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In vitro synthesis of full-length DNA transcripts of Rous sarcoma virus RNA by viral DNA polymerase

(Rous virus DNA polymerase/optimal Triton X-100 activation/formamide electrophoresis/alkaline sucrose gradients/RNA·DNA hybridization)

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ABSTRACT **Varying the concentration of Triton X-100, a nonionic detergent used to promote the DNA polymerase activity of Rous sarcoma virus in an endogenous reaction, showed a very sharp peak at about 0.02% (vol/vol) for optimal DNA synthesis. The yield of DNA at this concentration of Triton exceeded yields obtained at concentrations above the optimum by a factor of 2–5 for the 90-min reaction. At optimal Triton concentration, about 1–7% of the DNA made in the absence of actinomycin and about 4–10% of the DNA made in the presence of actinomycin was 2.5 × 10^6 daltons or greater, as estimated by formamide polyacrylamide gel electrophoresis and by alkaline sucrose gradient sedimentation. No large DNA was obtained at higher than optimal Triton concentrations. The large DNA molecules were rendered totally resistant to single-strand specific nuclease S1 after hybridization to an excess of viral RNA. It was concluded that at optimal detergent concentration, the viral DNA polymerase can synthesize full-size DNA transcripts of viral RNA.**

Avian tumor viruses contain 60–70S RNA, which consists predominantly of two 35S RNA subunits (1) with an approximate molecular mass of 3 × 10^6 daltons (2). The mass of avian tumor virus RNA has been estimated on the basis of genetic (3) and biochemical studies (4–7) to be around 3 × 10^6 daltons, implying that the 60–70S RNA is diploid, consisting of two very similar or identical subunits. In agreement with this, the minimal size of infectious proviral DNA, recovered from virus infected cells, was determined to be about 6 × 10^6 daltons (8). *In vitro* transcription of viral RNA by viral DNA polymerase was reported in many laboratories to lead only to short DNA transcripts ranging up to 200,000 daltons in size (9–11). Nevertheless the DNA made *in vitro* in an endogenous reaction [i.e., detergent-disrupted virus (9, 10)] includes all sequences of viral RNA, because it was shown to render viral RNA completely resistant to RNase after hybridization with excess DNA (11, 12). It would appear then that in the conditions described above, all sequences of viral RNA were transcribed to DNA by viral DNA polymerase although transcription of all viral RNA sequences into a continuous DNA strand of about 3 × 10^6 daltons was not observed.

The difficulties in obtaining full-length viral DNA transcripts *in vitro* may have several sources: (*i*) the detergents necessary to promote virion-associated DNA polymerase activity (9) may also permit virus penetration by virus-associated nucleases. Such nucleases have been shown to degrade the viral RNA template (13–16) and may also degrade DNA synthesized *in vitro*. (*ii*) The majority of the RNA molecules of conventionally prepared virus, which is harvested from infected cultures at 12- to 24-hr intervals was shown to be fragmented (17–19). Small RNA present in virus stocks would yield only short DNA molecules as transcripts. (*iii*) It is also conceivable that the viral DNA polymerase is not the sole catalyst to transcribe viral RNA to DNA and that cellular helper factors are required to obtain a full-length DNA transcript from 35S viral RNA (9).

To decide among these alternatives we have varied *in vitro* conditions of transcription of viral RNA by virus-associated DNA polymerase to improve the yield and size of the DNA product. A critical variable was found to be the concentration of the detergent, Triton X-100, necessary for expression of viral DNA synthesis. In optimal conditions an amount of DNA of the order of the weight of the RNA template was synthesized. About 1–10% of this DNA represented molecules with molecular masses between 2.5 and 3.5 × 10^6 daltons which were complementary to viral RNA. It was concluded that under optimal conditions the viral DNA polymerase can transcribe 35S viral RNA into a continuous DNA molecule of the same size.

MATERIALS AND METHODS

Cells and Virus. All virus was propagated on SPAFAS Co. gs^-chf^- chick embryo fibroblast cells. Cloned Prague Rous sarcoma virus (RSV) of subgroup B was used. Virus was harvested from infected cultures at intervals of 3 hr, purified according to published procedures (3, 22), and stored at 4° in 0.1 M NaCl, 0.01 M Tris·HCl (pH 7.4), 1 mM EDTA, 30% glycerol. Virus stored in this manner showed stable polymerase activity over a period of 6 months. The 35S RNA species of all virus preparations used were of size class *a*, which is typical of nondefective sarcoma virus free of contaminating leukosis virus (3).

Synthesis of Viral DNA. RSV was incubated for 18 hr at 41° in 4 ml of a mixture containing 10^{-4} M dATP, dGTP; 10^{-5} M dTTP, dCTP; 2 mM magnesium acetate; 30 mM dithiothreitol; 0.1 M Tris·HCl pH 7.6; 0.0225% Triton X-100; 0.825 μM [^{3}H]dTTP (20 Ci/mmol; New England Nuclear). The DNA made was calculated to have a specific activity of 200,000 cpm/μg. A parallel reaction was carried out in the presence of actinomycin D (gift of Merck, Sharpe, and Dohme) at 100 μg/ml. The reactions were terminated by the addition of EDTA to 15 mM, sodium dodecyl sulfate to 1%, NaCl to 0.1 M, and mercaptoethanol to 2%, and 80 μg of denatured salmon sperm DNA was added as carrier. The mixture was extracted twice with phenol and precipitated twice with ethanol. The product was then incubated with RNase A (Worthington) at 100 μg/ml for 1 hr at 37° in 5 mM Tris·HCl pH 7.4 and 0.5 mM EDTA to digest RNA from RNA·DNA hybrids (20), extracted twice with phenol and precipitated twice with ethanol. About 1.25 mg of viral protein, measured by the Lowry test (21), was used in each preparation. This corresponds to about 50 μg of viral RNA, as estimated from the known composition of RSV. The virus

Abbreviation: RSV, Rous sarcoma virus.

contains 60% protein and about 2% RNA (22) and was observed to have an A_{260}/A_{280} ratio of 1.05 in sodium dodecyl sulfate at pH 7.0, which is consistent with a protein/RNA ratio of 25:1 (23).

RESULTS

Viral DNA Synthesis as a Function of Different Concentrations of Triton X-100. Since both the viral DNA polymerase and the virus-associated RNase require detergent to become active on the viral RNA, it appeared possible that by varying the concentration of the detergent a condition could be found that favors the action of the polymerase over that of the RNase. Fig. 1 shows that increasing the Triton X-100 concentration for a given amount of virus increases the yield of DNA synthesized *in vitro* until it reaches a narrow peak at about 0.02%. At higher concentrations of detergent, DNA synthesis declines to a broad plateau of about half-maximal activity that extends to 0.5% Triton X-100. Under conditions more optimal for DNA synthesis (50 μM dTTP, 2 mM Mg^{++}, and 15 mM dithiothreitol; else as in Fig. 1), the difference between peak and plateau levels approached 5-fold (not shown). Narrow detergent optima have been shown previously for the endogenous polymerase activity of murine RNA tumor viruses (24, 25), but avian viruses have in the past been reported to have relatively broad optima with this variable (26, 27). The Triton X-100 concentration necessary to obtain maximal DNA synthesis increased linearly, but nonstoichiometrically with the concentration of virus used (Fig. 2).

Some characteristics of long-term (18 hr) viral DNA synthesis at optimal detergent concentrations are summarized in Table 1. Up to 17 μg of viral DNA was made in a reaction mixture containing about 50 μg of viral RNA with limiting concentration of dNTP at 10^{-5} M. With all four dNTPs at 10^{-4} M, synthesis levels were observed to increase 2- to 3-fold for the long-term reaction and a further 1.5-fold with all dNTPs at 10^{-3} M (data not shown). In accord with the results of others (12), the presence of actinomycin reduced the yield of DNA 3- to 4-fold (Table 1). The DNA made in the absence of actinomycin was over 60% double-stranded, as determined by its resistance to the single-strand specific nuclease S1, whereas the DNA made in the presence of actinomycin was less than 20% double-stranded (Table 1). Higher nucleotide concentrations were found to reduce the effectiveness of the actinomycin-mediated suppression of second (+) strand DNA synthesis, which results in higher fractions of double-stranded DNA (data not shown).

Table 1. DNA product of RSV endogenous reaction

	Preparation 1	Preparation 2
Actinomycin (100 μg/ml)	−	+
Synthesis	17 μg	5 μg
Recovery	12 μg (70%)	3 μg (60%)
Double-strandedness (S1 DNase resistance)	63%	19%

DNA syntheses in the absence and presence of actinomycin were with 1.25 mg of viral protein or about 50 μg of viral RNA (see *Materials and Methods*). Recoveries after purification were determined as described in *Materials and Methods*. Resistance to nuclease S1 was determined by incubating an aliquot of the [^{3}H]DNA product in 0.5 ml, 1 mM $ZnSO_4$, 0.1 M Na acetate, pH 4.5, for 1 hr at 37° with 2000 units of S1 nuclease (28) and 5 μg of denatured salmon sperm DNA. S1 nuclease-resistant DNA was recovered as trichloroacetic acid-precipitable material on Millipore filters, and its radioactivity determined in a scintillation counter. Backgrounds of about 1% resistance of alkali-denatured DNA were subtracted.

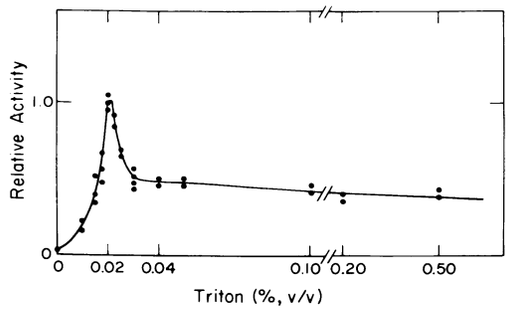

FIG. 1. DNA synthesis as a function of Triton concentration. DNA synthesis was with 10 μg of viral protein in 100-μl volume with 5 mM dithiothreitol, 5 mM magnesium acetate, dTTP as [^{3}H]dTTP at 2 μM, dCTP, dATP, and dGTP at 10^{-4} M, 0.1 M Tris, pH 8.0. The amount of Triton X-100 used is indicated in the figure. Incubation was at 37° for 90 min. (Synthesis was linear for 180 min and continued at high rates for over 24 hr.) Yields of DNA were determined as trichloroacetic acid-precipitable radioactivity.

Size Analysis of the DNA Products. The size of DNA products was determined under denaturing conditions by formamide-polyacrylamide gel electrophoresis and by alkaline sucrose gradient sedimentation. Electrophoretic analyses of DNA made at optimal Triton concentration in the absence and in the presence of actinomycin are shown in Figs. 3A and 3B, respectively. There was a relatively sharp upper size limit in the same positions of two parallel gels for the DNAs made in the absence (Fig. 3A) and in the presence of actinomycin (Fig. 3B). The largest DNA made in the absence of actinomycin had the same electrophoretic mobility as a 35S RSV [^{32}P]RNA standard, with an approximate molecular weight of 3×10^6. Calibration experiments were performed in parallel gels with RSV RNA coelectrophoresed with single-stranded DNA standards consisting of phage P4 DNA and specific fragments of P4 DNA derived by cleavage with *E. coli* restriction endonuclease RI (inset, Fig. 3A). The electrophoretic positions of the largest RSV DNA molecules synthesized *in vitro* and of the 35S RSV RNA marker coincided with that of authentic single-stranded DNA molecules of about 3- to 3.5×10^6 daltons. (Calibrations in this region of the gel are good only to about $\pm 0.3 \times 10^6$ daltons).

Fig. 4 compares the size distributions of DNA made at optimal Triton concentration (0.0225%, Fig. 4A) to that made

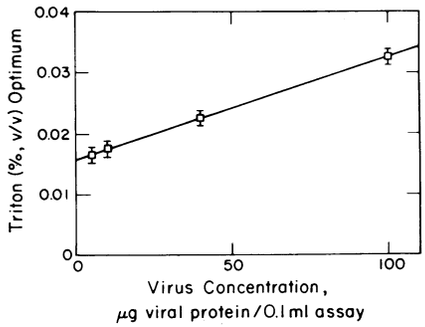

FIG. 2. Triton optimum as a function of virus concentration. DNA syntheses were as in legend of Fig. 1 except that the reaction mixtures contained 15 mM dithiothreitol, 2 mM magnesium acetate, and 50 μM dTTP, plus the indicated concentrations of virus and Triton X-100. Vertical error bars represent $\pm 0.00125\%$ Triton, the accuracy with which optima were determined.

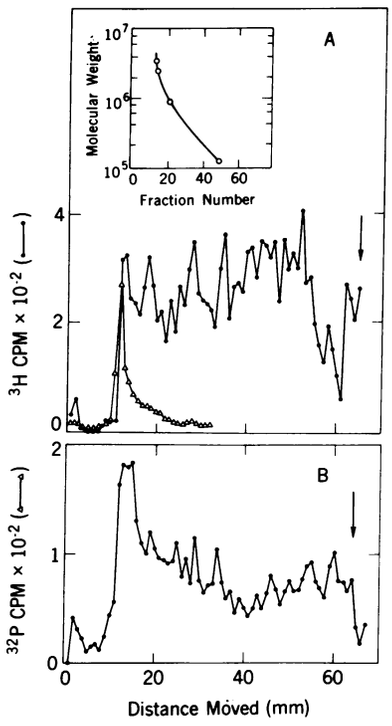

FIG. 3. Polyacrylamide gel electrophoresis of product DNAs in the presence of formamide. Electrophoresis was in 3.0% polyacrylamide gels 7 cm long containing 98% formamide for 3.5 hr at 100 V as described (2), except that 2,3-dibromo-1-propanol (d^{25} = 2.13) instead of glycerol was used to increase sample density. Electrophoresis was from left to right. (A) ●, −actinomycin [^{3}H]DNA (preparation 1, Table 1); △, RSV [^{32}P]RNA. (B) ●, +actinomycin [^{3}H]DNA (preparation 2, Table 1). Inset: The mobility-molecular weight relation was established in parallel experiments with 35S RSV RNA electrophoresed against P4 phage DNA or fragments of P4 phage DNA generated by the *E. coli* restriction endonuclease RI. The open circles (inset) indicate the positions of single-stranded P4 DNA or P4 DNA single-stranded fragments with molecular weights from left to right of 3.63, 2.48, 0.900, and 0.116 million (30). Arrows indicate positions of bromphenol blue dye markers.

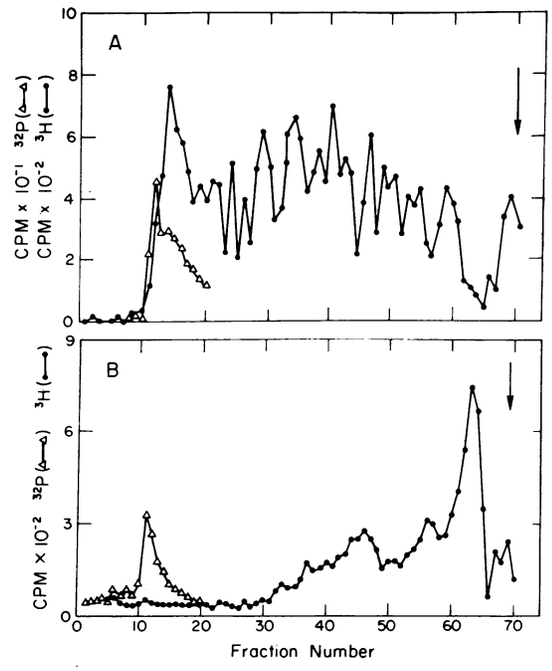

FIG. 4. Size analysis by formamide-polyacrylamide gel electrophoresis of DNA products made in endogenous reactions at optimal and higher than optimal Triton concentration. Virus at 30 μg of viral protein was incubated in the absence of actinomycin for 8 hr at 41° as described in *Materials and Methods*, except that all dNTPs were at 10^{-4} M. The Triton optimum was 0.0225% (see Fig. 2). DNA purification and electrophoresis were as in *Materials and Methods*. (A) DNA synthesized in the presence of 0.0225% Triton. (B) DNA synthesized in the presence of 0.1% Triton. ●, [^{3}H]DNA product; △, 35S RSV [^{32}P]RNA. The arrow indicates the position of a bromphenol blue dye marker.

at higher than optimal (0.1%, Fig. 4B) Triton concentration. The results indicate that only the reaction at optimal Triton concentration produced DNA molecules that comigrated with 35S RSV [^{32}P]RNA in formamide-polyacrylamide gels, whereas a parallel reaction at high Triton concentration did not produce any DNA molecules in the size range of viral RNA. It follows that Triton concentration is an important variable in synthesis of large DNA molecules in an endogenous reaction.

We also note by comparison of Figs. 3A and 4A that a 10-fold increase in limiting nucleotide concentration from 10^{-5} M to 10^{-4} M had relatively little effect on the size distribution of products when Triton is maintained at its optimum, and similar conclusions were derived from experiments with all dNTPs at 10^{-3} M. However, higher nucleotide levels did result in increased total yields of all DNA species synthesized up to a factor of 4 (see above).

Alkaline sucrose gradient sedimentation of a DNA preparation made at optimal Triton concentration in the absence of actinomycin, but in the same method as Preparation 1 of Table 1, is shown in Fig. 5A. About 2.5% of this DNA was 2.5 × 10^6 daltons or greater in size. It can be seen in Fig. 5A that most of this DNA was small, which is in agreement with the electrophoretic data of Fig. 3. However, some molecules sedimented faster than the bulk of the material, ahead of a single-stranded SV40 DNA marker (1.65 × 10^6 daltons) and up to the position of a phage P4 DNA marker (3.63 × 10^6 daltons) (Fig. 5B). Points corresponding to the larger DNA are replotted on a 5× scale, with arrows designating approximate molecular weight positions.

To confirm more directly the existence of large DNA molecules, DNA that was estimated to be approximately 2.5 × 10^6 daltons or greater was isolated from the preparative gradient of Fig. 5A and was subjected to further analysis. One aliquot of this DNA was resedimented in alkali with the same markers as in Fig. 5B. It can be seen to have retained

Table 2. Size distribution of large DNA made in the presence or absence of actinomycin (AM) according to method of analysis*

	Size				
	≥2.5 × 10^6		≥1.0 × 10^6		
	cpm	% Total	cpm	% Total	Total cpm
−AM DNA†					
Gel electrophoresis	710	5.1	2,685	19.1	14,050
Sedimentation	4,457	3.7	14,166	11.5	122,788
+AM DNA†					
Gel electrophoresis	480	10.1	1,504	31.7	4,749

* Data for the electrophoresis are derived from Fig. 3; sedimentation data not shown, but performed as in Fig. 5B. The DNAs in this set of analyses were those of preparations 1 and 2 (Table 1).

† −AM, +AM DNA is DNA made in the absence or presence of actinomycin, respectively.

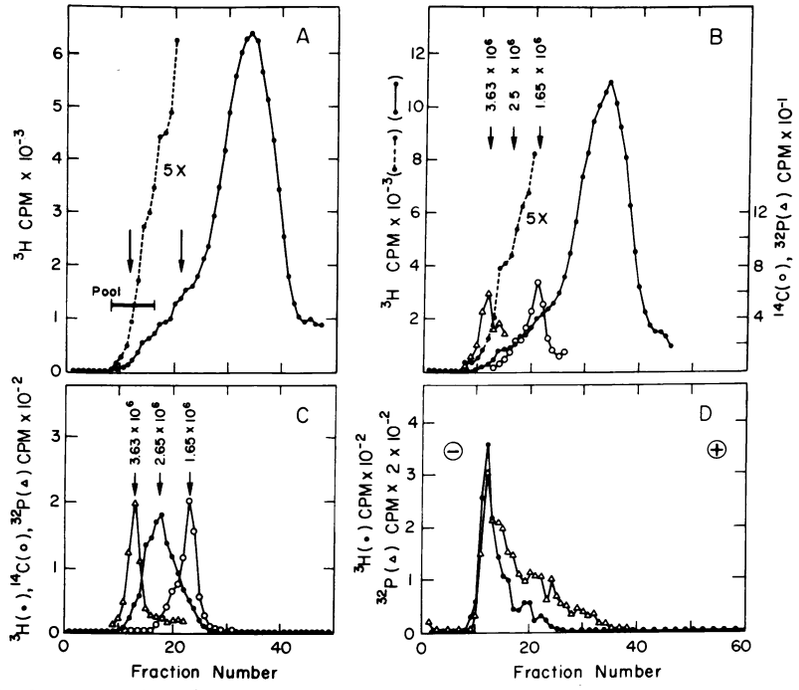

FIG. 5. Isolation and hydrodynamic and electrophoretic characterization of large DNA transcripts from reaction products *in vitro*. (A) DNA was prepared in the absence of actinomycin as described for Table 1. Sedimentation was performed in 5–20% sucrose gradients containing 0.33 M NaOH, 0.5 M NaCl, and 0.02 M EDTA for 10 hr at 40,000 rpm in an SW41 rotor without DNA markers. Molecular weight positions of phage P4 and SV40 DNAs which were centrifuged in parallel (arrows) are estimated from the data of (B). Fractions 8 through 16, indicated by horizontal bar, were combined as molecules of approximately 2.5 × 10^6 daltons and greater. This pooled material was used for analysis in (C) and (D). ●—●, RSV [^{3}H]DNA; ●--●, 5× replot of the RSV [^{3}H]DNA data. (B) Sedimentation was with DNA of the same preparation used in (A) except that P4 [^{32}P] and SV40 [^{14}C]DNA markers were included in the gradient. ●—●, RSV [^{3}H]DNA; ○, SV40 linear [^{14}C]DNA. [1.65 × 10^6 daltons is used as marker position for SV40 (34). The leading shoulder is contaminating single-stranded SV40 DNA circles.] △, P4 [^{32}P]DNA (3.63 × 10^6 molecular weight). ●--●, 5× replot of the RSV [^{3}H]DNA data. (C) DNA from the combined fractions of (A) was resedimented in alkali under the same conditions, except with DNA markers included in the gradient. ●—●, RSV [^{3}H]DNA; ○, SV40 [^{14}C]DNA; △, P4 [^{32}P]DNA. (D) DNA from the combined fractions of (A) was electrophoresed in formamide-polyacrylamide gels in the presence of RSV [^{32}P]DNA as marker. Conditions as in Fig. 3. ●—●. RSV [^{3}H]DNA; △, RSV [^{32}P]RNA.

its high sedimentation coefficient on resedimentation, and to have formed a peak at about 2.65 × 10^6 daltons (Fig. 5C). A second aliquot was electrophoresed in formamide in the presence of a 35S RSV [^{32}P]RNA marker. The peak position of DNA in the gel was 3 to 3.5 × 10^6 daltons and coincided with that of the marker RNA (Fig. 5D). The weight average of the gel profile was about 2.5 × 10^6 daltons, as with the sedimentation profile.

Of the two preparations listed in Table 1, approximately 3–5% of the DNA made in the absence of actinomycin was 2.5 × 10^6 daltons or greater in size and 12–19% of this DNA was greater than 1 × 10^6 daltons (Table 2). About 10% of the DNA made in the presence of actinomycin (Table 1) was 2.5 × 10^6 daltons or larger, and 30% was over 1 × 10^6 daltons in size (Table 2). The range of values for DNA greater than or equal to 2.5 × 10^6 daltons from several preparations were 1–7% for the DNA in the absence of and 4–10% for the DNA in the presence of actinomycin. Although the proportion of DNA ≥ 2.5 × 10^6 daltons was greater (Table 2), net synthesis of the largest DNA species was reduced by the presence of actinomycin since total DNA synthesis was decreased nearly 4-fold (Table 1).

Hybridization of the Large DNA, Synthesized *In Vitro*, with RSV RNA. It may be argued that the relatively low percentage of large DNA molecules synthesized under our conditions represents cellular DNA contaminants that may have functioned as templates in the endogenous reaction. A low level of such large DNA contaminants has been observed electron microscopically in purified virus preparations (29; H.-J. Kung and N. Davidson, personal communication). To test directly whether our large DNA was transcribed from viral RNA, a further aliquot of the DNA greater than or equal to 2.5 × 10^6 daltons isolated from the gradient of Fig. 5A was incubated under hybridization conditions with 35S RSV RNA. The data of Table 3 show that the large RSV DNA was 100% protected by the 35S viral RNA, and hence is RSV DNA and not an artifact of nucleotide addition to preexisting DNA molecules. The data also show that the DNA was 100% complementary to viral RNA, since no self-annealing was seen with the DNA alone (Table 3). We conclude that a small but significant fraction of the DNA transcribed *in vitro* from RSV RNA consists of molecules that have approximately the same molecular weight as the 35S viral RNA and a sequence complementary to it.

DISCUSSION

The experiments described here suggest that 35S viral RNA can be transcribed *in vitro* by viral DNA polymerase into an approximately full-length DNA complement, although at low efficiency. We attribute the low yield of very large DNA molecules primarily to the presence of RNase in preparations of purified virus, as mentioned in the introduction, which would degrade the RNA template as it is transcribed *in vitro* (13–16). Although preexisting nicks in the viral RNA, mentioned in the introduction, may also contribute to the small size of most of our DNA products, the virus used in these experiments was harvested at short intervals from infected cultures, and the RNA of such virus consisted of at least 30–50% physically intact RNA molecules (2, 3, 16).

Table 3. Hybridization of large ($\geq 2.5 \times 10^6$ daltons), *in vitro* synthesized DNA* with an excess of RSV RNA†

	Radioactivity (cpm)	% Resistance
Control	1,213	103
(minus S1 nuclease)	1,137	97
Self-annealing	−2	0
(plus S1 nuclease)		
RNA-DNA hybridization	1,175	100
(plus S1 nuclease)	1,225	104

* [^{3}H]DNA made in an endogenous reaction without actinomycin was isolated from the leading region of the gradient of Fig. 5A.

† Incubations were in 60 μl of 3.3 × SSC (SSC = 0.15 M NaCl, 0.015 M Na citrate, pH 7.0) at 68° for 10 hr. The [^{3}H]DNA used had a specific activity of 5×10^5 cpm/μg, and assuming a size and complexity of about 3×10^6 daltons (3–8), this predicts a $C_0t_{1/2}$ (C_0 = concentration of DNA of one polarity, $t_{1/2}$ = time at which the reaction is half complete; refs. 16, 31, and 32) of about 4×10^{-4} M-sec. However, if one strand is significantly in excess of the other, the $C_0t_{1/2}$ is 3×10^{-4} M-sec, in which C_0 now is the concentration of the major strand (33). At reaction termination, the C_0t was 2×10^{-3} M-sec if the DNA is treated as equal amounts of (+) and (−) strand, or about 4×10^{-3} M-sec if the DNA is predominantly of one polarity. The RNA·DNA hybridizations included 2 μg of 35S RSV RNA. The $R_0t_{1/2}$ (R_0 = concentration of RNA; ref. 33) would be of the order of 3×10^{-4} to 3×10^{-3} M-sec, given the generally slower rates of RNA·DNA annealing relative to DNA·DNA annealing (16, 35). At reaction termination, the R_0t was 4 M-sec under our conditions. Resistance to nuclease S1 was determined as described for Table 1. S1 resistant backgrounds of about 12 cpm or 1% of the input radioactivity were subtracted.

Under conditions of detergent concentration optimal for DNA synthesis, the viral RNA and DNA polymerase are retained within a core, permeable to ingredients of the reaction mixture. In this state the virus structure is sufficiently intact to retain its capacity to restrict but not eliminate access of virus-associated RNase to the viral RNA (viral RNA is slowly degraded) (16). The failure to observe large DNA transcripts at higher than optimal detergent concentrations may, according to this hypothesis, be attributed to the action of RNase on the template.

It is not yet clear whether the large, and full-sized DNA molecules made in our experiments represent complete copies of viral 35S RNA rather than a large molecule with redundant sequences. However, experiments carried out with unfractionated DNA made under our conditions indicate that this DNA has a high complexity and renders viral RNA completely resistant to RNase after hybridization with a low 2- to 5-fold excess of complementary DNA (16). This and the finding that none of our DNA transcripts was larger than the 35S viral RNA template suggest that these large DNA molecules probably represent transcripts that are colinear with viral RNA.

It would follow that the viral DNA polymerase is sufficient to transcribe viral RNA into a full-size DNA complement and that full-size viral DNA copies synthesized *in vitro* could be used for heteroduplex mapping of viral RNAs or for the specific detection of all viral RNA or DNA sequences in virus-infected and transformed cells.

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1. Mangel, W. F., Delius, H. & Duesberg, P. H. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4541–4545.
2. Duesberg, P. H. & Vogt, P. K. (1973) *J. Virol.* **12**, 594–599.
3. Duesberg, P. H. & Vogt, P. K. (1973) *Virology* **54**, 207–219.
4. Billeter, M. H., Parsons, J. T. & Coffin, J. M. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 3560–3564.
5. Beemon, K., Duesberg, P. & Vogt, P. (1974) *Proc. Nat. Acad. Sci. USA* **71**, 4254–4258.
6. Quade, K., Smith, R. E. & Nichols, J. L. (1974) *Virology* **61**, 287–291.
7. Duesberg, P. H., Vogt, P. K., Beemon, K. & Lai, M. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 847–857.
8. Cooper, G. M. & Temin, H. M. (1974) *J. Virol.* **14**, 1132–1141.
9. Temin, H. M. & Baltimore, D. (1972) *Adv. Virus Res.* **17**, 129–186.
10. Green, M. & Gerard, S. F. (1974) in *Progress in Nucleic Acid Research and Molecular Biology*, eds. Davidson, J. N. & Cohn, W. E. (Academic Press, New York), Vol. 14, pp. 187–334.
11. Duesberg, P. H. & Canaani, E. (1970) *Virology* **42**, 783–788.
12. Garapin, A. C., Varmus, H. E., Faras, A. J., Levinson, W. E. & Bishop, J. M. (1973) *Virology* **52**, 264–274.
13. Rosenbergova, M. F., Lacour, F. & Huppert, D. (1966) *C. R. Hebd. Seances Acad. Sci.* **260**, 5145–5748.
14. Quintrell, N., Fanshier, L., Evans, B., Levinson, W. & Bishop, J. M. (1971) *J. Virol.* **8**, 17–27.
15. Hung, P. P. (1973) *Virology* **51**, 287–296.
16. Junghans, R. P. (1975) Ph.D. Dissertation, University of California, Berkeley.
17. Duesberg, P. H. & Cardiff, B. (1968) *Virology* **36**, 696–700.
18. Bader, J. P. & Steck, T. C. (1969) *J. Virol.* **4**, 454–459.
19. Erikson, R. L. (1969) *Virology* **37**, 124–131.
20. Nygaard, A. P. & Hall, B. O. (1964) *J. Mol. Biol.* **9**, 125–142.
21. McDonald, C. & Chen, L. (1965) *Anal. Biochem.* **10**, 175–177.
22. Duesberg, P. H. (1970) *Current Topics Microbiol. & Immunol.* **51**, 79–104.
23. Layne, E. (1957) in *Methods in Enzymology*, eds. Colowick, S. P. & Kaplan, N. O. (Academic Press, New York), Vol. 3, pp. 447–454.
24. Scolnick, E. M., Rands, E., Aaronson, S. A. & Todaro, G. J. (1970) *Proc. Nat. Acad. Sci. USA* **67**, 1789–1796.
25. Wu, A. M. & Cetta, A. (1975) *Biochemistry* **14**, 789–795.
26. Garapin, A. C., McDonnell, J. P., Levinson, W., Quintrell, N., Fanshier, L. & Bishop, J. M. (1970) *J. Virol.* **6**, 589–598.
27. Bishop, D. H. L., Ruprecht, R., Simpson, R. W. & Spiegelman, S. (1971) *J. Virol.* **8**, 730–741.
28. Sutton, W. D. (1971) *Biochim. Biophys. Acta* **240**, 522–531.
29. Weber, G. H., Heine, U., Cottler-Fox, M., Garon, E. F. & Beaudreau, G. S. (1975) *Virology* **64**, 205–216.
30. Goldstein, L., Thomas, M. & Davis, R. (1975) *Virology* **66**, 420–427.
31. Britten, R. J. & Kohne, D. E. (1968) *Science* **161**, 529–540.
32. Wetmur, J. G. & Davidson, N. (1968) *J. Mol. Biol.* **31**, 349–370.
33. Fan, H. & Baltimore, D. (1973) *J. Mol. Biol.* **80**, 93–117.
34. Crawford, L. V., Follett, E. A. C. & Crawford, E. M. (1966) *J. Microsc. (Paris)* **5**, 597–604.
35. Straus, N. A. & Bonner, T. I. (1972) *Biochim. Biophys. Acta* **277**, 87–95.