Synthesis *in vitro* by Bacterial RNA Polymerase of Simian Virus 40-specific RNA: Multiple Transcription of the DNA Template into a Continuous Polyribonucleotide

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**SUMMARY**

The mol. wt. of the single-stranded RNA molecules synthesized in a cell-free system on simian virus 40 (SV40) DNA by *Escherichia coli* RNA polymerase was determined at intervals after the initiation of the reaction. Chain elongation of the RNA, under the experimental conditions used, took place at an average rate of 5 nucleotides/s. Thus single-stranded RNA molecules of $5 \times 10^6$ mol. wt. resulting from continuous transcription of one turn of the circular SV40 genome did not appear until about 20 min after initiation of the reaction. After 40 to 80 min, molecules with mol. wt. equal to or greater than the mol. wt. of the DNA template strand ($> 5 \times 10^6$) accounted for 25 to 50% of the mass of the RNA population. Up to 25% of the RNA synthesized at that time had mol. wt. equal to or larger than $3 \times 10^6$. The results indicate that the cell-free system used does not contain specific factors or signals which would cause the *E. coli* RNA polymerase to terminate transcription.

**INTRODUCTION**

This study was undertaken to determine the mol. wt. of the single-stranded RNA molecules synthesized on simian virus 40 (SV40) DNA by *Escherichia coli* RNA polymerase at intervals after initiation of the reaction. RNA synthesized *in vitro*, which is complementary to SV40 DNA (cRNA), has been used extensively to detect, by hybridization techniques, SV40 DNA in transformed cells (Westphal & Dulbecco, 1968; Tai & O'Brien, 1969; Levine *et al.* 1970). In addition, transcription of SV40 DNA by *E. coli* RNA polymerase has been included in more complete *in vitro* systems in attempts to synthesize virus-coded proteins (Bryan, Gelfand & Hayashi, 1969; Crawford *et al.* 1970). The molecular size of the virus-specific messenger RNA is important in such studies.

We were also interested to find out whether cRNA as long as, or longer, than the DNA template, i.e. than one strand of SV40 DNA, can be synthesized and isolated in amounts sufficient for investigation by RNA–DNA hybridization of the molecular size distribution of virus DNA integrated into the cellular genome.

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METHODS

Preparation of SV40 DNA. The LP-4 clone of the RH11 strain of SV40 was propagated in monolayer cultures of CV-1 or African green monkey kidney cells, as described previously (Carp, Sauer & Sokol, 1969). To prepare labelled virus, either 5 Ci/ml of [3H]-thymidine (specific activity, 30 Ci/m-mol; Schwarz-Mann, Orangeburg, N.Y.) or 0.1 Ci/ml of [14C]-thymidine (specific activity, 35 mCi/m-mol; Schwarz-Mann) was added to cultures 20 h after infection. The virus was purified by treatment with sodium desoxycholate and centrifuging in a steep KBr density gradient, as described previously (Carp et al. 1969), except that the trypsin treatment was omitted from the procedure. As an additional step, the virus was centrifuged to equilibrium in CsCl solution (Hummeler, Tomassini & Sokol, 1970). Only virus particles banding at 1.34 to 1.35 g/cm³ were used for isolation of virus DNA.

DNA was extracted from the virus by treatment with phenol, as has been described (Carp et al. 1969), except that the removal of phenol from the aqueous phase by extraction with ether was omitted. Superhelical, closed circular SV40 DNA (component I SV40 DNA) was isolated by equilibrium sedimentation in CsCl solution containing ethidium bromide (Radloff, Bauer & Vinograd, 1967). The dye was removed by extraction with isopropanol, which had previously been saturated with CsCl solution of the same density as the buoyant density of component I SV40 DNA (Cuzin et al. 1970). The virus DNA was then dialyzed at 4 °C against three changes of KET buffer (0.02 M-KCl, 0.001 M-EDTA, 0.01 M-tris, pH 8.0) for 24 h and stored at -20 °C. The DNA isolated by this procedure sedimented at 21 S, as expected for component I SV40 DNA (Crawford & Black, 1964), when analysed by velocity centrifugation in a linear gradient of sucrose in NTES buffer (0.13 M-NaCl, 0.05 M-tris (pH 7.8), 0.001 M-EDTA, 0.5 % (w/w), SDS).

Evidence for absence of contaminating cellular DNA in preparations of SV40 DNA was obtained by hybridization experiments, carried out as described previously (Carp et al. 1969). About 500 ct/min hybridized to as little as 5 x 10⁻³ µg of denatured component I SV40 DNA when saturated with [3H]-cRNA, which was synthesized as described below. However, no detectable amounts of [3H]-cRNA were hybridized to as much as 25 µg of denatured monkey-cell DNA under identical conditions.

The concentration of virus DNA was determined by spectrophotometry, assuming that 50 µg/ml of non-denatured DNA gives E_{260}^1cm = 1.

Preparation of E. coli RNA polymerase. E. coli RNA polymerase purified by the method of Chamberlin & Berg (1962) was further concentrated by precipitation with 1.5 vol. of saturated ammonium sulphate solution and dialyzed against 0.01 M-tris (pH 7.9), 0.01 M-MgCl₂, 0.0001 M-EDTA, 0.0001 M-dithiothreitol, containing 5 % (v/v) glycerol. Finally, it was centrifuged in the same buffer in a 10 to 30 % (v/v) glycerol gradient (Burgess, 1969a). The tetramer enzyme aggregate, with a sedimentation coefficient of 21 S, was isolated and used in the experiments described below. It was stored in 50 % glycerol at -70 °C.

In vitro synthesis of cRNA and its purification. The reaction mixture contained in 1 ml. 2.4 µg component I SV40 DNA, 1.8 µg RNA polymerase, 150 µ-mol KCl, 10 µ-mol tris (pH 8.0), 10 µ-mol β-mercaptoethanol, 150 n-mol each of cytidine 5'-triphosphate, adenosine 5'-triphosphate, guanosine 5'-triphosphate, and 150 n-mol of [3H]-uridine 5'-triphosphate (specific activity: 0.5 or 2.5 Ci/m-mol; Schwarz-Mann). The four nucleoside triphosphates, in 200 µl of 0.2 M-tris (pH 8.0), were not added until the rest of the mixture had been allowed to stand for 10 min at 20 °C (Westphal, 1970). After addition of the nucleoside triphosphates, the reaction mixture was incubated at 37 °C.

Synthesis of cRNA was terminated by the addition of SDS to a final concentration of
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0.5% (w/w). *E. coli*-soluble RNA (500 μg/ml; Schwarz-Mann; pre-purified by phenol treatment) was added and the solution was extracted twice with phenol containing 0.1% 8-hydroxyquinoline. RNA and the template DNA were then precipitated by 2.5 vol. of ethanol and stored at −20°C. Before any analysis, the nucleic acid precipitate was washed twice with 95% ethanol. About 90% of both the cRNA and the DNA in the reaction mixture was recovered by this extraction procedure.

When SV40 DNA was omitted from the reaction mixture, the acid-precipitable radioactivity recovered after 80 min incubation was less than 1% of that obtained from similarly incubated complete reaction mixtures. Thus, the amount of contaminating bacterial DNA possibly present in the enzyme preparation was negligible.

Fractionation of cRNA by velocity centrifugation in sucrose density gradient. In one method used, cRNA was dissolved in low salt-formaldehyde solution (LSF) (0.0045 M-Na₂HPO₄, 0.0005 M-NaH₂PO₄, 0.001 M-EDTA, 0.1 M-HCHO, pH 7.7), heated to 80°C for 5 min and cooled to 25°C. The composition of the solvent was then changed from LSF to high salt-formaldehyde solution (HSF) (0.09 M-Na₂HPO₄, 0.01 M-NaH₂PO₄, 0.001 M-EDTA, 1.1 M-HCHO, pH 7.7) before the RNA was fractionated. About 1 ml of RNA solution in HSF was then layered on 28 ml of 7 to 27% (w/v) linear gradient of sucrose in HSF and centrifuged at 24,000 rev/min for 19 h at 20°C in the SW 25.1 rotor of a Spinco centrifuge. The E₆₀₀ of 1 ml fractions collected from the bottom of the tube was determined. Each fraction then received 100 μg of carrier yeast RNA, followed by 1.2 ml of 20% trichloroacetic acid (TCA). The precipitates were collected in the cold by filtration through nitrocellulose membranes and subsequently washed with 5% TCA. The dried filters were placed into 10 ml of Liquiflor (New England Nuclear, Boston, Mass.) toluene mixture, and the radioactivity was counted.

In a second method of fractionation the cRNA was dissolved in 1.0 ml of NTES buffer, layered on 28 ml of 8 to 34% (w/v) linear gradient of sucrose in NTES buffer, and centrifuged at 24,000 rev/min for 12 h at 20°C in a SW 25.1 rotor. The fractions were then analysed in the same way as described above.

Equilibrium density gradient sedimentation. Equilibrium density gradient sedimentation of cRNA was done in a manner similar to that described previously (Lozeron & Szybalski, 1966). cRNA (460 μl) in 3.5 M-HCHO, 0.01 M-EDTA, 0.52 M-Na₂HPO₄, 0.0058 M-NaH₂PO₄ (pH 7.7) (FES buffer) was mixed with 3080 μl of saturated CsCl solution in distilled water (saturated at 25°C) and 880 μl of saturated Cs₂SO₄ solution. In the experiment involving purified virus DNA, SV40 DNA in 460 μl of FES buffer was mixed with 1220 μl of water, 2130 μl of saturated CsCl solution and 610 μl of saturated Cs₂SO₄ solution. Centrifuging was done in polycarbonate tubes at 44,000 rev/min for 40 h at 20°C in a No. 50 angle-head rotor of a Spinco centrifuge. The density and the radioactivity of fractions collected from the bottoms of the tubes were then determined.

**RESULTS**

Fractionation of cRNA and template DNA by velocity centrifugation in sucrose density gradient

[³H]-cRNA preparation obtained after 80 min of reaction in the *in vitro* system, and from which the component ¹⁴C]-SV40 DNA was not removed, was deproteinized by treatment with SDS and phenol and fractionated by velocity sedimentation on a sucrose gradient in NTES buffer (Fig. 1). The template DNA was evidently dissociated from the cRNA by this procedure, as about 85% of the DNA had the same sedimentation coefficient (21 S) as that
Fig. 1. Velocity sedimentation in a sucrose density gradient of untreated $[^{3}H]$-cRNA and $[^{14}C]$-SV40 DNA template. $[^{3}H]$-cRNA was synthesized for 80 min (specific activity of $[^{3}H]$-uridine 5'-triphosphate: 0.5 Ci/mmol), extracted together with the template DNA, mixed with 130 μg hamster kidney cell ribosomal RNA, and analyzed by velocity sedimentation in a sucrose density gradient in NTES buffer, as described in Methods. Fractions were collected and assayed for: $E_{280}$ (●—●); $[^{3}H]$-radioactivity (■—■); $[^{14}C]$-radioactivity (□—□). Panels a and b of this Fig. refer to the same experiment.

of component I SV40 DNA that had not been incubated in the in vitro system. About 15% of the DNA sedimented at 16S, indicating that these molecules had a break introduced in at least one of their backbone phosphodiester bonds (Crawford & Black, 1964). A single-stranded cRNA molecule as long as one strand of the circular SV40 DNA would have a mol. wt. of $1.5 \times 10^9$ (Crawford & Black, 1964; Crawford, Follett & Crawford, 1966) and a sedimentation coefficient of about 27S in this solvent (Kurland, 1960; Petermann & Pavlovec, 1966; McConkey & Hopkins, 1969). About 50% of the cRNA sedimented faster than 27S. In all calculations of mol. wt. it is assumed that the 28S and 18S ribosomal RNA molecules isolated from baby hamster kidney (BHK) cells have a mol. wt. of $1.8 \times 10^9$ and $7 \times 10^8$, respectively (McConkey & Hopkins, 1969; Petermann & Pavlovec, 1966).

To make an unequivocal interpretation of the molecular size of the cRNA molecules, however, we had to be sure that we were not dealing with aggregates of single-stranded cRNA molecules. So that any multi-stranded aggregates present would be eliminated, cRNA preparations were heated in the presence of formaldehyde prior to sedimentation. Heating at 90 °C in 0.33 M-HCHO and 0.01 M-Na phosphate buffer was sufficient to dissociate the synthetic double-stranded RNA composed of polynucleotides with the highest melting temperature (Spirin, 1963; Haselkorn &
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Fig. 2. Velocity sedimentation in a sucrose density gradient of formaldehyde-treated [3H]-cRNA and [14C]-SV40 DNA template. The cRNA synthesized in 80 min (specific activity of [3H]-uridine 5'-triphosphate; 0.5 Ci/mmol) and the SV40 DNA template, were isolated from the reaction mixture, mixed with hamster kidney cell ribosomal RNA, heated in formaldehyde solution, cooled, and analysed by velocity sedimentation in a sucrose density gradient. (For details see Methods.) Fractions were collected and assayed for: $E_{260}$ (○ ---○); [14C]-radioactivity (■ --- ■); [3H]-radioactivity (□ --- □). Panels a and b in this Fig. refer to the same experiment. The values over the arrows indicate mol. wt.

Fox, 1965). In addition, increasing the formaldehyde concentration from 0.33 M to 1.1 M in a similar buffer solution should have decreased the melting point of poly rG-rC to about 60 °C (Haselkorn & Doty, 1961). As a result, we chose heating at 80 °C in 1.1 M-formaldehyde in 0.005 M-phosphate buffer as a method to ensure dissociation of multi-stranded aggregates of cRNA molecules. The presence of the formaldehyde also prevented any subsequent base pairing between single-stranded molecules which have been cooled (Freifelder & Davidson, 1962).

A sample of the same [3H]-cRNA preparation that was analysed in Fig. 1 was deproteinized by SDS and phenol, heated at 80 °C in LSF for 5 min and analysed by velocity sedimentation in a sucrose gradient in HSF (Fig. 2). To interpret the results, we used the equation (Boedtker, 1968) that relates the sedimentation coefficient ($s_{20,w}$) in HSF of a single-stranded RNA molecule, which was allowed to react to formaldehyde, to its mol. wt. ($M_w$): $s_{20,w} = 0.05 M_w^{0.40}$. It can be calculated that the ribosomal RNA, which sediments in NTES buffer at 28S before formaldehyde treatment, will have its sedimentation coefficient decreased to 16S in HSF, after formaldehyde treatment. Correspondingly cRNA molecules of $1.5 \times 10^6$ and $3 \times 10^6$ mol. wt. will sediment in HSF at 15S and 19S, respectively. Thus, a cRNA molecule of $1.5 \times 10^6$ mol. wt. will sediment only about $\frac{3}{16}$ as far on the gradient
Fig. 3. Analysis by equilibrium density gradient sedimentation of cRNA fractions.

(a) A portion of the unfractionated [3H]-cRNA preparation, the sedimentation pattern of which is shown in the inset of panel b, was heated at 80 °C in LSF for 5 min and cooled before being centrifuged in CsCl-Cs2SO4 solution. Fractions were collected and assayed for radioactivity (■—■) and density (○—○).

(b) 28S pool [3H]-cRNA (see inset) was freed of sucrose by dialysis against HSF buffer, heated in the same buffer and sedimented to equilibrium. BHK-cell ribosomal RNA (50 μg) heated in the presence of formaldehyde was also analysed on this gradient (E_{260}, ■—■). Inset: [3H]-cRNA was synthesized on unlabelled component I SV40 DNA for 80 min (specific activity of [3H]-uridine 5'-triphosphate: 2.5 Ci/m-mol), extracted, and dissolved in LSF solution together with BHK cell ribosomal RNA. The mixture was heated in LSF and analyzed by velocity sedimentation in a sucrose density gradient in HSF buffer (for details see Methods). Only 30 μl samples of each fraction were used for determination of radioactivity. The remainder of fractions 3 to 8 and that of fractions 9 to 13 were pooled and referred to as 35S and 28S pool cRNA, respectively. Abbreviations: B = bottom; T = top.

(c) 35S pool [3H]-cRNA (see inset, panel b) treated with formaldehyde.

(d) [3H]-thymidine-labelled component I SV40 DNA which was heated at 80 °C in LSF for 5 min and cooled before centrifuging.
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as the large ribosomal RNA molecule and a cRNA molecule of $3 \times 10^6$ mol. wt. will sediment about $1\frac{1}{3}$ farther. We can conclude, therefore, on the basis of the distribution of cRNA on the gradient (Fig. 2), that about 30% of the cRNA population had a mol. wt. equal to or greater than $1.5 \times 10^6$. In other experiments (Fig. 3b, inset and Fig. 4c) 45 to 50% of cRNA had molecular size larger than one SV40 DNA strand.

Heating of RNA molecules at 80 °C introduces breaks in the backbone phosphodiester bonds of some of the molecules. Heating for 5 min will induce one such break in 15% of the molecules of $1.5 \times 10^6$ mol. wt. and in 30% of the molecules of $3 \times 10^6$ mol. wt. (Eigner, Boedtker & Michaels, 1961). In experiments not shown here, we confirmed this reported rate of bond breakage by calculating the % of 28S ribosomal RNA molecules that remained intact after different periods of heating at 80 °C in LSF. It will become evident that the above amount of degradation of cRNA molecules does not affect the basic conclusions derived from this study.

The results presented in Fig. 2 also support the previous conclusion that most of the component I SV40 DNA template remained intact during the incubation of the reaction mixture. About 90% of the $[^{14}C]$-labelled DNA sedimented as a homogeneous component to a point in the gradient midway between the 18S and 28S ribosomal RNAs. Purified component I SV40 DNA that had not been incubated in the reaction mixture, and that had been heated to 80 °C in LSF, sedimented to the identical position on the gradient (not shown here). Essentially all of the cRNA and template DNA layered on the gradients was recovered in the fractions collected at the end of the centrifugation.

Equilibrium density gradient sedimentation of cRNA

To determine whether heating in the presence of formaldehyde resulted in dissociation of all cRNA into single-stranded molecules, formaldehyde-treated cRNA was analysed by equilibrium density gradient sedimentation in CsCl-Cs$_2$SO$_4$ solution. $[^3H]$-cRNA was synthesized for 80 min on unlabelled component I SV40 DNA. After deproteinization, part of the cRNA preparation was heated at 80 °C in LSF for 5 min and fractionated by velocity sedimentation in a sucrose density gradient in HSF. About 40% of cRNA had mol. wt. equal to or larger than $1.5 \times 10^6$ and about half of these large RNA molecules exceeded in mol. wt. the double-stranded virus DNA template. cRNA was pooled separately from the 28S and 35S regions of the gradient (Fig. 3, panel b, inset) and centrifuged to equilibrium in CsCl-Cs$_2$SO$_4$ solution (Fig. 3, panels b and c). A sample of unfractionated cRNA was treated similarly by formaldehyde and centrifuged to equilibrium in parallel (Fig. 3, panel a). BHK cell ribosomal RNA, heated in the presence of formaldehyde, was used as single-stranded RNA marker (Fig. 3, panel b) and purified component I SV40 DNA, which was allowed to react with formaldehyde under identical conditions, was also included as a control (Fig. 3, panel d).

Each of the three cRNA samples banded as an essentially homogeneous population of molecules with a buoyant density of 1.837 to 1.843 g/cm$^3$. In each case between 80 and 100% of the cRNA added to the gradient was recovered in this component. The buoyant density of ribosomal RNA was 0.005 g/cm$^3$ lower than that of cRNA, when the two RNAs were centrifuged together in the same tube. Formaldehyde-treated component I SV40 DNA had a buoyant density of 1.61 g/cm$^3$ in CsCl-Cs$_2$SO$_4$ solution. Since the buoyant density of double-stranded RNA in CsCl-Cs$_2$SO$_4$ solution is by 0.05 g/cm$^3$ lower than that of single-stranded RNA (Sakuma & Watanabe, 1971), these results confirmed that cRNA heated in the presence of formaldehyde is single-stranded.
Table 1. Time dependence of total cRNA synthesis*

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<thead>
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<th>Reaction time (min)</th>
<th>Amount of acid-precipitable cRNA (ct/min/ml)</th>
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<tbody>
<tr>
<td>6</td>
<td>$2.28 \times 10^3$</td>
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<tr>
<td>20</td>
<td>$5.10 \times 10^3$</td>
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<tr>
<td>40</td>
<td>$8.80 \times 10^3$</td>
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<td>80</td>
<td>$1.50 \times 10^3$</td>
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* For composition of the reaction mixture and conditions of incubation see Methods. The specific activity of $[^{3}H]$-uridine 5'-triphosphate used was $2.5 \text{ Ci/m-mol}$. 

Fig. 4. Kinetics of cRNA elongation. Samples of $[^{3}H]$-cRNA, synthesized in 6 min (panel a), 20 min (panel b), and 40 min (panel c) of transcription, respectively, were heated together with BHK cell ribosomal RNA, in LSF, at $80^\circ \text{C}$ for 5 min, cooled, and then fractionated by velocity sedimentation in a sucrose density gradient in HSF. Fractions were then collected and assayed for radioactivity (■—■) and $E_{260}$ (○—○). The specific activity of $[^{3}H]$-uridine 5'-triphosphate used for synthesis of cRNA was $2.5 \text{ Ci/m-mol}$. The ribosomal RNA components are marked with coefficients (arrows) characteristic for their sedimentation in absence of formaldehyde.

Kinetics of cRNA synthesis

The amount of cRNA synthesized increased with increasing time of reaction in a nearly linear way (Table 1), the deviation from linearity being more pronounced with progressing reaction time. The kinetics of elongation of the cRNA strand during transcription was examined by determining the size distribution of single-stranded cRNA molecules at intervals after initiation of transcription (Fig. 4). cRNA synthesized within 6 min was moderately heterogeneous, the majority of the molecules sedimenting slightly faster or
somewhat slower than the 18S marker ribosomal RNA. The heterogeneity of cRNA molecules became evident after 20 min of transcription and at that time the predominant molecular size corresponded to that of 28S ribosomal RNA. These results indicated that under the experimental conditions used, SV40 cRNA molecules were elongated at an average rate of approximately 5 nucleotides/s. This was in good agreement with the rate at which E. coli RNA polymerase transcribes lambda bacteriophage DNA in vitro under similar conditions (Hayward & Green, 1970).

By 40 min the sedimentation rate of the cRNA became extremely heterogeneous. cRNA synthesized within this interval exhibited a molecular size distribution similar to that observed after 80 min of reaction (cf. Figs. 2, 3 b (inset) and 4 c). RNA with mol. wt. equal to or larger than $1.5 \times 10^8$ or $3.0 \times 10^8$ accounted for 50 and 25% of total RNA, respectively.

**DISCUSSION**

The results of the present study are consistent with the following model of in vitro transcription of superhelical SV40 DNA by bacterial RNA polymerase. The cRNA chain is elongated, under the experimental conditions used, at an average rate of about 5 nucleotides/s, i.e. one complete turn of one SV40 DNA strand is transcribed in approximately 17 min. The rate of cRNA elongation is dependent on the reaction conditions. Therefore, it is not surprising that other investigators reported a substantially higher elongation rate of SV40-specific RNA transcribed in vitro by the bacterial RNA polymerase (Westphal & Kiehn, 1970). The transcription reaction does not stop after the enzyme reaches the initiation point again. Instead, the elongation of cRNA continues, until the length of the transcribed RNA sometimes reaches the equivalent of two or more contour lengths of template DNA. This means that on at least one of the two SV40 DNA strands there are no nucleotide sequences that cause the polymerase to terminate transcription. Failure of a substantial amount of the cRNA molecules to increase in size after 40 min is probably caused by non-specific events, such as inactivation of the enzyme or degradation by trace amounts of ribonuclease. Nicking or degradation of the DNA template is not responsible for artificial termination of transcription, as 85% of the template molecules are shown to remain intact through the entire time of the reaction. Preparations of E. coli RNA polymerase, purified by the method of Chamberlin & Berg (1962), do not contain the bacterial protein factor rho ($\rho$) which causes the termination of transcription in vitro (Richardson, 1970). It is possible that the absence of this factor from our enzyme preparation allowed the RNA polymerase to pass the termination site on the DNA template.

Supercoiled SV40 DNA possessed four binding sites for bacterial RNA polymerase, of which at least three are significantly non-random (Herzberg & Winocour, 1970). Since the enzyme-to-DNA template ratio used in the present study is relatively high, 0.75:1 on weight basis and 3:1 on a molar basis (Burgess, 1969 b), it is anticipated that the superhelical virus DNA bound several enzyme molecules at different sites. The results indicate, therefore, that the presence of several RNA polymerase molecules on one circular SV40 DNA molecules does not prevent a large proportion of the polymerases from synthesizing cRNA molecules that are longer than the DNA template.

The synthesis of this 'giant' cRNA is efficient, since molecules exceeding in molecular size the DNA template strand represented 30 to 50% of the total RNA product, provided the reaction has proceeded for a sufficient time. Previous reports on the molecular size of in vitro synthesized SV40-specific RNA indicate either absence or presence in only minute amounts of such cRNA molecules (Westphal, 1970; Westphal & Kiehn, 1970). cRNA
transcribed in this type of in vitro system has been demonstrated to compete with ‘late’ SV40-specific RNA, derived from productively infected cells, for at least 90% of the binding sites on SV40 DNA (Levin et al. 1971). ‘Late’ SV40-specific RNA is most likely transcribed from 100% of one SV40 DNA strand (Martin & Axelrod, 1969). Thus, one could conclude from these results that E. coli RNA polymerase is able to transcribe all nucleotide sequences contained in one of the SV40 DNA strands. Our results are consistent with this and virtually rule out the explanation that this complete transcription must be done in segments, with each polymerase molecule transcribing only a portion of the DNA strand. The transcription of SV40 DNA by E. coli RNA polymerase is asymmetric if component I SV40 DNA is used as a template (Westphal, 1970). As a result, the long cRNA molecules synthesized in our system are probably transcribed specifically off only one of the two strands of DNA.

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