Proc. Nat. Acad. Sci. USA
Vol. 70, No. 8, pp. 2304–2308, August 1973

In Vitro Transcription of Simian Virus 40 Sequences in SV3T3 Chromatin

(transformed mouse cells/hybridization/*Escherichia coli* RNA polymerase)

SUSAN M. ASTRIN

The Salk Institute, La Jolla, California 92112; and * The Institute for Cancer Research, Fox Chase Center for Cancer and Medical Sciences, 7701 Burholme Avenue, Philadelphia, Pennsylvania 19111

Communicated by Thomas F. Anderson, May 17, 1973

ABSTRACT **The template properties of chromatin were studied in a system in which transcription of unique genes could be examined. Chromatin and DNA were isolated from SV3T3 cells, a line of simian virus 40 (SV40)-transformed mouse cells, and used as templates for *Escherichia coli* RNA polymerase. The SV40 sequences in the RNA transcribed *in vitro* and in RNA isolated from the transformed cells were analyzed by hybridization to radioactive RNA transcribed from SV40 DNA. The results indicate that the same sequences of the SV40 "−" strand (DNA strand which serves as a template for early RNA) are transcribed *in vitro* from chromatin as are found in transformed cells. In contrast, when the DNA isolated from SV3T3 chromatin by removal of proteins is used as a template, the entire SV40 "−" strand is transcribed. It is concluded that the specific template restriction observed with chromatin is due to the presence of proteins associated with the DNA.**

Much of the DNA of eukaryotic cells is bound to proteins (1). One mechanism for regulation of gene expression *in vivo* may be that these proteins prevent transcription of the region of DNA to which they are bound. Indeed, the proteins bound to the DNA of purified chromatin do seem to inhibit the ability of specific sequences in the DNA to act as template for transcription by *Escherichia coli* RNA polymerase *in vitro* (5, 7, 12, 13), presumably by rendering these sequences inaccessible to the polymerase. Moreover, the DNA sequences of chromatin that are available for transcription *in vitro* are the same sequences that are transcribed *in vivo*, at least with respect to families of related or reiterated sequences (8, 12, 15). Whether such a mechanism operates to repress transcription of unique sequences is not known. If so, it may represent a general mechanism by which gene expression is regulated in eukaryotic cells. Chromatin would thus be an appropriate system for studying eukaryotic gene regulation in cell-free systems.

We have attempted to clarify the template properties of chromatin by examining the transcription *in vitro* of the simian virus (SV)40 genome in SV3T3 cells, a line of mouse fibroblasts transformed by SV40 virus. The use of this system to study chromatin has the following unique advantages. (*1*) The SV40 genome is integrated into cellular DNA (9) at a frequency of about one copy per cell (4) and is not part of a family of related sequences. Consequently, RNA that hybridizes to SV40 DNA must be a transcript of the integrated SV40 genome, and not of a related sequence. (*2*) Although the entire viral genome is present (17), less than 70% of the genome is transcribed in transformed cells (10). Consequently, one may determine whether the same regions of the genome are available for transcription in chromatin as are transcribed *in vivo*. (*3*) When purified SV40 DNA (covalently closed double-stranded circles) is used as a template for *E. coli* RNA polymerase, the entire "−" strand (DNA strand that serves as a template for early RNA) is transcribed (19). Therefore, an inability of the polymerase to transcribe regions of the SV40 "−" strand in chromatin cannot be a property of the SV40 DNA. (*4*) Since SV40 DNA can be easily purified, nucleic-acid hybridization can be used to assay specifically for RNA transcribed from the SV40 genome.

Abbreviations: SV, simian virus; c, complementary.

* Address reprint requests to this address.

MATERIALS AND METHODS

Cells. The cultivation of cell lines 3T3 (16) and SV3T3 (9) has been described.

RNA Was Extracted from SV3T3 Cells by the procedure of Weinberg *et al.* (18). Any contaminating DNA was digested with electrophoretically purified DNase (Worthington Biochemical), which had been previously treated with iodoacetate to remove traces of RNase (20). RNA concentrations were determined by absorbance at 260 nm.

Chromatin Was Prepared from isolated nuclei by the method of Seligy and Miyagi (11) and sedimented through 1.7 M sucrose. Chromatin (at a DNA concentration of 1 mg/ml) was sheared by sonication for 5 sec at a setting of 3 with a Branson S-75 sonifier equipped with a standard 0.5-inch tip. The DNA:protein:RNA ratio in purified chromatin was 1:2.6:0.05.

SV3T3 DNA Was Prepared from Sheared Chromatin by addition of sodium perchlorate to a final concentration of 1 M and extraction four times with phenol–chloroform (1:1) and once with chloroform. The DNA was precipitated with ethanol and dissolved in 10 mM Tris·HCl (pH 7.9)–1 mM EDTA. DNA concentration was determined by absorbance at 260 nm.

Preparation of RNA Transcribed In Vitro from SV3T3 Chromatin and SV3T3 DNA. RNA was synthesized from a chromatin template by use of equal weight ratios of DNA and *E. coli* DNA-dependent RNA polymerase [purified according to Burgess (2) with glycerol gradients] in a standard reaction mixture containing 150 mM KCl, 40 mM Tris·HCl (pH 7.9), 0.1 mM dithiothreitol, 5 mM $MgCl_2$, and 1 mM each ATP, GTP, CTP, and UTP (P-L Biochemicals). At the end of the reaction, chromaint was removed by centrifugation at 10,000

× *g* for 5 min and the supernatant was extracted three times with phenol:chloroform (1:1) and once with chloroform. The RNA was precipitated with cold 5% trichloroacetic acid to remove triphosphates, redissolved in 1 M Tris·HCl (pH 7.9)–0.1 M NaCl, and precipitated with ethanol overnight at −20°. The precipitate was dissolved in 0.1 M Na acetate (pH 5.3)–0.01 M $MgCl_2$ and treated with DNase as described above for *in vivo* RNA. RNA transcribed from SV3T3 DNA was synthesized and purified as described above except that the DNase treatment was done in the reaction mixture at the end of the reaction, and the centrifugation to remove chromatin was omitted.

Preparation of Radioactive RNA Complementary to SV40 DNA. Highly radioactive RNA complementary to SV40 DNA ([^{3}H]cRNA) was prepared by transcribing purified SV40 DNA with *E. coli* RNA polymerase (6). The specific activity of [^{3}H]cRNA calculated from the specific activity of the tritiated triphosphates that were used as substrates was 1.4×10^8 dpm/μg. [^{3}H]cRNA was sedimented through dimethylsulfoxide gradients and the 28S fraction was isolated. 28S RNA corresponds to a single-stranded RNA molecular weight of 1.5×10^6 (14) and is the equivalent of a total transcript of the SV40 genome [molecular weight 3.2×10^6 (3)]. 28S [^{3}H]cRNA consists predominately (90–97%) of transcripts of the "−" strand of the DNA and will be referred to as asymmetric [^{3}H]cRNA and defined as "+" RNA strands. A portion of 28S [^{3}H]cRNA was self-annealed and digested with RNase. The RNase-resistant product contained equal amounts of "+" and "−" RNA strands (data not shown) and will be referred to as symmetric [^{3}H]cRNA.

Preparation of Unlabeled RNA Complementary to SV40 DNA. Unlabeled asymmetric cRNA was synthesized in a 2-ml standard reaction described above containing 100 μg of SV40 closed-circular DNA and 50 μg of *E. coli* RNA polymerase and purified as described above for RNA transcribed from SV3T3 DNA. Unlabeled asymmetric cRNA was self annealed to prevent contaminating "−" strands from participating in subsequent hybridization reactions.

Hybridizations were performed in 50% formamide, 0.9 M NaCl, 0.01 M Tris·HCl (pH 7.4), and 0.05% sodium dodecylsulfate at 37° or 50°. Hybridization proceeded at about the same rate at 37°, 45°, and 50°, and the pleatau values of RNase resistance were the same (data not shown). More than 70% of the [^{3}H]cRNA remained hybridizable after 5 weeks at 37° or 1 week at 50°.

Assay for RNase Resistance. After hybridization, samples were diluted into 4 volumes of 0.3 M NaCl–0.03 M Na_3 citrate and the single-stranded RNA was digested to completion with 50 μg/ml of pancreatic RNase and 37 units/ml of T1 RNase for 30 min at 37°. 25-μl Aliquots were applied to glass-fiber discs containing an equal volume of cold 10% trichloroacetic acid, washed with cold 5% trichloroacetic acid followed by ethanol, dried, and counted. Aliquots of each sample were removed before RNase treatment and precipitated as above to determine total radioactivity present before RNase treatment. RNase resistance was calculated from each pair of count values.

Hybridization: Correction for Self-annealing of [^{3}H]cRNA. The data for all hybridizations in which symmetric [^{3}H]cRNA was annealed with unlabeled RNA were corrected for the amount of self-annealing of the [^{3}H]cRNA. The rate of self-annealing was measured by incubation of denatured, symmetric [^{3}H]cRNA alone at the same concentrations used in each experiment. The values for self-annealing of the [^{3}H]-cRNA observed in these control hybridizations were then corrected for the amount of [^{3}H]cRNA that had been prevented from self-annealing in the experiment because it had annealed to unlabeled RNA. These corrected self-annealing values have been subtracted from all data on hybridization to symmetric [^{3}H]cRNA. In no case was the magnitude of the correction greater than 3% RNase resistance.

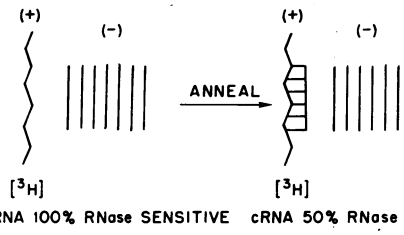

FIG. 1. Schematic representation of the annealing of asymmetric (+) [^{3}H]cRNA with a sample of RNA containing 50% of the "−" strand sequences.

Asymmetric (+) [^{3}H]cRNA was pre-annealed in order to prevent all the contaminating "−" strand sequences from participating in subsequent hybridization reactions. This pre-annealed [^{3}H]cRNA (8% RNase resistant) was used in all experiments calling for asymmetric [^{3}H]cRNA. The hybridization values observed in the experiments were then corrected for the initial 8% RNase resistance of the cRNA.

RESULTS

Detection of virus-specific RNA

SV40-specific sequences in a sample of nonradioactive RNA were assayed in the following manner. The sample was annealed to small amounts of [^{3}H]cRNA (described in *Methods*), and the RNase resistance of the [^{3}H]cRNA was monitored. The unlabeled SV40-specific RNA, present in 5- to 10-fold excess, protects from RNase digestion all regions of the [^{3}H]-cRNA to which it anneals. The annealing of asymmetric [^{3}H]cRNA ("+" strands) with a sample of RNA, as depicted in Fig. 1, thus serves as an assay for the proportion of the sequences in the "−" RNA strand present in the sample. In an analogous fashion, the annealing of denatured symmetric [^{3}H]cRNA to the sample serves to detect both "+" and "−" strand sequences present in the sample.

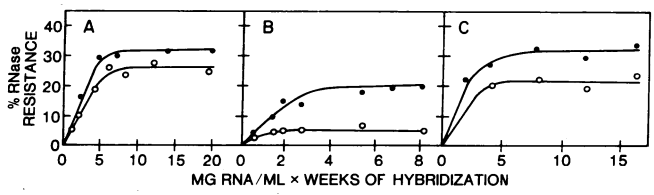

FIG. 2. (*A*) Annealing of RNA extracted from SV3T3 cells with [^{3}H]cRNA. Symmetric cRNA (●): 0.5 ng (10^4 cpm) of 28S symmetric [^{3}H]cRNA, which had been denatured by boiling for 8 min in water, and various concentrations (2, 4, and 6 mg/ml) of SV3T3 RNA were annealed in a volume of 0.5 ml at 37° for 8 and 24 days. 0.25-ml Samples were assayed for RNase resistance. Asymmetric cRNA (○): 0.5 ng (10^4 cpm) of 28S asymmetric

[^{3}H]cRNA and various concentrations (1, 4.6, and 7 mg/ml) of SV3T3 RNA were annealed in a volume of 0.5 ml of 37° for 6, 12, and 20 days. 0.25-ml Samples were assayed for RNase resistance. (*B*) Annealing of RNA transcribed from SV3T3 chromatin with symmetric (●) and asymmetric (○) [^{3}H]cRNA. RNA was transcribed from chromatin in a 30-ml standard reaction containing 8 mg of DNA as chromatin and 8 mg (3000 units) of RNA polymerase. The reaction mixture was incubated at 37° for 2 hr and then the *in vitro* RNA was extracted. 1.6 mg of RNA were recovered from the reaction. 0.5 ng (10^4 cpm) of denatured 28S symmetric [^{3}H]cRNA, and various concentrations (0.450, 1.8, 2.2, and 2.7 mg/ml) of *in vitro* RNA were annealed in a volume of 0.2 ml at 37° for 7 and 21 days. 0.1-ml Samples were assayed for RNase resistance. 0.5 ng (10^4 cpm) of 28S asymmetric [^{3}H]cRNA and various concentrations (0.9, 1.8 and 2.7 mg/ml) of *in vitro* RNA were annealed in a volume of 0.1 ml. 0.05-ml Samples were assayed for RNase resistance. (*C*) Annealing of RNA transcribed from SV3T3 chromatin with symmetric (●) and asymmetric (○) [^{3}H]cRNA. RNA was transcribed from chromatin in a 15-ml standard reaction containing 6 mg of DNA as chromatin and 5 mg of RNA polymerase (2500 units). The reaction was incubated at 37° for 2 hr and then the *in vitro* RNA was extracted. 2.7 mg of RNA was recovered from the reaction. 0.5 ng (10^4 cpm) of denatured 28S symmetric [^{3}H]cRNA and various concentrations (4 and 2 mg/ml) of *in vitro* RNA were annealed in a volume of 0.2 ml at 37° for 1,2, 3, and 4 weeks. 0.025-ml Samples were assayed for RNase resistance.

Annealing of SV3T3 RNA transcribed *in vivo* with asymmetric and symmetric cRNA

In order to compare RNA transcribed *in vitro* from chromatin with RNA transcribed *in vivo* it was necessary to determine what portion of the total viral sequences was present in the RNA transcribed *in vivo*.

Unlabeled RNA was extracted from SV3T3 cells and annealed with asymmetric (+) [^{3}H]cRNA, as described in Fig. 2*A*. At saturation 26% of the cRNA was resistant to RNase digestion. This experiment was repeated once and gave a value of 25%. Thus, about 26% of the RNA "−" strand sequences are present in SV3T3 cells; i.e., 26% of the "+" strand of SV40 DNA is expressed as RNA in these cells.

SV3T3 RNA was also annealed to symmetric [^{3}H]cRNA (Fig. 2*A*). At saturation 32% of the cRNA is resistant to RNase digestion. This experiment was repeated three times with the same RNA preparations, and maximum values of 32%, 36%, and 37% RNase resistance at saturation were obtained. Protection of an average of 34% of symmetric [^{3}H]cRNA is equivalent to 68% protection of one strand. Thus, the equivalent of 68% of a single strand of SV40 DNA is expressed as RNA in SV3T3 cells. Since 26% of the "+" strand of SV40 is expressed (as shown by its hybridization to 26% of asymmetric (+) [^{3}H]cRNA), 42% (68% minus 26%) of the "−" strand must be expressed. This calculation is based on the assumption that the same number and distribution of sequences are present in asymmetric (+) [^{3}H]cRNA as are present in the "+" strand of symmetric [^{3}H]cRNA. Since both cRNAs were derived from RNA that has the length of a total transcript of the SV40 genome, the above assumption seems warranted. Expression of 42% of the "−" DNA strand and 26% of the "+" DNA strand is in general agreement with the data of Sambrook *et al.* (10), who found that 50% of the "−" strand and 20% of the "+" strand of SV40 DNA are expressed as RNA in SV3T3 cells. No hybridization was detected between [^{3}H]cRNA and RNA from nontransformed 3T3 cells.

TABLE 1. *Summary of results*

Source of RNA	% "−" DNA strand expressed	% "+" DNA strand expressed
SV3T3 cells	42	26
Transcribed from:		
SV3T3 chromatin I	38	4
Sv3T3 chromatin II	41	21
SV3T3 DNA	100	30

Annealing of the RNA transcribed from SV3T3 chromatin with [^{3}H]cRNA

Nonradioactive RNAs transcribed from two preparations of SV3T3 chromatin were annealed to symmetric and asymmetric (+) [^{3}H]cRNA. The first preparation of RNA gave the results shown in Fig. 2*B*. Unlabeled RNA transcribed *in vitro* protected about 21% of the symmetric [^{3}H]cRNA from RNase digestion but very little (about 4%) of the asymmetric (+) [^{3}H]cRNA. From the virtual lack of hybridization to asymmetric (+) [^{3}H]cRNA, it appears as if this *in vitro* RNA preparation contained very few "−" strand sequences. However, it did contain 38% (2 times 21 minus 4) of the "+" strand sequences, in good agreement with the data presented above on RNA transcribed *in vivo*.

Nonradioactive RNA was transcribed from a second preparation of SV3T3 chromatin and annealed to symmetric and asymmetric [^{3}H]cRNA. The results are shown in Fig. 2*C*. A maximum of 31% of symmetric [^{3}H]cRNA and 21% of asymmetric (+) [^{3}H]cRNA were protected by annealing to the *in vitro* RNA. From these data it was calculated that 21% of the "+" DNA strand and 41% (31 times 2 minus 21) of the "−" DNA strand were transcribed. These numbers are in good agreement with the data on *in vivo* RNA and also with the value for the transcription of the "−" DNA strand calculated for the first chromatin preparation (see Table 1). However, transcription of the "+" SV40 DNA strand in chromatin appears to be variable.

In order to determine whether the same RNA sequences are present in the *in vitro* RNA as are contained in the *in vivo* RNA, *in vivo* RNA was mixed with *in vitro* RNA from the second chromatin preparation and annealed to symmetric [^{3}H]cRNA. The annealing of this mixture was compared to the annealing to symmetric [^{3}H] CRNA of *in vivo* RNA alone. If the *in vitro* RNA contained additional sequences, the percent RNase resistance at saturation would be higher for the mixture than for the *in vivo* RNA alone. The results shown in Fig. 3 indicate that the *in vitro* RNA contains no additional sequences to those present in *in vivo* RNA.

The double-saturation experiment described above was also performed with *in vitro* RNA transcribed from a third preparation of chromatin. In this experiment, *in vivo* RNA alone hybridized to 37% of symmetric [^{3}H]cRNA and the mixture of *in vivo* and *in vitro* RNAs hybridized to 39% of symmetric [^{3}H]cRNA. These results confirm the conclusion drawn from Fig. 3.

Several conclusions can be drawn from the above data. The same percentage of the SV40 "−" DNA strand appears to be

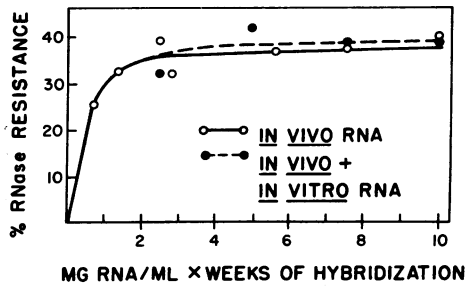

FIG. 3. Annealing of a mixture of *in vitro* and *in vivo* RNA to symmetric [^{3}H] cRNA. SV3T3 *in vitro* RNA was described in the legend of Fig. 2. 0.5 ng (10^4 cpm) of denatured, symmetric [^{3}H]-cRNA was annealed with 1.26 mg of SV3T3 *in vitro* RNA and 0.81 mg of SV3T3 *in vitro* RNA in a volume of 0.2 ml for 1, 2, 3, and 4 weeks, as described in the legend of Fig. 2. 0.05-ml Samples were assayed for RNase resistance. 0.5 ng (10^4 cpm) of symmetric [^{3}H]cRNA was also annealed with various amounts (0.35, 0.7, and 1.26 mg) of *in vivo* RNA in a volume of 0.2 ml for 1–4 weeks, as described in the legend of Fig. 2.

transcribed *in vitro* from SV3T3 chromatin as is found in SV3T3 cells. In addition, since *in vitro* RNA contains no additional sequences to those present in *in vivo* RNA, it can be concluded that the same sequences of the SV40 "−" strand are transcribed *in vitro* as are found *in vivo*.

Annealing of the RNA transcribed from SV3T3 DNA with [^{3}H]cRNA

The experiments described above show that some of the SV40 sequences in SV3T3 chromatin are not transcribed *in vitro*. If this restriction is due to the presence of proteins bound to the SV3T3 DNA, then removal of these proteins should increase the fraction of the SV40 sequences that is transcribed *in vitro*. Alternatively, if the restriction derives from the state of the DNA (i.e., length of fragments, nicks) or from the presence of termination signals in the DNA that are recognized by *E. coli* polymerase, DNA extracted from chromatin should behave like chromatin itself. Therefore, SV3T3 DNA was prepared from chromatin and transcribed, and the resulting RNA was characterized by hybridization to [^{3}H]cRNA.

As shown in Fig. 4, RNA transcribed from SV3T3 DNA anneals to about 65% of symmetric and 30% of asymmetric (+) [^{3}H]cRNA. From these data it was calculated that 30% of the "+" SV40 DNA strand and 100% (65 times 2 minus 30) of the "−" DNA strand were transcribed. Thus, RNA transcribed from SV3T3 DNA contains 30% of the "−" RNA sequences and 100% of the "+" RNA sequences.

The existence of all the "+" strand sequences in the RNA made from DNA was substantiated in the following way. An excess of nonradioactive SV40 "+" RNA strands (nonradioactive, asymmetric cRNA synthesized as described in *Methods*) was added to symmetric [^{3}H]cRNA that had already been annealed to RNA made from SV3T3 DNA. The original plateau value of 65% RNase resistance did not increase upon incubation with the unlabeled "+" strand RNA. The fact that no additional annealing of the [^{3}H]cRNA was observed indicates that all the sequences of [^{3}H]cRNA complementary to "+" strands had already been hybridized.

The hybridization to asymmetric (+) [^{3}H]cRNA suggests that only 30% of the "−" strand sequences are present in the RNA transcribed from SV3T3 DNA. It is also possible, however, that there is a stoichiometric excess of "+" RNA strands in the RNA transcribed from SV3T3 DNA which competes with the asymmetric (+) [^{3}H]cRNA for annealing to "−" RNA strands in the sample. This would prevent complete hybridization of the asymmetric (+) [^{3}H]cRNA even though its entire complement was present in the sample. Therefore, any possible excess of "+" RNA strands was eliminated by pre-annealing the RNA transcribed from SV3T3 DNA and digesting the excess nonannealed strands with RNase. When this RNA was melted and assayed for the presence of "−" RNA sequences a maximum of only 30% protection of the asymmetric (+) [^{3}H]cRNA was observed. Thus, only 30% of the "−" RNA sequences are present in the RNA made from DNA.

The hybridization results are summarized in Table 1. The data indicate that transcription *in vitro* of the "−" strand of SV40 DNA in SV3T3 chromatin is restricted by the presence of DNA-associated protein in those sequences expressed *in vivo*. Upon removal of these proteins, the entire "−" strand becomes available for transcription *in vitro*.

The finding that the "+" strand of SV40 DNA can be transcribed *in vitro* from either SV3T3 chromatin or SV3T3 DNA is interesting because *E. coli* RNA polymerase does not efficiently transcribe the "+" strand when viral DNA is used as template. Introduction of nicks into the viral DNA template does not increase transcription from the +" strand (unpublished data); thus, enhanced initiation of RNA synthesis at nicks in the "+" strand in SV3T3 chromatin or DNA cannot account for transcription of the "+" DNA strand *in vitro*. Moreover, since certain regions (30%) of the "+" DNA strand are preferentially transcribed, it seems likely that this transcription is due to enhanced initiation at a specific site or sites. Such would be the case if transcription of the "+" DNA strand were initiated at a promoter in the adjacent cellular DNA. Thus, the viral sequences adjacent to the cellular

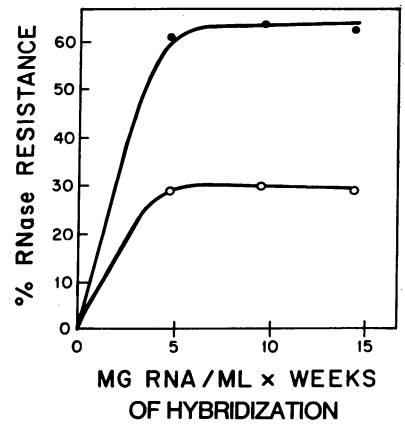

FIG. 4. Annealing of RNA transcribed from SV3T3 DNA with symmetric (●) and asymmetric (○) [^{3}H]cRNA. *In vitro* RNA was transcribed from SV3T3 DNA in a 20-ml standard reaction containing 4.8 mg of SV3T3 DNA and 6 mg of RNA polymerase (3000 units). The reaction mixture was incubated for 2 hr at 37° and then treated for 1 hr with 50 μg/ml of DNase 1. The RNA was purified. 10 mg of RNA was isolated from the reaction. 0.5 ng (10^4 cpm) of denatured, symmetric [^{3}H]cRNA was mixed with *in vitro* RNA at a concentration of 33 mg/ml in a volume of 0.1 ml, sealed in a capillary, and boiled for 12 min to denature the RNA. 0.025-ml Samples of RNA solution were then sealed into capillaries, annealed at 50° for 24, 48, and 72 hr, and assayed for RNase resistance. Annealing with asymmetric [^{3}H]cRNA was performed in exactly the same manner, as described above.

sequences would be transcribed preferentially. Variability in the portion of the "+" DNA strand transcribed by "read through" could be accounted for by variation in the size of the DNA template or the RNA transcripts.

For the above reasons, it is an attractive possibility that the DNA sequences on the "+" strand of SV40 that are transcribed *in vitro* (and *in vivo*) are located adjacent to the cellular DNA sequences and in a position such that their transcription can be controlled by a cellular promoter.

DISCUSSION

Two major conclusions can be drawn from these studies. First, there is restricted transcription *in vitro* of the chromatin template as compared to the DNA template. 100% of the "−" DNA strand of SV40 is transcribed from the SV3T3 DNA template, whereas only 38–41% of that strand is transcribed from the chromatin template. Since DNA was prepared from chromatin by removal of proteins, proteins complexed with the DNA must be involved in restricting the transcription. The conclusion is consistent with the many published reports that the *in vitro* template restriction of chromatin is due to the presence of bound protein (5, 7, 12, 13). The experiments reported here demonstrate that such restriction is not confined to reiterated DNA sequences, but operates at the level of transcription of unique sequences of the DNA.

Second, RNA transcription *in vitro* of the "−" DNA strand of SV40 in SV3T3 chromatin is restricted to those sequences that are expressed *in vivo*. This finding indicates that chromatin as a template for *E. coli* RNA polymerase allows transcription of only those sequences transcribed *in vivo* and is thus appropriate for study of gene regulation in cell-free systems.

A model for transcriptional regulation in eukaryotes implied by these findings is that, *in vivo*, certain regions of the DNA are specifically bound to protein, making them unavailable for transcription by cell RNA polymerase. However, it is possible that the regions of DNA that are transcribed by *E. coli* RNA polymerase are free from protein merely by virtue of their being transcribed *in vivo* at the time the chromatin was isolated. That is, the mammalian RNA polymerase, or other proteins, might be capable of recognizing regions of the chromatin and dissociating the proteins from those regions in the process of transcription. The latter alternative is inconsistent, however, with experiments that indicate that chromatin that has been reconstituted *in vitro* regains the template specificity of natural chromatin (5, 8).

In either of the above cases, however, the experiments presented here imply that there is transcriptional regulation of the SV40 genes in SV3T3 cells since only a fraction of the SV40 DNA sequences in chromatin are available for transcription *in vitro*. Moreover, all the viral sequences transcribed *in vitro* from chromatin are also found in the cell. Thus, it would not be necessary to postulate any post-transcriptional controls to account for the sequences of viral RNA observed in SV40-transformed cells.

I thank Janet MacDowell and June Hatley for technical assistance and Dr. Jesse Summers for his encouragement and helpful advice throughout the course of this work, which was supported by Grants CA-07592, CA-13506, and RR-05539 from the National Institutes of Health and by an appropriation from the Commonwealth of Pennsylvania.

1. Bonner, J., Dahmus, M. E., Fambrough, D., Huang, R. C., Marushige, K. & Tuan, D. Y. H. (1968) *Science* **159**, 47–56.
2. Burgess, R. R. (1969) *J. Biol. Chem.* **244**, 6160–6167.
3. David, I. B. & Wolstenholme, D. R. (1967) *J. Mol. Biol.* **28**, 233–245.
4. Gelb, L. D., Kohne, D. E. & Martin, M. A. (1971) *J. Mol. Biol.* **57**, 129–145.
5. Huang, R. C. C. & Huang, P. C. (1969) *J. Mol. Biol.* **39**, 365–378.
6. Lindstrom, D. M. & Dulbecco, R. (1972) *Proc. Nat. Acad. Sci. USA* **69**, 1517–1520.
7. Paul, J. & Gilmour, R. S. (1966) *Nature* **210**, 992–993.
8. Paul, J. & Gilmour, R. S. (1968) *J. Mol. Biol.* **34**, 305–316.
9. Sambrook, J., Westphal, H., Srinivasan, P. R. & Dulbecco, R. (1968) *Proc. Nat. Acad. Sci. USA* **60**, 1288–1295.
10. Sambrook, J., Sharp, P. A. & Keller, W. (1972) *J. Mol. Biol.* **70**, 57–71.
11. Seligy, V. & Miyagi, M. (1969) *Exp. Cell Res.* **58**, 27–34.
12. Smith, K. D., Church, R. B. & McCarthy, B. J. (1969) *Biochemistry* **8**, 4271–4277.
13. Spelsberg, T. C., Hnilica, L. S. & Ansevin, A. T. (1971) *Biochim. Biophys. Acta* **228**, 550–562.
14. Strauss, J. H., Jr., Kelly, R. B. & Sinsheimer, R. L. (1968) *Biopolymers* **6**, 793–807.
15. Tan, C. H. & Miyagi, M. (1970) *J. Mol. Biol.* **50**, 641–653.
16. Todaro, G. & Green, H. (1966) *Virology* **28**, 756–759.
17. Watkins, J. R. & Dulbecco, R. (1967) *Proc. Nat. Acad. Sci. USA* **58**, 1396–1403.
18. Weinberg, R. A., Ben-Ishai, Z. & Newbold, J. E. (1972) *Nature New Biol.* **238**, 111–113.
19. Westphal, H. (1970) *J. Mol. Biol.* **50**, 407–420.
20. Zimmerman, S. B. & Landeen, G. (1966) *Anal. Biochem.* **14**, 269–277.