therefore heated 70S RNA sequentially at 63 and 80 C, isolated the 4S RNAs released at each temperature, and analyzed these RNAs by two-dimensional electrophoresis in polyacrylamide (Fig. 3). The set of 4S RNAs released from RSV 70S RNA at 63 C was representative of total 70Sa-4S RNA (compare Fig. 3a and b), although the relative recovery of the rapidly migrating RNA which we have denoted "spot 1" (26) was greatly reduced (Fig. 3b). The remainder of spot 1 RNA (and only spot 1 RNA) was released from high-molecular-weight subunits during subsequent heating at 80 C (Fig. 3c). Each of the individual 4S RNAs identified by electrophoresis of total 70Sa-4S RNA (Fig. 3a) yielded a characteristic and unique set of oligonucleotides when hydrolyzed with T1 RNase (26); the 4S RNAs of spot 1 obtained at 63 and 80 C are identical by this criterion (unpublished observations and ref. 13).

We conclude that all but one of the 70Sa-4S RNAs (and 5S RNA as well) are released from 70S RNA at a temperature (63 C) which does not reduce template activity. By contrast, the bulk of the RNA contained in spot 1 can only be removed by conditions which disrupt the template-primer complex. It is therefore likely that the RNA of spot 1 includes primer molecules.

We also examined the electrophoretic mobilities of 70Sa-4S RNAs obtained after initiating DNA synthesis in the presence of araUTP (Fig. 4). The denatured RNAs were subjected to a single dimension of electrophoresis in 20% polyacrylamide, a procedure which permits direct comparison of several samples in the same gel. The 4S RNAs released at 63 and 80 C were analyzed separately, with results identical to those obtained with RNA which had not participated in a polymerase reaction (Fig. 4). As before, a single species of 4S RNA was obtained in the 80-C fraction, and the presence of deoxynucleotides on an appreciable portion of this RNA (about 50%; see ref. 13) had no effect on its electrophoretic mobility when compared with the 4S RNA released from 70S RNA in a

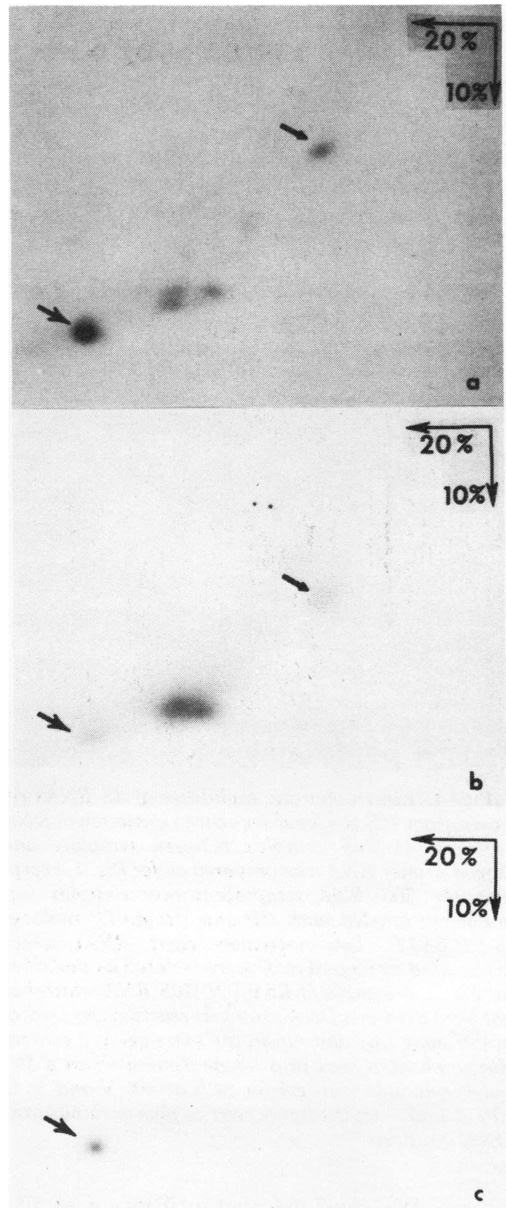

FIG. 3. *Analysis of low-molecular-weight RNAs by two-dimensional electrophoresis in polyacrylamide gels. RSV [³²P]70S RNA was heated for 2 min in 0.01 M EDTA-0.02 M Tris-hydrochloride, pH 7.4, at the indicated temperatures. Two-dimensional electrophoresis was performed as described under Materials and Methods. The first dimension (top to bottom in the figure) was in 10% polyacrylamide, the second dimension (right to left in the figure) was in 20% polyacrylamide. Only low-molecular-weight RNAs (7S and smaller) enter the 10% gel of the first dimension. In each panel, the upper, smaller arrow marks 5S RNA, and the lower, larger arrow marks "spot 1" 4S RNA. (a) Total 70S-associated 4-5S RNA, released by heating to 95 C; (b) low-molecular-weight (4-5S) RNA released from 70S RNA at 63 C and isolated by rate zonal centrifugation; (c) low-molecular-weight RNA released from high-molecular-weight subunits at 80 C; subunits were prepared by heating 70S RNA at 63 C and subsequent rate zonal centrifugation.*

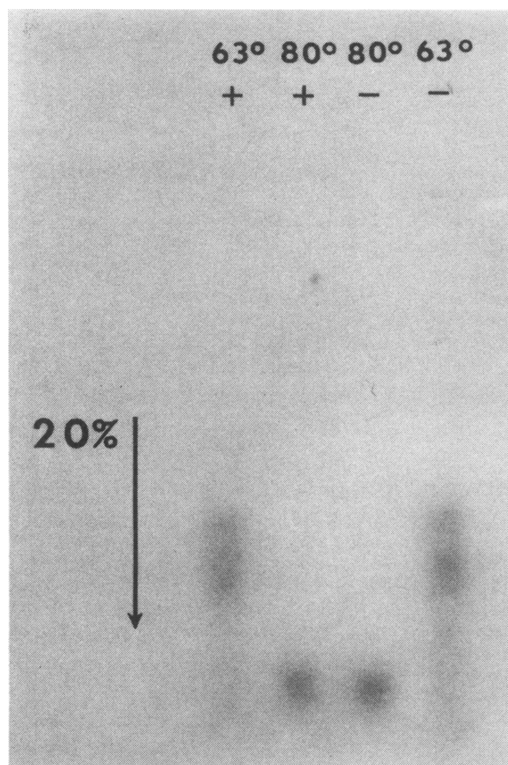

FIG. 4. *Electrophoretic mobilities of 4S RNAs released from 70S RNA subsequent to initiation of DNA synthesis. A 70S complex between template and tagged primer RNA was prepared as for Fig. 1, except that the 70S RNA template-primer complex was uniformly labeled with ^{32}P and [^{3}H]dATP replaced [α-^{32}P]dATP. Low-molecular-weight RNAs which dissociated at 63 and 80 C were isolated as described for Fig. 3. A sample of RSV [^{32}P]70S RNA which had not been used in a DNA synthesis reaction was treated in the same way and run in the same gel as a control. Electrophoresis was in a single dimension in a 20% polyacrylamide slab gel for 14 h at 400 V and 15 C. The + and − on the figure refer to plus or minus prior DNA synthesis.*

control. We conclude that utilization of 70S RNA as template for DNA polymerase and initiation of DNA synthesis on primer molecules has no demonstrable effect on either the thermal dissociation of the 70S complex or the electrophoretic mobilities of its low-molecular-weight constituents.

Identification of 4S RNAs which serve as primers for DNA synthesis. Primers were tagged with [^{32}P]dAMP in the presence of araUTP, released from 70S RNA by denaturation, and subjected to two-dimensional electrophoresis (Fig. 5). The bulk of the [^{32}P]deoxynucleotides was found in a single population which had the same electrophoretic mobility as the 70Sa-4S RNA of spot 1 (Fig. 5a). This conclusion was substantiated by demonstrating that the major species of tagged primer and the independently isolated 70Sa-4S RNA of spot 1 migrated as a single population when mixed and analyzed by electrophoresis in two dimensions (Fig. 5b).

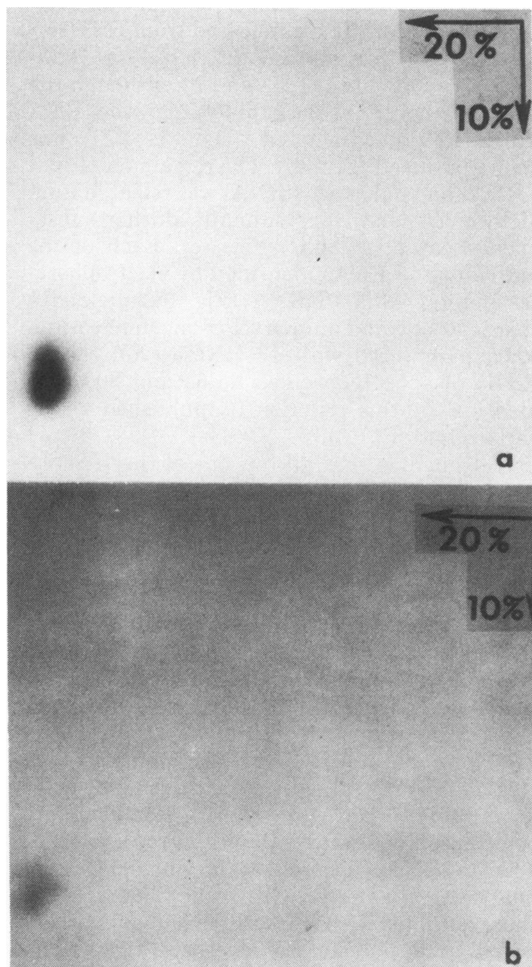

FIG. 5. *Two-dimensional electrophoresis of primer RNAs labeled with [^{32}P]dAMP. A 70S complex of template and tagged primer was prepared as described in Materials and Methods by using purified polymerase, unlabeled RSV 70S RNA (3 μg/ml), [α-^{32}P]dATP, and araUTP. (a) The isolated complex was denatured by treatment with 85% dimethylsulfoxide and subjected to two-dimensional electrophoresis as in Fig. 3. A sample of ^{32}P-labeled RSV 70S RNA was also denatured with dimethysulfoxide and analyzed in a separate gel. The results were identical to those in Fig. 3. (b) The RNA contained in spot 1 was eluted from the two gels, mixed in equal amounts (measured as counts per minute), and subjected to a second two-dimensional electrophoresis.*

Several other species of low-molecular-weight RNA were also tagged by [^{32}P]dAMP, albeit at a relatively low level (not visible in Fig. 5a). The extent of this tagging at various ratios of template to polymerase, as well as in a reaction carried out with detergent-activated virus, was analyzed by a single dimension of electrophoresis in 20% polyacrylamide (Fig. 6). Similar results were obtained with two-dimensional electrophoresis (data not shown). (The numbering system in Fig. 6 does not conform to previous numberings of two-dimensional gels, and the several species of RNA migrating more rapidly than spot 1 in Fig. 6 have not been previously identified.) A minimum of five distinct RNAs were tagged, with spot 1 constituting the bulk of the primer (85 to 99%) (Table 1) in reactions with either disrupted virions or purified polymerase, and irrespective of the ratio template to polymerase (Table 1). The conclusions were based on the assumption that all of the primer RNAs are tagged by approximately the same lengths of DNA. We validated this assumption by analyzing the DNA attached to each of the tagged primers illustrated in Fig. 6. The nucleic acids were eluted from individual spots and hydrolyzed with alkali, and the [^{32}P]DNAs were subjected to sequential electrophoresis at pH 3.5 on Whatman 540 and DE 81 papers. Each of the primer species released pdA, pdApdA, and pdApdAparaU in approximately the same proportions as those found with unfractionated primer (unpublished data). Thus, the predominant labeling of spot 1 RNA cannot be attributed to a longer chain of nascent DNA.

We conclude that DNA synthesis in vitro with RSV 70*S* RNA as template for the DNA polymerase of the virus initiates predominately on a single species of the 70*S*a-4*S* RNAs. Several other low-molecular-weight RNAs also serve infrequently as primers, but the amounts of these RNAs are inadequate to permit further characterization at this time.

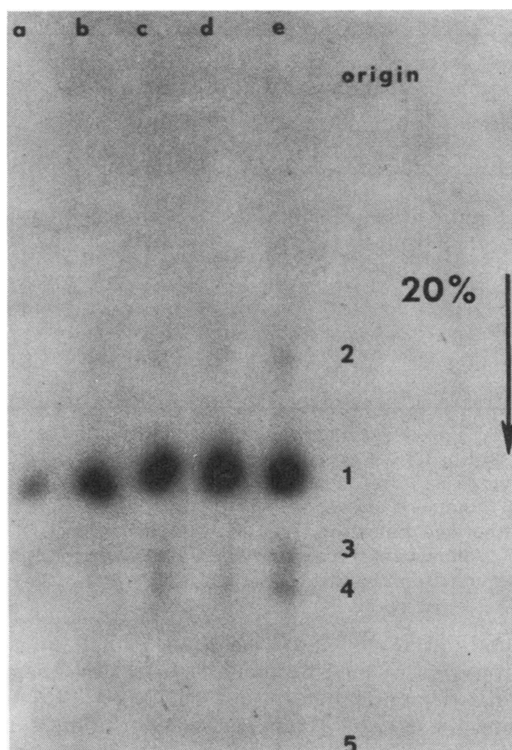

FIG. 6. *Utilization of 4S RNAs as primers for DNA synthesis at different ratios of enzyme to template. Enzymatic reactions (1 h) were carried out with purified polymerase as described in Materials and Methods by using [α-^{32}P]dATP, araUTP, and varying amounts of unlabeled RSV 70S RNA as template. An identical reaction was also carried out with detergent-disrupted virions (200 μg of protein/ml) in place of the purified polymerase and RNA template. The 70S complexes between template and [^{32}P]dAMP-tagged primer were isolated by rate zonal centrifugation, denatured by treatment with dimethylsulfoxide, and analyzed by a single dimension of electrophoresis in 20% polyacrylamide (400 V, 14 h, 15 C). The samples analyzed were as follows: (a–d) Primer tagged in a reaction with purified polymerase, [α^{32}P]dATP, araUTP, and unlabeled 70S RNA as template at 1, 9, 27, and 3 μg/ml, respectively. The preparation of polymerase used here was saturated by template at about 6 μg of RNA per ml; i.e., the rate of DNA synthesis was unchanged at all higher concentrations of template with the polymerase at the usual concentration of 0.2 U/ml (15). (e) Primer tagged with [^{32}P]dAMP in a reaction using detergent-activated virions of RSV, [α-^{32}P]dATP, and araUTP.*

DISCUSSION

These data confirm and extend previous demonstrations that 4*S* RNA serves as a primer for the synthesis of DNA in vitro with RSV 70*S* RNA as template (4, 14). The bulk of this primer (85 to 99%) migrates as a single population in two-dimensional gels of polyacrylamide and is homogeneous as judged by analysis of its nucleotide composition and sequence (13). This RNA serves as the principal primer for DNA synthesis by both virions disrupted with Nonidet-P40 and purified DNA polymerase of RSV with 70*S* RNA as template. We have not identified the primer(s) used in either virions disrupted by other means (eg., ether; cf. ref. 16) or virions newly released from infected cells (5, 6, 16).

Several other species of low-molecular-weight RNA also serve as primers (Fig. 6). These RNAs are responsible for only a small fraction of the

TABLE 1. *Utilization of 4S primers in Rous sarcoma virus*[a]

Sample	Method of analysis[b]	Percent of total counts/min recovered from spot number:					Total counts/ min recovered from gel[c]
		1	2	3	4	5	
Detergent-activated virions	1-D	88	2	5	5		1355
	2-D	97					114
Purified polymerase with 70*S* RNA template (μg/ml)							
1	1-D	85		10			125
	2-D	89	2	5	4		645
3	1-D	97	1		2		1484
9	1-D	94	3	2	1		748
	2-D	85	5	7	3		1047
27	1-D	91	2	4	3		1406

[a] Pieces of gel containing the spots shown in Fig. 6 were cut out, and their radioactivity was determined by counting Cerenkov radiation for 10 min. A background value of 23 counts/min was subtracted from the data in every case. Negligible radioactivity is denoted by a minus sign.

[b] Radioactively tagged [^{32}P]dAMP primers were fractionated by electrophoresis through polyacrylamide in either one dimension (1-D) or two dimensions (2-D), as illustrated in Fig. 6 and 5, respectively.

[c] Amounts of radioactivity in different samples were not constant. This accounts for the variation in counts per minute recovered.

total initiations, and occur in such small quantities that we have been unable to identify them with demonstrable species of 70*S*a-4*S* RNA. The role of either the major or minor primers in transcription of the RSV genome during the infectious cycle cannot be ascertained from presently available data.

The major primer fraction in vitro (i.e., spot 1 RNA) constitutes approximately 15 to 30% of the total 70*S*a-4*S* RNA (26), or an average of two to four primer molecules per viral genome (12, 25, and present data). The DNA polymerase of RSV can transcribe the entire viral genome into DNA chains which are only 100 to 500 nucleotides long (8, 19). This is true of both detergent-activated virions (which may or may not contain DNase activity; see ref. 22 and 23) and purified polymerase free of DNase (2), and implies that DNA synthesis initiates at a large number of sites on the RNA template. Thus, there appear to be insufficient primers on any single 70*S* molecule to provide the required number of initiation sites. We cannot presently resolve this paradox.

We identify primer molecules by virtue of their covalent attachment to deoxynucleotides. The terminal addition of tags up to three nucleotides in length has no apparent effect on either the electrophoretic mobility of 4*S* RNA or its selective release from 70*S* RNA by thermal denaturation. It should therefore be possible to use tagging with the appropriate radioactive deoxynucleotide in combination with two-dimensional electrophoresis to identify primer molecules from other strains of RNA tumor viruses.

There are now three means by which to purify RNA primer from 70*S* RNA of RSV: direct isolation from covalent complexes with nascent DNA (14), selective release from 70*S* RNA by thermal denaturation (Fig. 2), and two-dimensional electrophoresis in polyacrylamide after denaturation (Fig. 5). By analysis of nucleotide composition and primary structure, we have determined that the 4*S* primer RNAs prepared by each of these procedures are homogeneous and similar if not identical to each other (13). These observations validate the use of the relatively convenient second and third procedures for the preparation of primer RNA in bulk for further characterization.

ACKNOWLEDGMENTS

We thank L. Levintow and H. Temin for editorial assistance, and J. Jackson, K. Smith, B. Evans, S. Heasley, and B. P. Stark for excellent technical assistance.

This study was supported by grants from the Public Health Service (CA 12380, CA 12705, and CA 14026 from the National Cancer Institute; and AI 06862, AI 00299, and AI 08804 from the National Institute of Allergy and Infectious Diseases), the National Science Foundation (GB-32152X), the American Cancer Society (VC-70 and NP-112A), and by contract number NO1 CP 33293 within The Virus Cancer Program of the National Cancer Institute. J. E. D. has Public Health Service research career development award K04-GM-32770 from the National Institute of General Medical Sciences. J. M. T. acknowledges the support by Senior Dernham Fellowship (D-201) of the American Cancer Society, California Division. R. C. S. is supported by Public Health Service training grant 5-T01-GM-00302-14 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Baltimore, D. 1970. RNA-dependent DNA polymerase in virions of RNA tumour viruses. Nature (London) **226**:1209-1211.
2. Bishop, J. M., C. T. Deng, A. J. Faras, H. M. Goodman, W. E. Levinson, J. M. Taylor, and H. E. Varmus. 1973. Transcription of the Rous sarcoma virus genome by RNA-directed DNA polymerase. *In* C. F. Fox (ed.), Virus research, proceedings of second ICN-UCLA symposium.
3. Bishop, J. M., W. Levinson, N. Quintrell, L. Fanshier, and J. Jackson. 1970. The low molecular weight RNA's of Rous sarcoma virus. I. The 4S RNA. Virology **42**:182-195.
4. Canaani, E., and P. H. Duesberg. 1972. Role of subunits of 60 to 70*S* avian tumor virus ribonucleic acid in its template activity for the viral deoxyribonucleic acid polymerase. J. Virol. **10**:23-31.
5. Canaani, E., K. V. D. Helm, and P. Duesberg. 1973. Evidence for 30-40S RNA as precursor of the 60-70S RNA of Rous sarcoma virus. Proc. Nat. Acad. Sci. U.S.A. **72**:401-405.
6. Cheung, K.-S., R. E. Smith, M. P. Stone, and W. K. Joklik. 1972. Comparison of immature (rapid harvest) and mature Rous sarcoma virus particles. Virology **50**:851-864.
7. Duesberg, P. H. 1968. Physical properties of Rous sarcoma virus RNA. Proc. Nat. Acad. Sci. U.S.A. **60**:1511-1518.
8. Duesberg, P. H., and E. Canaani. 1970. Complementarity between Rous sarcoma virus RNA and the *in vitro* synthesized DNA of the virus-associated DNA polymerase. Virology **42**:783-788.
9. Duesberg, P. H., K. V. D. Helm, and E. Canaani. 1971. Comparative properties of RNA and DNA templates for the DNA polymerase of Rous sarcoma virus. Proc. Nat. Acad. Sci. U.S.A. **68**:2505-2509.
10. Erikson, E., and Erikson, R. L. (1971) Association of 4*S*-ribonucleic acid with oncornavirus ribonucleic acids. J. Virol. **8**:254-256.
11. Erikson, R. L. 1969. Studies on the RNA from avian myeloblastosis virus. Virology **37**:124-131.
12. Faras, A. J., A. C. Garapin, J. M. Bishop, and H. Goodman. 1973. The characterization of low-molecular-weight RNAs associated with 70*S* RNA of Rous sarcoma virus. J. Virol. **12**:334-342.
13. Faras, A. J., J. E. Dahlberg, R. C. Sawyer, F. Harada, J. M. Taylor, I. M. Bishop, and H. M. Goodman. 1974. Transcription of DNA from the 70*S* RNA of Rous sarcoma virus. II. Structure of a 4*S* RNA primer. J. Virol. **13**:1134-1142.
14. Faras, A. J., J. M. Taylor, W. Levinson, H. Goodman, and J. M. Bishop. 1973. RNA-directed DNA polymerase of Rous sarcoma virus: initiation of synthesis with 70S viral RNA as template. J. Mol. Biol. **79**:163-183.
15. Faras, A. J., J. M. Taylor, J. McDonnell, W. Levinson, and J. M. Bishop. 1972. Purification and characterization of the DNA polymerase associated with RSV. Biochemistry **11**:2334-2342.
16. Flügel, R. M., U. Rapp, and R. D. Wells. 1973. RNA-DNA covalent bonds between the RNA primers and the DNA products formed by RNA tumor virus DNA polymerase. J. Virol. **12**:1491-1502.
17. Flügel, R. M., and R. D. Wells. 1972. Nucleotides at the RNA-DNA covalent bonds formed in the endogenous reaction by the avian myeloblastosis virus DNA polymerase. Virology **48**:394-401.
18. Garapin, A. C., J. P. McDonnell, W. E. Levinson, N. Quintrell, L. Fanshier, and J. M. Bishop. 1970. Deoxyribonucleic acid polymerase associated with Rous sarcoma virus and avian myeloblastosis virus: properties of the enzyme and its product. J. Virol. **6**:589-598.
19. Garapin, A. C., H. E. Varmus, A. Faras, W. E. Levinson, and J. M. Bishop. (1973) RNA-directed DNA synthesis by virions of Rous sarcoma virus: further characterization of the templates and the extent of their transcription. Virology **52**:264-274.
20. Ikemura, T., and J. E. Dahlberg. 1973. Small RNAs of *E. coli*. I. Characterization by polyacrylamide gel electrophoresis and fingerprint analysis. J. Biol. Chem. **248**:5024-5032.
21. Katz, L., and S. Penman. 1967. The influence of coprecipitants on the solvent-denaturation of double-stranded RNA. Biochem. Biophys. Res. Commun. **27**:456-461.
22. Mizutani, S., S. Boettiger, and H. M. Temin, 1970. A DNA-dependent DNA polymerase and a DNA endonuclease in virions of Rous sarcoma virus. Nature (London) **228**:424-427.
23. Quintrell, N., L. Fanshier, B. Evans, W. E. Levinson, and J. M. Bishop. 1971. The DNA polymerase(s) of Rous sarcoma virus: effects of virion-associated endonuclease on the enzymatic product. J. Virol. **8**:17-27.
24. Rosenthal, L. J., and P. C. Zamecnik. 1973. Amino-acid acceptor activity of the "70S-associated" 4S RNA from avian myeloblastosis virus. Proc. Nat. Acad. Sci. U.S.A. **70**:1184-1185.
25. Rosenthal, L. J., and P. C. Zamecnik. 1973. Minor base composition of "70S-associated" 4S RNA from avian myeloblastosis virus. Proc. Nat. Acad. Sci. U.S.A. **70**:865-869.
26. Sawyer, R. C., and J. E. Dahlberg. 1973. Small RNAs of Rous sarcoma virus: characterization by two-dimensional polyacrylamide gel electrophoresis and fingerprint analysis. J. Virol. **12**:1226-1237.
27. Spiegelman, S., A. Burny, M. B. Das, J. Keydar, J. Schlom, M. Travnicek, and K. Watson. 1970. Characterization of the products of RNA-directed DNA polymerases in oncogenic RNA viruses. Nature (London) **227**:563-567.
28. Symons, R. H. 1966. A rapid and improved method for the synthesis of ^{32}P-labeled ribonucleoside 5'-monophosphates. Biochem. Biophys. Res. Commun. **24**:872-876.
29. Taylor, J. M., A. J. Faras, H. E. Varmus, H. M. Goodman, W. E. Levinson, and J. M. Bishop. 1973. Transcription of RNA by the RNA-directed DNA polymerase of Rous sarcoma virus and DNA polymerase I of *E. coli*. Biochemistry **12**:460-467.
30. Temin, H. M., and S. Mizutani. 1971. RNA-dependent DNA polymerase in virions of Rous sarcoma virus. Nature (London) **226**:1211-1213.
31. Verma, I. M., N. L. Meuth, and D. Baltimore. 1972. Convalent linkage between ribonucleic acid primer and deoxyribonucleic acid product of the avian myeloblastosis virus deoxyribonucleic acid polymerase. J. Virol. **10**:622-627.