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# *In Vitro* Synthesis of Rous Sarcoma Virus-Specific RNA is Catalyzed by a DNA-Dependent RNA Polymerase

**(actinomycin D/α-amanitin/assay of virus-specific RNA)**

L. RYMO, J. T. PARSONS, J. M. COFFIN, AND C. WEISSMANN

Institut für Molekularbiologie I, Universität Zürich, Zürich, Switzerland

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**ABSTRACT** **Synthesis of Rous sarcoma virus RNA was examined *in vitro* with a new assay for radioactive virus-specific RNA. Nuclei from infected and uninfected cells were incubated with ribonucleoside [α-$^{32}$P]triphosphates, $Mn^{++}$, $Mg^{++}$ and $(NH_4)_2SO_4$. Incorporation into total and viral RNA proceeded with similar kinetics for up to 25 min at 37°. About 0.5% of the RNA synthesized by the infected system was scored as virus-specific, compared to 0.03% of the RNA from the uninfected system and 0.005% of the RNA synthesized by monkey kidney cell nuclei. Preincubation with DNase or actinomycin D completely suppressed total and virus-specific RNA synthesis. α-Amanitin, a specific inhibitor of eukaryotic RNA polymerase II, completely inhibited virus-specific RNA synthesis, while reducing total RNA synthesis by only 50%. We conclude that tumor virus-specific RNA is synthesized on a DNA template, most probably by the host's RNA polymerase II.**

Temin's model for tumor virus RNA replication postulates that the infecting viral RNA is transcribed into DNA, which is then integrated into the host genome. Progeny RNA would subsequently be produced by transcription of the DNA (1–3). While the first part of the hypothesis is supported by a number of findings (1–3), the only evidence for DNA-dependent viral RNA synthesis is provided by the observations that actinomycin D inhibits virus formation (4, 5) and that virus mutants are generated by exposure of infected cells to bromodeoxyuridine (6). Although the inhibition experiment demonstrates the requirement for a DNA-dependent step, it does not show that the inhibitor is acting directly on viral RNA synthesis, rather than, for instance, by preventing synthesis of protein(s) required for viral RNA replication. Failure to find tumor virus-specific minus strands in infected cells (J. Duffy, H. Diggelmann, and C. Weissmann, unpublished results; see however ref. (7) for an opposite result) argues against the alternative pathway of RNA-directed RNA replication.

In order to clarify the nature of template and enzyme involved in Rous sarcoma virus RNA replication we developed a cell-free system of RNA synthesis in which about 0.5% of the RNA synthesized is virus-specific. Virus-specific RNA synthesis is as sensitive to actinomycin D and DNase as cellular RNA synthesis, supporting the view that the template involved is DNA. The high sensitivity of virus RNA synthesis to α-amanitin suggests that DNA-dependent RNA polymerase II is responsible for tumor virus RNA synthesis.

Abbreviations: RSB, 10 mM Tris·HCl (pH 7.4)–10 mM NaCl–1.5 mM $MgCl_2$; SRV, Schmidt–Ruppin strain of Rous sarcoma virus; AMV, avian myeloblastosis virus.

## MATERIALS AND METHODS

DNase I (electrophoretically purified, from Worthington Biochemical Corp., Freehold, N.J.) was treated with iodoacetate to eliminate RNase activity (8). RNase $T_1$ was from Calbiochem; crude RNase $T_2$ was prepared as described (9). α-Amanitin was a gift from Prof. T. Wieland. Ribonucleoside [α-$^{32}$P]triphosphates were synthesized by a method based on published procedures (10, 11). All information regarding cells, cell lines, viruses, labeled viral nucleic acids, and other materials has been published (12, 16). Chicken embryo fibroblast cultures infected with the Schmidt–Ruppin strain of Rous sarcoma virus (SRV) were transferred 2–4 days after infection and used to prepare nuclei 3–4 days later.

*Preparation of nuclei.* Cells were suspended at 1 to 3 × $10^6$ cells per ml in cold 0.5 × RSB where RSB is 10 mM Tris·HCl (pH 7.4)–10mM NaCl–1.5 mM $MgCl_2$, (12). After 30 min on ice the cells were homogenized by 10–15 strokes in a Dounce homogenizer (Kontes Glass Co., Vineland, N.J.) with a tight-fitting glass pestle. More than 90% of the cells were ruptured by this procedure. The nuclei were collected by centrifugation for 5 min at 1300 × *g* and washed twice with 20 volumes of cold RSB. Portions of about $10^7$ nuclei were centrifuged as above and the nuclei were either stored at −70° or resuspended in 50 μl of RSB (unless specified otherwise) for immediate use. After thawing, frozen nuclei were largely disrupted, but synthesis of total and virus-specific RNA was not impaired. Storage at −70° for 6 months did not affect activity.

RNA (13), DNA (14), and protein (15) were determined on five independent preparations; typically, protein-to-DNA ratios of 6 and RNA-to-DNA ratios of 0.7 were found. The preparations did not contain endogenous ribonucleoside triphosphates in detectable amounts.

*Synthesis of $^{32}$P-Labeled RNA by Isolated Nuclei and Purification of RNA.* The standard reaction mixture contained in 0.1 ml: 8 μmoles of Tris·HCl (pH 8.0), 20 μmoles of $(NH_4)_2SO_4$, 0.4 nmoles of dithiothreitol, 0.3 μmoles of $MgCl_2$, 0.2 μmoles of $MnCl_2$, 100 nmoles each of unlabeled and 5 nmoles of an α-$^{32}$P-labeled ribonucleoside triphosphate (2 to 13 × $10^7$ cpm/nmole), and nuclei from about $10^7$ cells. After 15 min at 37°, 0.1 ml of 0.5 M NaCl–50 mM $MgCl_2$–2 mM $CaCl_2$–10 mM Tris·HCl (pH 7.5) and 50 μg/ml of purified DNase I

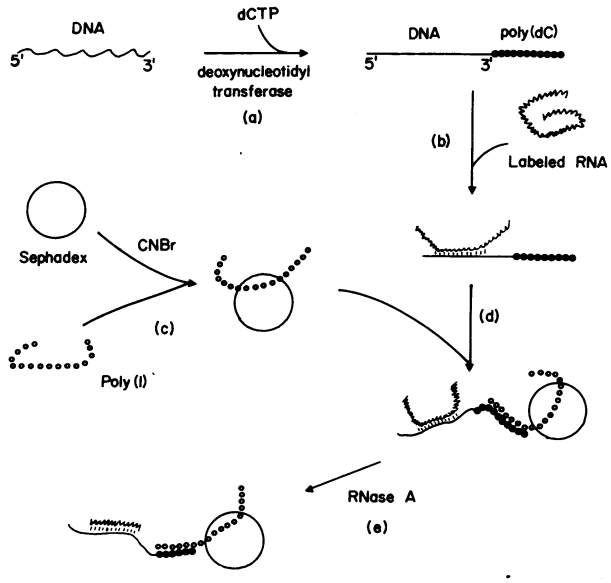


FIG. 1. Scheme for the detection and isolation of viral RNA by hybridization to complementary poly(dC)-elongated DNA. (*a*) DNA complementary to tumor virus RNA is elongated at its 3′ terminus with a poly(dC) sequence. (*b*) Labeled nucleic acid is annealed with the elongated DNA to form a hybrid. (*c*) Poly(I) is coupled to Sephadex. (*d*) The annealed nucleic acids are passed through a column of poly(I)-Sephadex; the virus-specific hybrids are retained by their poly(dC) extension. (*e*) The column is washed with RNase A to degrade nonhybridized RNA, and the RNA retained on the column is eluted and its radioactivity determined. To reduce the background, oligo(C) is added prior to hybridization, and poly(U) prior to chromatography, as explained elsewhere (16). A known amount of viral RNA homologous to the RNA being assayed for (labeled with a different isotope) is added prior to hybridization as an internal marker and its yield is used to correct for incomplete recovery of the unknown (12, 16).

were added and incubation was continued for 20 min. The mixture was then incubated for 30 min at 37° with 0.5% sodium dodecyl sulfate and 0.1% Pronase. Total RNA synthesis was monitored by determining the acid-insoluble $^{32}P$ radioactivity of an aliquot. Two milliliters of 10 mM Tris·HCl (pH 7.5)–10 mM ethylenediaminetetraacetate–0.1% sodium dodecyl sulfate were added and the solution was extracted with 2 ml of phenol at 20°. The phenol phase was reextracted at 66° with an equal volume of the same buffer. The RNA was precipitated from the pooled aqueous phases with ethanol, dissolved in 0.5 ml of 10 mM Tris·HCl (pH 7.5)–50 mM NaCl–10 mM ethylenediaminetetraacetate–0.1% sodium dodecyl sulfate, and chromatographed on a 0.6 × 10-cm column of Sephadex G-100 in the same buffer. The [$^{32}P$]RNA was precipitated with ethanol.

*Hybridization Techniques.* Radioactive SRV RNA was determined according to Coffin *et al.* (16). Samples (1–20 μg total RNA) were annealed with 0.05–0.1 μg of poly(dC)-DNA in 50 μl of 0.5 M NaCl–10 mM Tris·HCl (pH 7.5) containing 1 μg of oligo$(C)_{5-20}$. After 4 hr at 66°, 2 μg of poly(U) (Miles Laboratories, Elkhart, Ind.) were added and labeled hybrid was determined by poly(I)-Sephadex chromatography.

## RESULTS

(*1*) *Detection and Characterization of Tumor Virus-Specific RNA Synthesized by a Cell-Free System.* The major problem in studying tumor virus RNA synthesis *in vitro* was the detection of the newly formed virus-specific RNA, since it constitutes only 1% or less of the total RNA synthesized. This difficulty was overcome by the use of the assay (16) outlined in Fig. 1.

Since earlier work had identified the nucleus as the site of viral RNA synthesis (12), nuclei from uninfected or SRV-infected chicken fibroblasts, or from monkey kidney (Vero) cells were used as source of enzymatic activity. Incubation with the four ribonucleoside triphosphates (one of which was $^{32}P$-labeled) was carried out under conditions similar to those established previously for nuclear RNA synthesis (17–21). The RNA was purified, mixed with an internal standard of SRV [$^{3}H$]RNA, and annealed with poly(dC)-AMV DNA (AMV is avian myeloblastosis virus), which can hybridize to more than 50% of SRV RNA (12, 16). Table 1 shows that [$^{32}P$]RNA from the SRV-infected *in vitro* system contained 0.4–0.5% virus-specific RNA (measured value 15–18 times

TABLE 1. *Virus-specific RNA sequences in RNA synthesized in vitro*

| Exp. | Source of nuclei | Input [$^{32}P$]RNA (cpm × $10^{-3}$) | $^{32}P$ radioactivity in hybrid fraction after hybridization with: No DNA (cpm) | Poly(dC)-T4 DNA (cpm) | Poly(dC)-AMV DNA (cpm) | Hybridization efficiency of $^{3}H$-labeled internal standard (%) | Virus-specific [$^{32}P$]RNA corrected (% of input) |
|---|---|---|---|---|---|---|---|
| I | SRV-infected fibroblasts | 487 | 12 | 17 | 302 | 13 | 0.45 |
| | uninfected fibroblasts | 520 | 2 | 8 | 47 | 26 | 0.029 |
| II | SRV-infected fibroblasts | 349 | Not done | 30 | 454 | 26 | 0.47 |
| | uninfected fibroblasts | 654 | Not done | 9 | 87 | 35 | 0.034 |
| III | Vero cells | 655 | 6 | 9 | 15 | 17 | 0.005 |

RNA synthesis by nuclei was as described in *Materials and Methods,* using [$\alpha$-$^{32}P$]UTP (0.05 mM) in experiments I (20 × $10^{6}$ cpm/nmole) and III (60 × $10^{6}$ cpm/nmole). In experiment II all four substrates (0.05 mM each) were $\alpha$-$^{32}P$-labeled at 15 × $10^{6}$ cpm/nmole each. Aliquots of the purified, labeled RNAs were mixed with a known amount of SRV [$^{3}H$]RNA (2–4000 cpm) and annealed with 0.1 μg of either poly(dC)-AMV DNA or poly(dC)-T4 bacteriophage DNA, or without DNA. The hybridized radioactive RNA was determined by the poly(I)-Sephadex method (16). A machine background of 20 cpm was subtracted from all $^{32}P$ radioactivities. The content of SRV [$^{32}P$]RNA was calculated from the amount of $^{32}P$ radioactivity hybridized to poly(dC)-AMV DNA after subtraction of the value from the control hybridization with poly(dC)-T4 DNA. The values were corrected by dividing by the "hybridization efficiency" as monitored by the yield of the SRV [$^{3}H$]RNA added as internal standard.

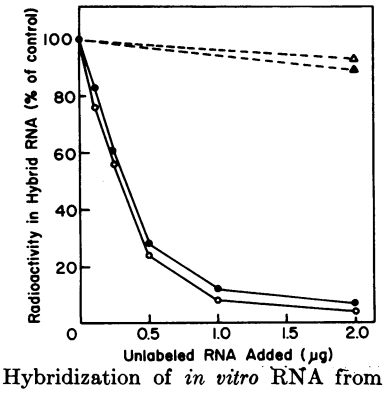


FIG. 2. Hybridization of *in vitro* RNA from SRV-infected chicken cells to poly(dC)-AMV DNA: Competition with unlabeled 70S AMV RNA. A mixture of [$^{32}$P]UMP-labeled *in vitro* RNA (780,000 cpm) and 70S SRV [$^{3}$H]RNA (2400 cpm) was annealed with 0.1 μg of poly(dC)-AMV DNA and unlabeled 70S AMV RNA as indicated. The amount of $^{3}$H-labeled (○) and $^{32}$P-labeled (●) hybrid formed was determined by the poly(I)-Sephadex method. Background hybridization values, obtained by annealing parallel samples with 0.1 μg of poly(dC)-T4 DNA (3 cpm of $^{3}$H and 24 cpm of $^{32}$P), were subtracted. In a control 2 μg of Qβ RNA were added instead of AMV RNA and the $^{3}$H-labeled (△) and $^{32}$P-labeled (▲) hybrids were determined as above. All values are in percent of the values obtained in the absence of AMV RNA (1200 cpm of $^{3}$H and 1200 cpm of $^{32}$P).

over background). In the case of the noninfected system the corresponding value was 0.03–0.035% (measured value 6–10 times over background). The [$^{32}$P]RNA from the Vero system gave a value of 0.005% (measured value 1.5 times above background, not significant).

The labeled RNA scored as hybrid was further characterized by hybridization competition. Fig. 2 shows that adding increasing amounts of unlabeled AMV RNA to a mixture of *in vitro* [$^{32}$P]RNA and SRV [$^{3}$H]RNA prior to annealing diminished the hybridization of both labeled RNAs to the same extent. With 2 μg of unlabeled AMV RNA (an estimated 20- to 40-fold excess over the total virus-specific RNA present) the hybridization values were less than 10% of those of the controls not subjected to competition; 2 μg of bacteriophage Qβ RNA showed no significant effect.

The *in vitro* product isolated by the poly(I)-Sephadex method was further analyzed both by rehybridization and by nucleotide analysis. RNA uniformly labeled with the four [α-$^{32}$P]triphosphates was mixed with SRV [$^{3}$H]RNA and annealed with poly(dC)-AMV DNA; the hybrid was bound to poly(I)-Sephadex, treated with RNase, and eluted. 0.14% of the $^{32}$P-labeled product and 30% of the [$^{3}$H]RNA were recovered and purified by a procedure including DNase treatment. Samples of the purified RNA were annealed with increasing amounts of AMV DNA and the RNase-resistant radioactivity was determined. Up to 64% of the [$^{32}$P]RNA was scored as hybrid, as compared to 80% of the SRV [$^{3}$H]-RNA (Fig. 3). Competition hybridization analysis of the purified product showed that RNase-resistant [$^{32}$P]-and [$^{3}$H]RNA were reduced to background values by addition of 2 μg of AMV RNA. Taking into account the hybridization efficiency of the internal standard and the background value in the dilution experiment, the purified *in vitro* RNA was at least 76% virus-specific. Table 2 shows that the nucleotide composition of the purified [$^{32}$P]RNA was similar to that of the RNase-resistant fraction of SRV [$^{32}$P]RNA hybridized to

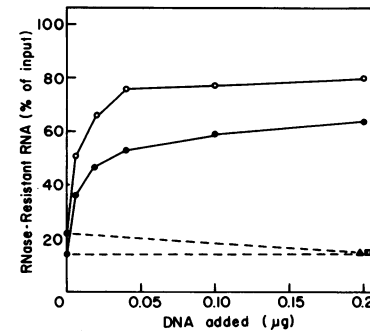


FIG. 3. Rehybridization of *in vitro* RNA purified by annealing to poly(dC)-AMV DNA and recovery from poly(I)-Sephadex. A mixture of $^{32}$P-labeled *in vitro* RNA ($7.0 \times 10^{6}$ cpm) and 70S SRV [$^{3}$H]RNA (28,000 cpm) was annealed with 0.36 μg of poly-(dC)-AMV DNA in the presence of 9 μg of oligo$(C)_{5-20}$. After 4 hr at 66°, 5 μg of poly(U) were added and the mixture was applied to a poly(I)-Sephadex column. The column was washed, treated with RNase A, and the hybrid eluted as usual, except that the last washing buffer contained 0.5% sodium dodecyl sulfate. Qβ RNA (20 μg) was added as carrier; the RNA was precipitated with ethanol, dissolved in 0.1 ml of 10 × RSB containing 0.1 mg/ml of purified DNase, and incubated for 10 min at 37°. After digestion with Pronase, phenol extraction, and chromatography on Sephadex G-50, the RNA was precipitated with ethanol (*Materials and Methods*). Aliquots of the purified RNA (470 cpm of $^{32}$P and 550 cpm of $^{3}$H) were annealed for 14 hr at 66° with AMV DNA (○;●) or T4 DNA (△;▲) as indicated. Another annealing mixture with AMV DNA contained in addition 2 μg of unlabeled 70S AMV RNA (□;■). Hybrid formation was determined by digesting in 0.1 ml of 0.5 M NaCl–10 mM Tris·HCl (pH 7.5) for 30 min at 37° with 400 units/ml of RNase $T_1$ and 40 μg/ml of RNase A in the presence of 0.25 mg/ml of *Escherichia coli* RNA and measuring acid-insoluble radioactivity (16). The results are given as the percentage of input RNA. *Open symbols* (○,△,□), $^{3}$H; *closed symbols* (●,▲,■), $^{32}$P.

AMV DNA and differed from that of total [$^{32}$P]RNA synthesized by infected or noninfected nuclei.

*(2) Properties of the In Vitro System of RNA Synthesis.* The effect of ammonium sulfate concentration on nucleotide incorporation into both total and SRV-specific RNA in the presence of either 8 mM $Mg^{++}$ or 3 mM $Mn^{++}$ is shown in Fig. 4. The highest absolute and relative incorporation into virus-specific RNA was at 3 mM $Mn^{++}$ and 150 mM $(NH_4)_2SO_4$. Slightly better incorporation was obtained by using both 3 mM $Mg^{++}$ and 2 mM $Mn^{++}$, and 200 mM $(NH_4)_2SO_4$. Substrates were added at 1 mM, which was close to saturation for UTP and CTP (data not shown), except for the α-$^{32}$P-labeled nucleotide, which for practical reasons was used at 0.05 mM. At this level incorporation was about 20% of that at 1 mM. Under our standard conditions incorporation was not linear but continued for at least 25 min, both into total and virus-specific RNA (Fig. 5). The specific enzymatic activity of nuclear preparations (total RNA synthesis) was 1.40 ± 0.35 pmoles of UMP per μg of DNA per 15 min (9 determinations); of this, 0.43 ± 0.06% was in SRV-specific RNA (10 determinations).

*(3) Effect of Inhibitors on Total and SRV-Specific RNA Synthesis.* Preincubation of nuclei at various levels of actinomycin D reduced total and SRV-specific RNA synthesis to equal extents, down to 7% of control at 50 μg/ml (Fig. 6*A*). This effect was not due to degradation induced by actinomycin, as

TABLE 2. *Nucleotide composition of SRV [$^{32}$P]RNA and [$^{32}$P]RNAs synthesized in vitro*

| Source of [$^{32}$P]RNA | Fraction examined | $^{32}$P radioactivity (%) | | | |
|---|---|---|---|---|---|
| | | CMP | AMP | GMP | UMP |
| 70S SRV RNA | Total | 25.8 | 23.8 | 29.1 | 21.3 |
| 70S SRV RNA | RNase-resistant after hybridization to poly(dC)-AMV DNA | 24.3 | 24.6 | 27.8 | 23.3 |
| *In vitro* RNA from SRV-infected cells | Total | 26.6 | 20.2 | 28.2 | 25.0 |
| *In vitro* RNA from SRV-infected cells | RNase-resistant after hybridization to poly(dC)-AMV DNA | 23.2 | 24.4 | 28.8 | 23.7 |
| *In vitro* RNA from uninfected cells | Total | 26.8 | 19.6 | 28.7 | 24.9 |

RNA was synthesized *in vitro* using the 4-ribonucleoside [$\alpha$-$^{32}$P]triphosphates at the same specific activities ($15 \times 10^6$ cpm/nmole each). *In vitro* product from SRV-infected cell nuclei or SRV [$^{32}$P]RNA was annealed to poly(dC)-AMV DNA; the hybrid was bound to poly(I)-Sephadex, treated with RNase, eluted by formamide, and recovered by ethanol precipitation (compare *legend* to Fig. 3). Aliquots of [$^{32}$P]RNA (2400 cpm or more) in 10 $\mu$l of a solution containing 10 $\mu$g of *E. coli* RNA, 0.4 mg/ml of RNase A, and 100 units/ml of crude RNase $T_2$ (9) were incubated for 90 min at 37°. The nucleotides were separated by electrophoresis (12). All values are the average of two determinations.

shown by the following experiment: Labeled RNA was synthesized under standard conditions for 3 min, then 50 $\mu$g/ml of actinomycin and a 200-fold excess of unlabeled over labeled substrate were added. After further 15 min of incubation total and virus-specific labeled RNA were not reduced as compared to a control without actinomycin (data not shown).

Both total and virus-specific RNA synthesis were progressively reduced after preincubation with increasing levels of DNase, reaching 15% and 7% of control, respectively, at 100 $\mu$g/ml (Fig. 6*B*). RNA synthesis by Q$\beta$ RNA-directed Q$\beta$ replicase (22) was not affected by either actinomycin D or DNase under conditions similar to those above except that $(NH_4)_2SO_4$ (which is inhibitory to Q$\beta$ replicase) was omitted. Fig. 6*C* shows that increasing levels of $\alpha$-amanitin preferentially inhibited virus-specific RNA synthesis as compared to total RNA synthesis. At 0.01 $\mu$g/ml of $\alpha$-amanitin SRV-specific RNA synthesis was reduced to less than 5% while total RNA synthesis was 46% of control; this value was only slightly reduced by increasing the $\alpha$-amanitin concentration 5-fold.

## DISCUSSION AND SUMMARY

An *in vitro* system containing nuclei from SRV-infected chicken fibroblasts synthesizes RNA of which about 0.5% is virus-specific, a value only slightly lower than that found *in vivo*, namely 0.4–0.8% (12, 16). Preparations from noninfected cells gave a 14-fold lower value which was still significantly above background. Since virus-specific DNA sequences are present in uninfected chicken cells (23–25) and virus-specific RNA occurs in noninfected fibroblasts from certain chicken embryos (26), these *in vitro* results may be meaningful.

Both DNase and actinomycin D (compare review ref. 27), which act on the DNA itself, inhibited virus-specific RNA synthesis even more effectively than total RNA synthesis. Although the actinomycin D concentration required for 90% inhibition of *in vitro* RNA synthesis (50 $\mu$g/ml) is far higher than that required for an equivalent inhibition of total and SRV-specific RNA synthesis *in vivo* (about 2 $\mu$g/ml) (J. M. Coffin *et al.*, in preparation) the actinomycin to DNA ratio was similar in both cases. SRV-specific RNA synthesis is completely inhibited by $\alpha$-amanitin while total RNA synthesis is only reduced by half. This is of particular significance since $\alpha$-amanitin specifically inhibits nucleoplasmic DNA-dependent RNA polymerase II (28–30), the enzyme thought to be responsible for the transcription of messenger and heterogeneous nuclear RNA (29, 31), and does not affect nucleolar polymerase I, which synthesizes ribosomal RNA (29, 31). *In vitro* synthesis of adenovirus-specific RNA by nuclei is also strongly inhibited by $\alpha$-amanitin (20, 21). Inhibition of Rous sarcoma virus production after treatment of infected chicken cells with high concentrations of $\alpha$-amanitin has been reported (32).

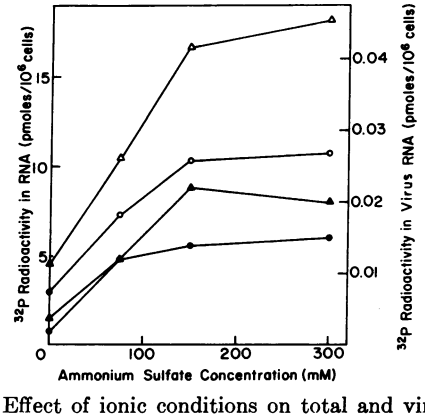


FIG. 4. Effect of ionic conditions on total and virus-specific RNA synthesis. Total (O,△; *left ordinate*) and SRV-specific (●,▲; *right ordinate*) RNA syntheses were determined at the concentrations of $(NH_4)_2SO_4$ indicated, in the presence of either 8 mM $MgCl_2$ (O,●) or 3 mM $MnCl_2$ (△,▲). Conditions were otherwise as described in *Materials and Methods*.

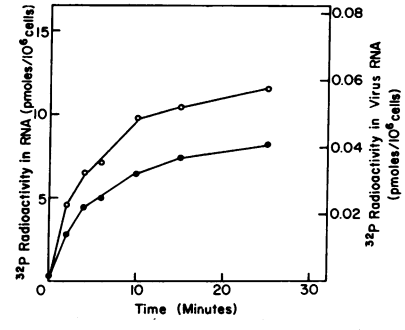


FIG. 5. Time course of total and virus-specific *in vitro* RNA synthesis. Standard reaction mixtures (*Materials and Methods*) were incubated for the times indicated. Total (O; *left ordinate*) and SRV-specific (●; *right ordinate*) RNA syntheses were determined.

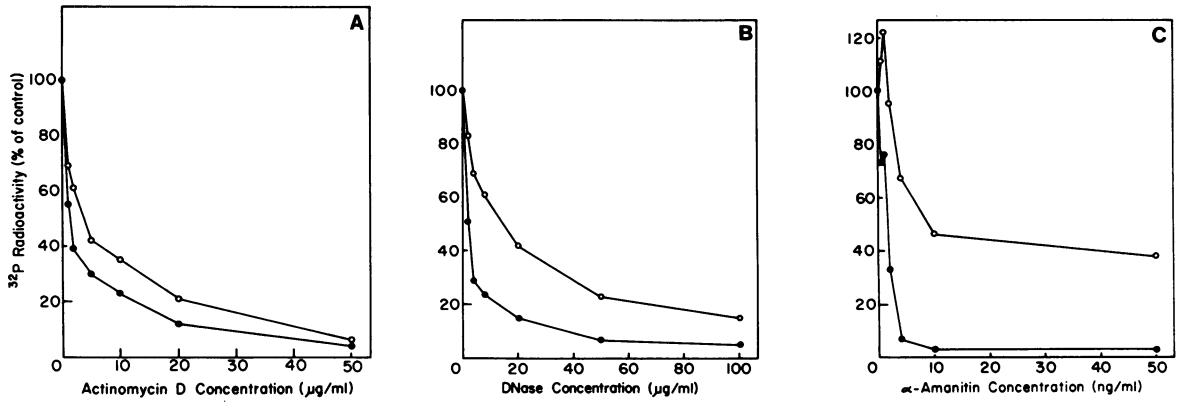


FIG. 6. Effect of inhibitors on total and virus-specific *in vitro* RNA synthesis. Nuclei from about $10^7$ SRV-infected cells were suspended in 50 μl of 10 mM Tris·HCl (pH 7.5)–3 mM $MgCl_2$–2 mM $MnCl_2$ containing (*A*) actinomycin D as indicated (and 3% dimethylsulfoxide as solvent), (*B*) DNase as indicated and, (*C*) α-amanitin as indicated and 200 mM ammonium sulfate. The nuclear suspensions were incubated for 15 min at 0° (*A* and *C*) or 6° (*B*), adjusted to the standard conditions for *in vitro* RNA synthesis described in *Materials and Methods* (final volume 0.1 ml) and incubated for 15 min at 37°. Aliquots corresponding to (*A*) $10^5$, (*B*) $1.4 \times 10^5$, and (*C*) $1.2 \times 10^5$ cells were taken to determine total RNA synthesis (○) and aliquots corresponding to (*A*) $1.5 \times 10^6$–$2.5 \times 10^6$, (*B*) $5.6 \times 10^6$, and (*C*) $4.8 \times 10^6$ cells for the determination of SRV-specific RNA synthesis (●). The results are expressed as percent of control incubations without inhibitor. Control values, total RNA: (*A*) 715,000, (*B*) 284,000, (*C*) 199,000 cpm/$10^6$ cells; SRV-specific RNA (corrected values): (*A*) 3180, (*B*) 1170, (*C*) 660 cpm/$10^6$ cells; hybridization efficiencies 8–21%.

Since the enzyme activity of our *in vitro* system is essentially due to nuclei that had been subjected to a minimum of handling, we believe that our results reflect normal *in vivo* processes. We therefore conclude that most if not all tumor virus RNA is synthesized on a DNA template, most probably by the host-specific DNA-dependent RNA polymerase II.

**Note added in proof.** Similar conclusions have been reached by M. Jacquet, Y. Groner, G. Monroy and J. Hurwitz, *Proc. Nat. Acad. Sci. USA*, in press.

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