Proc. Natl. Acad. Sci. USA
Vol. 77, No. 6, pp. 3297–3301, June 1980
Biochemistry

5,6-Dichloro-1-β-ribofuranosylbenzimidazole enhances premature termination of late transcription of simian virus 40 DNA

(simian virus transcriptional control/transcription complexes/isolated nuclei/simian virus 40 minichromosomes)

ORGAD LAUB, EDWARD B. JAKOBOVITS, AND YOSEF ALONI

Department of Genetics, Weizmann Institute of Science, Rehovot, Israel

Communicated by Igor Tamm, March 20, 1980

ABSTRACT **Short RNA chains initiating at the major promoter sites for simian virus 40 (SV40) late transcription are elongated to approximately 450 nucleotides in a molar amount greater than that from any other region of the viral DNA. This conclusion is based on the following observations: (*i*) Transcriptional complexes isolated by Sarkosyl and by hypotonic leaching (minichromosomes) from nuclei of cells infected with SV40 as well as intact nuclei were pulse labeled *in vitro* with [α-^{32}P]UTP and were observed to synthesize short RNA transcripts that hybridized predominantly to a SV40 DNA fragment spanning between 0.67 and 0.76 map units. (*ii*) In the presence of 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), a drug known to accentuate premature transcriptional termination, accumulation of these short SV40 RNA chains was enhanced. When SV40-infected cells were pretreated with DRB and then labeled *in vivo* or *in vitro*, they synthesized short labeled viral RNAs that hybridized almost exclusively with the DNA fragment spanning between 0.67 and 0.76 map units. These observations suggest a mechanism in the regulation of SV40 late transcription.**

One of the basic questions that must be answered before we understand how cells and viruses transcribe and process their mRNAs is where and how the primary transcripts are initiated and terminated. Biochemical and electron microscopic studies have indicated that transcription of simian virus 40 (SV40) DNA initiates at late times after infection at several alternative sites in a region of the genome that spans between 0.59 and 0.73 map units (m.u.) (1, 2). This conclusion is supported by studies in which the 5′ ends (2–6) and the cap structures (7, 8) of SV40 mRNAs were localized in this region of the physical map of SV40 DNA. Prokaryotes not only control the initiation of transcription at promoter sites, but also they carry out premature termination of transcription at attenuation regions located a few hundred nucleotides downstream (i.e., 3′) from promoters (9–11). The process of attenuation has also been observed in eukaryotes and has been studied mainly in two systems: whole nuclear RNA (12, 13) and adenovirus type 2 (Ad-2) RNA (14, 15). "Attenuators" in eukaryotic systems may be regions of DNA that limit the frequency of transcription of a complete primary transcription unit through a process of premature termination. Although the operational use of this term follows closely its original usage in prokaryotic systems, there is not yet any experimental basis to suggest that the mechanism of attenuation in eukaryotes is either similar to or different from that of prokaryotes.

It has been proposed that 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) blocks the synthesis of heterogeneous nuclear RNA (hnRNA) and Ad-2 RNA by accentuation of premature termination at attenuation regions on the DNA (12–17). Indeed, in the presence of DRB, Ad-2-infected cells continue to synthesize short RNA chains complementary to regions of the viral genome containing start sites for both early and late RNA synthesis (14, 15). DRB, however, was found to inhibit the synthesis of Ad-2 RNAs downstream from the first 400–800 nucleotides (14).

In this paper we describe experiments indicating that a similar attenuation mechanism is operative during SV40 late transcription. Accordingly, there is premature termination of RNA synthesis a few hundred nucleotides downstream from the late transcriptional initiation sites. This mechanism is enhanced in DRB-treated cultures.

MATERIALS AND METHODS

Growth of plaque-purified SV40 on BSC-1 monkey cells as well as concentration and purification of the virus from the tissue culture lysates and preparation of SV40 DNA component I have been described (18). In these experiments BSC-1 cells were infected with 50 to 100 plaque-forming units per cell of stock 777 SV40.

Nuclear fractions were prepared from infected cells with Nonidet-P40 and sodium deoxycholate as described (19), and RNAs were extracted from nuclei with phenol/chloroform/isoamyl alcohol (18).

Purified nuclei capable of cell-free transcription were resuspended in buffer containing 0.1 M $(NH_4)_2SO_4$, 6 mM KCl, 5 mM $CaCl_2$, 1.5 mM $MnCl_2$, 1 mM dithiothreitol, 30 mM Hepes–NaOH at pH 8.0, 12.5% (vol/vol) glycerol, 0.5 mM each of ATP, CTP, and GTP, and 0.1 mCi of [α-^{32}P]UTP (80–250 Ci/mmol, Amersham; 1 Ci = 3.7×10^{10} becquerels). Incubation was at 26°C. [^{32}P]RNA products were extracted as described (18). For *in vitro* RNA synthesis of Sarkosyl-extracted viral transcriptional complexes (VTC) (1, 20) the standard reaction mixture contained 1.0–2.0 ml of Sarkosyl supernatant containing VTC as well as final concentrations of 0.15 M $(NH_4)_2SO_4$, 5 mM KCl, 30 mM Hepes–NaOH at pH 8.0, 1 mM $CaCl_2$, 1.5 mM $MnCl_2$, 1 mM dithiothreitol, 25 μg/ml each of CTP, ATP, and GTP, 0.1 mCi of [α-^{32}P]UTP, and 0.25% Sarkosyl. Incubation was at 26°C. For *in vitro* RNA synthesis of SV40 minichromosomes the reaction mixture contained, in a final volume of 0.3 ml, 2 mM $MnCl_2$, 1 mM dithiothreitol, 80 mM $(NH_4)_2SO_4$, 0.5 mM each of CTP, ATP, and GTP, and 0.03 mCi of [α-^{32}P]UTP (2000–3000 Ci/mmol), to which was added 0.25 ml of nuclear extract (21) containing 50 mM Tris·HCl at pH 7.9, 1 mM $MgCl_2$, and 5 mM 2-mercaptoethanol. Incubation was at 37°C. Radioactive RNA products were extracted from both complexes as described (18).

For the preparation of restriction endonuclease fragments, SV40 DNA was cleaved with *Eco*RI, *Hpa* I, and *Bgl* I, and the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: SV40, simian virus 40; m.u., map unit (1 m.u. is the equivalent of 0.01 of the viral DNA length); Ad-2, adenovirus type 2; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; VTC, viral transcriptional complexes; hnRNA, heterogeneous nuclear RNA.

fragments were separated by 1.4% agarose gel electrophoresis (22). Transfer from the gel onto nitrocellulose paper (Schleicher and Schuell BA85) was carried out by using the technique of Southern (1975) (23). A typical separation of the fragments and their positions on the physical map of SV40 DNA are shown in Fig. 1. Alternatively, for isolation of DNA fragments from the gel, the agarose in the corresponding bands was crushed and the DNA was eluted by shaking in buffer containing 0.2 M NaCl, 10 mM Tris·HCl at pH 7.4, and 5 mM EDTA. After 18 hr at room temperature, the suspension was filtered and phenol extracted and the DNA was precipitated with ethanol. The recovery was 70–90%. The separated DNA fragments were immobilized on nitrocellulose membrane filters and were used for hybridization as described (20).

RESULTS

RNA Synthesized by SV40 VTC and Transcriptionally Active Minichromosomes Initially Is Complementary to an SV40 DNA Fragment Spanning Between 0.67 and 0.76 m.u. *Pulse labeling experiments.* Sarkosyl-extracted VTC (20) and SV40 minichromosomes leached from infected nuclei in the presence of a hypotonic buffer at elevated temperature (21) were incubated *in vitro* in the presence of [α-^{32}P]UTP to allow elongation of preinitiated nascent RNA chains (20). At various periods, [^{32}P]RNA was extracted from both transcriptionally active molecules, purified, and hybridized with the five DNA fragments as shown in Fig. 1. After hybridization, the blots were washed, treated with RNase, and exposed to x-ray films. The results shown in Figs. 2 and 3 indicate that the labeled RNA synthesized during short labeling times (up to 15 min) hybridized mainly with fragment e (0.67–0.76 m.u.) The [^{32}P]RNA synthesized after longer times of incubation *in vitro* hybridized less with fragment e and more with fragment b, which is immediately downstream from fragment e. Low levels of hybridization were noticed also with the other fragments.

Because no initiation of transcription occurs *in vitro* with purified VTC and SV40 minichromosomes (i.e., they elongate preinitiated nascent RNA chains *in vitro*) (20, 24), we estimate from the radioactivity associated with the various bands that, *in vivo*, a greater molar amount of RNA was synthesized from the first 450 nucleotides of the transcriptional unit (the length of fragment e) than from any other region of the viral DNA. Similar accumulation of short labeled RNA chains was observed also after an [α-^{32}P]UTP pulse (2 min) in isolated nuclei (see Fig. 5A). Because linear incorporation of [α-^{32}P]UMP by VTC continues during at least the first 60 min of incubation *in vitro*

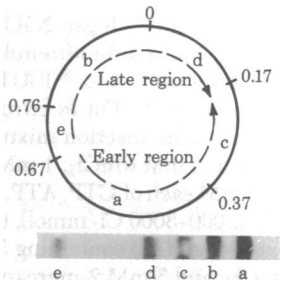

FIG. 1. Map positions of restriction endonuclease fragments produced by cleavage of form I SV40 DNA with *Eco*RI, *Hpa* I, and *Bgl* I. The cleavage sites of the restriction enzymes are: *Eco*RI, 0.0; *Hpa* I, 0.17, 0.37, and 0.76; *Bgl* I, 0.67. The strip below the map shows the result of fractionating the fragments on a 1.4% agarose gel (22), blotting the DNA onto a nitrocellulose membrane filter (23), and then hybridizing ^{32}P-labeled nick-translated SV40 DNA to the DNA filter. After hybridization the membrane filter was washed, dried, and exposed to an x-ray film. The intensities of the bands are approximately proportional to the length of the DNA fragments.

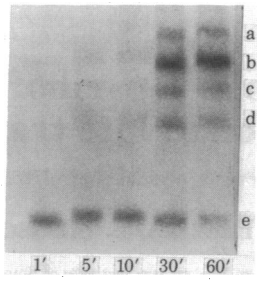

FIG. 2. Analysis of VTC RNA elongated *in vitro* in the presence of [α-^{32}P]UTP for various lengths of time. At indicated times (min) [^{32}P]RNA was purified and hybridized to a Southern blot (23) containing the five SV40 DNA fragments as shown in Fig. 1. After hybridization the blots were washed, treated with RNase, dried, and exposed to an x-ray film. After preliminary experiments the input radioactivity on each blot was adjusted to give similar intensities with fragment e. This allows a better comparison of the relative hybridization with the other fragments.

(20), we conclude that RNA polymerase can elongate *in vitro* the prematurely terminated RNA chains. Also, it appears from this experiment that elongation proceeds *in vitro* at a slow rate (about 10–30 nucleotides per minute) (25).

"Chase-pulse" experiment. Additional evidence indicating that RNA polymerase can transcribe DNA sequences downstream from fragment e (0.67–0.76 m.u.) (fragments b and d, see Fig. 1) *in vitro* was obtained by the following experiment. VTC were incubated *in vitro* for 5 min in the presence of [α-^{32}P]UTP either with or without preincubation for 20 min with unlabeled ribonucleotides. [^{32}P]RNA was then extracted, purified, and hybridized to Southern blots containing the five DNA fragments as shown in Fig. 1. The results summarized in Fig. 4 show that the RNA elongated during the first 5 min hybridized primarily to fragment e (0.67–0.76 m.u.), whereas the labeled RNA elongated during the last 5 min hybridized primarily to fragment b. Hybridization to fragment a would indicate cell-free transcription from the viral E-strand (0.67–0.37 m.u.) in a counterclockwise direction.

Effect of DRB on *in Vivo* and *in Vitro* Transcription of SV40. *Effect of DRB on* in vivo *transcription of SV40 RNA.* If DRB has the same effect on SV40 transcription as it has on nuclear and Ad-2 transcription (for review see refs. 17 and 26), then we expect that the addition of DRB to SV40-infected cells will have a less inhibitory effect on the transcription of viral sequences adjacent to initiation sites than on viral sequences downstream from the sites of initiation.

SV40-infected cells were pretreated 48 hr after infection for 30 min with DRB at 25 μg/ml and then labeled with [5,6-

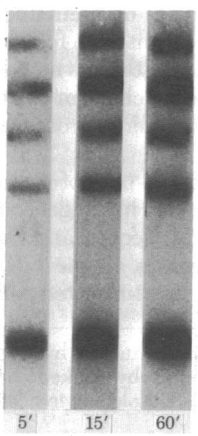

FIG. 3. Analysis of viral RNA elongated *in vitro* on SV40 minichromosomes in the presence of [α-^{32}P]UTP for various lengths of time. At the indicated times (min) [^{32}P]RNA was purified and hybridized to a Southern blot as in Fig. 2. In this experiment, equal volumes of assay mix were hybridized to the blot in order to show the kinetics of incorporation of the reaction.

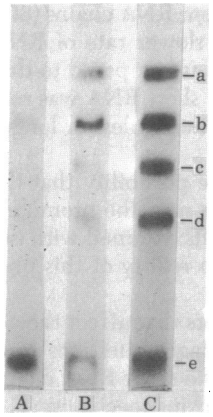

FIG. 4. Analysis of [^{32}P]RNA synthesized by VTC after a pulse and a "chase-pulse." Preparations of VTC were pulse labeled for 5 min, using 0.1 mCi of [α-^{32}P]UTP with or without preincubation for 20 min with 0.4 mM CTP, 0.4 mM GTP, 0.4 mM ATP, 0.02 mM UTP. Labeled RNAs were extracted, purified, and hybridized to blots (see Fig. 1). Strip A is the result of a control experiment without preincubation with unlabeled ribonucleotides. Strip B was obtained after preincubation with unlabeled ribonucleotides. Strip C is a control with nick-translated SV40 [^{32}P]DNA.

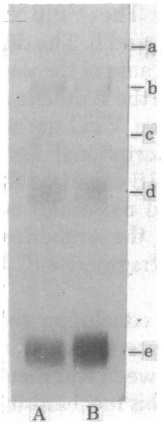

FIG. 5. Effect of DRB on transcription of SV40 in isolated nuclei. SV40-infected cells (10^7 cells) were treated with DRB at 25 μg/ml for 30 min 48 hr after infection. A control infected culture was treated with a solution containing no DRB. Nonidet-P40 was added to the medium and nuclei were purified by low-speed centrifugation. The nuclei were incubated *in vitro* for 2 min in the presence of [α-^{32}P]UTP and then [^{32}P]RNA was extracted. [^{32}P]RNA from the control culture (A) and from the DRB-treated culture (B) was hybridized to a Southern blot containing the five fragments shown in Fig. 1. After hybridization the strips were washed, treated with RNase, and exposed to an x-ray film.

^{3}H]uridine for 20 min in the continued presence of the drug. Control cultures containing an identical number of infected cells were labeled under the same conditions in the absence of DRB. The labeled RNA was extracted from the nuclei of the DRB-treated and untreated cultures.

Form I SV40 DNA was cleaved with the restriction endonucleases *Eco*RI, *Hpa* I, and *Bgl* I, and the five fragments were separated on an agarose gel (see Fig. 1) and eluted from the gel, and each fragment was bound to a nitrocellulose filter. The [^{3}H]RNAs of DRB-treated and untreated cultures then were hybridized with these filters. The results obtained are summarized in Table 1. It is evident that DRB inhibited the synthesis of viral RNA complementary to fragments a, b, c, and d by more than 90%, while the synthesis of RNA complementary to fragment e was inhibited by only 50%. We have previously shown that the major initiation sites for late transcription are contained within fragment e (1). Therefore we could conclude that, as in high molecular weight nuclear RNA and Ad-2 mRNA (13), DRB inhibited synthesis of late SV40 RNAs at a site(s) only a few hundred nucleotides downstream from their initiation sites.

Effect of DRB on in vitro *transcription of SV40 RNA.* Because it is known that the effect of DRB is reversible (17, 26), we avoided extensive washing of nuclei and VTC isolated from DRB-treated cells. In addition, the preparations were carried out as rapidly as was possible. In the first experiment, isolated nuclei prepared from either DRB-treated or untreated cultures were incubated *in vitro* in the presence of [α-^{32}P]UTP for only 2 min. [^{32}P]RNA was extracted, purified, and hybridized to filters containing the five restriction endonuclease fragments shown in Fig. 1. Fig. 5 shows that the [^{32}P]RNA from both DRB-treated and untreated cultures hybridized mainly to the three late fragments (e, b, and d). It is also evident that in both cases the amount of radioactivity associated with fragment e was relatively higher than that associated with the other two late fragments, similar to the results obtained when RNA from isolated VTC and transcriptionally active minichromosomes were analyzed (see Figs. 2 and 3). Finally, it is apparent that the relative amount of [^{32}P]RNA obtained from the DRB-treated cultures hybridizing with fragment e was higher (about 2-fold) than the [^{32}P]RNA of untreated cultures.

The enhancement of termination effected by DRB was also apparent in experiments in which isolated nuclei and VTC from DRB-treated and untreated cultures were incubated *in vitro* with [α-^{32}P]UTP for longer times. Fig. 6 shows that the [^{32}P]RNA synthesized within 10 min in nuclei isolated from untreated cultures hybridized to all five fragments, but mainly to the late fragments e, b, and d. This observation presumably reflects the continued transcription of the late region of the genome and the beginning of the accumulation of the nuclear viral RNA species (27). The hybridization observed with fragments a and c (Fig. 6) is because of the transcription, in a counterclockwise direction, of the E-strand (27, 28). It is apparent, however, that the [^{32}P]RNA synthesized in the nuclei of the DRB-treated cultures hybridized mainly with fragment e (A in Fig. 6). A similar effect of DRB on hnRNA in nuclei isolated from HeLa cells has recently been reported (13).

A similar pattern of hybridization was observed with [^{32}P]RNA synthesized *in vitro* by VTC. In this case, the accumulation of short nascent chains was noticeable even after

Table 1. Effect of DRB on SV40 transcription

[^{3}H]RNA source	cpm hybridized to SV40 DNA fragments				
	e	b	d	c	a
DRB-treated cultures	60	80	50	30	25
Control cultures	120	1150	600	440	295
DRB resistant, %	50	7	8	7	8

SV40-infected cells (10^7 cells) were treated with DRB at 25 μg/ml for 30 min, 48 hr after infection, and then were labeled for 20 min with 1 mCi of [5,6-^{3}H]uridine in the continued presence of the drug. Nuclear [^{3}H]RNA was extracted and purified. Control cultures received no DRB. The [^{3}H]RNA preparations were hybridized to the five SV40 DNA fragments (shown in Fig. 1) which were immobilized on nitrocellulose membrane filters. The control filters displayed about 20 cpm, and this amount was subtracted from the cpm that hybridized to the filters containing SV40 DNA. Each filter was counted for 30 min. Similar results were obtained in three independent experiments.

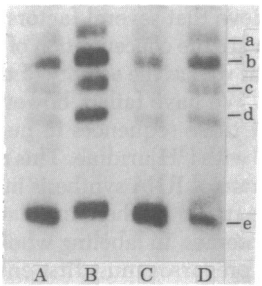

FIG. 6. Effect of DRB on SV40 transcription in isolated nuclei and VTC. SV40-infected cells (10^7 cells) were treated with DRB at 25 μg/ml for 30 min 48 hr after infection. Control infected cultures were treated with a solution not containing DRB. Isolated nuclei and VTC were incubated at 26°C in the presence of 0.1 mCi of [α-^{32}P]UTP for 10 min (nuclei) or 30 min (VTC). [^{32}P]RNAs were extracted and hybridized with the five DNA fragments shown in Fig. 1. After hybridization the filters were washed, treated with RNase, and exposed to an x-ray film. A and C are DRB-treated nuclei and VTC, respectively. B and D are the controls for nuclei and VTC, respectively.

longer durations of *in vitro* synthesis (30 min). The [^{32}P]RNA synthesized hybridized mainly to fragments e and b. The difference between isolated nuclei and VTC is because of the slow rate of RNA synthesis on VTC as compared to the synthesis in isolated nuclei (25) and, presumably, because VTC do not contain the machinery controlling posttranscriptional RNA processing (20, 24). It is clear, however, that DRB enhanced the accumulation of the prematurely terminated molecules because, even after 30 min of incubation *in vitro*, the synthesized [^{32}P]RNA hybridized almost exclusively with fragment e (C in Fig. 6).

The above experiments thus support the conclusion that during late transcription of SV40 there is premature transcriptional termination near the junction between fragments e and b (0.76 m.u.) and that DRB enhances this mechanism.

DISCUSSION

In the present communication, we describe experiments that demonstrate that RNA chains initiating at the major initiation sites of SV40 late transcription are synthesized up to approximately 450 nucleotides in length, in greater molar amounts than from any other region of the viral DNA. Moreover, in the presence of DRB (29), a drug known to accentuate premature transcriptional termination (17, 26), the accumulation of these short RNA chains is enhanced. Similar observations were reported previously for the transcription of hnRNA in mammalian cells and for late transcription of Ad-2 (12–17).

The accumulation of short SV40 RNA was evidenced in the analysis of the nascent chains attached to Sarkosyl-extracted transcriptional complexes (VTC) and to SV40 minichromosomes and during *in vitro* transcription in nuclei isolated from SV40-infected cells. The fact that the analysis of the RNA extracted from isolated nuclei gave results similar to those obtained with the nascent RNA attached to the transcriptional complexes indicates that the complexes were not a selective class of molecules. We have shown the accumulation of short nascent chains previously by electron microscopy analysis of SV40 transcriptional complexes (1). We have observed a variation in the exact time during *in vitro* incubation after which the large amount of labeled RNA complementary to fragment e (0.67–0.76 m.u.) starts to be reduced. In all our kinetic experiments the excess molar amount of the short RNA was noticeable after 0.5–1 min of *in vitro* incubation, whereas the reduction of the molar excess of e fragment sequences occurred after 3–15 min of *in vitro* incubation. This variation may explain why in our previous study (1) the hybridization with fragment e of RNA synthesized *in vitro* for 3 min was not as pronounced as in Fig. 1. We believe that several factors can affect this timing—among them are the concentration of nucleotides and salt, temperature, etc, as well as the activity of the endogenous RNA polymerase itself. We have failed, however, to detect higher molar amounts of these sequences in nuclear RNA purified from cells labeled with [^{3}H]uridine. This may result from several factors: The rate of RNA synthesis in whole cells is faster as compared to the rate of synthesis in isolated nuclei, VTC, and SV40 minichromosomes. In labeling whole cells with [^{3}H]uridine, the labeled precursor must first enter into equilibrium with the cellular pool before it is incorporated into RNA. This is accentuated even more by the fact that the original specific activity of the [^{3}H]uridine is lower than that of the [^{32}P]UTP used in our studies. In addition, the accumulation of mature nuclear RNA complicates the *in vivo* analysis. We estimate, therefore, that *in vivo* a very short pulse is needed, in the range of several seconds, in order to enrich significantly for nascent RNA molecules, and such experiments are difficult to perform. Indeed, in a similar experiment with the larger Ad-2 genome, a 45-sec pulse was needed to detect nascent RNA chains (30). Finally, we believe that because of the slower rate of RNA synthesis in VTC and minichromosomes as compared to that in isolated nuclei, the enrichment of the short RNA was recognizable in the transcriptionally active molecules for longer periods of *in vitro* incubation.

We have not completely excluded the possibility that the molar excess of the short RNA molecules near the promoters is an *in vitro* artifact. However, the results obtained with isolated nuclei strongly support the *in vivo* reality of this phenomenon.

In previous studies investigating factors that affect the accumulation of short RNA (12–17) two main questions were discussed: (*i*) whether the synthesis of the short RNA chains results from a pausing effect or from a blocking mechanism (14) and (*ii*) whether DRB terminates chain growth *in vivo* at sites of no physiological significance or accentuates a normal mechanism for transcription termination (17, 26).

When phage $Q\beta$ replicase polymerized midvariant RNA, a relatively small number of specific sites in the RNA were identified at which oligonucleotide chain elongation halted (31). Once such a pause site was encountered by the replicase, there was a finite probability that elongation continued. It was noticed that the progress of the replicase was slowed at the end of a hairpin structure. Thus it was concluded (31) that, in all processive nucleic acid–polymerase reactions, chain elongation occurs at a markedly variable rate and that the kinetics of polymerization are determined by the nature of the template. In studies with hnRNA in mammalian cells and Ad-2 RNA, (reviewed in refs. 17 and 26) it was postulated that the attenuator might function as a termination signal by which the cell governs the fraction of initiated RNA polymerase II molecules that will transcribe an entire transcriptional unit and that DRB enhances the premature termination. It is difficult to determine whether the accumulation of the short chains of SV40 late RNA is the result of a pausing effect or premature termination, because it is difficult to perform a meaningful pulse-chase experiment in eukaryotic cells. However, for the following reasons, we favor the possibility of premature termination: (*i*) The observation that DRB enhances the accumulation of the short nascent RNA chains in the present study suggests a similar mechanism of premature termination observed for transcription of hnRNA in mammalian cells and Ad-2 (13, 14). (*ii*) A termination signal lies in the e fragment at about 0.74 m.u. (32). It is composed of a stretch of five dA residues preceded by four d(G·C) pairs; furthermore, RNA copied from the template preceding these nine nucleotides can form a terminal hairpin loop structure similar to that in the terminal regions of bacterial transcripts. This site is therefore a potential signal for termination of transcripts of either short RNA or, as suggested by Lebowitz and Weissman (32), of transcripts of about unit genome length.

To explain the results indicating that DRB acts not at the point of initiation but a short distance downstream, Tamm and Sehgal (26) proposed that RNA polymerase II may function in two forms, of which the DRB-resistant form initiates transcripts and the sensitive form carries out a specific step in transcription a short distance downstream. Support for this hypothesis came from the observation that RNA polymerase II in lysates of Ehrlich ascites cells appears heterogeneous, in that it exhibits two salt optima (33). It is possible, however, that DRB may not interact with the RNA polymerase molecule itself, but with a component that plays a regulatory role in RNA transcription (26). It is interesting to note that the sites at which DRB blocks transcription of SV40 late RNA are approximately the sites where the 3′ ends of the leader sequences of late mRNAs are

located (5, 34–38). This region is also the end of a region in the SV40 minichromosome that is devoid of nucleosomes (39, 40) and is a region of the chromosome that was found to be highly sensitive to nuclease digestion (41–43). The correlation between the structure of the SV40 minichromosome and premature transcriptional termination is attractive.

We thank Drs. D. Roufa and Y. Groner for critical reading of the manuscript. This research was supported by U.S. Public Health Service Research Grant CA 14995.

1. Laub, O., Bratosin, S., Horowitz, M. & Aloni, Y. (1979) *Virology* **92,** 310–323.
2. Horowitz, M., Laub, O., Bratosin, S. & Aloni, Y. (1978) *Nature (London),* **257,** 558–559.
3. Lai, C. J., Dhar, R. & Khoury, G. (1978) *Cell* **14,** 971–982.
4. Villarreal, L., White, R. & Berg, P. (1979) *J. Virol.* **29,** 209–219.
5. Ghosh, P. K., Reddy, V. B., Swinscoe, J., Lebowitz, P. & Weissmann, S. M. (1978) *J. Mol. Biol.* **126,** 813–846.
6. Bina-Stein, M., Thoren, M., Salzman, N. & Thompson, J. A. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 731–735.
7. Canaani, D., Kahana, C., Mukamel, A. & Groner, Y. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 3078–3082.
8. Aloni, Y., Dhar, R. & Khoury, G. (1979) *J. Virol.* **32,** 52–60.
9. Zurawski, G., Brown, K., Killingly, D. & Yanofsky, C. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4271–4275.
10. Di Nocera, P. P., Blasi, F., De Lauro, R., Frunzio, R. & Bruni, C. B. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4276–4280.
11. Barnes, M. W. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 4281–4285.
12. Tamm, I. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5011–5015.
13. Tamm, I. & Kikuchi, T. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5750–5754.
14. Fraser, N. W., Sehgal, P. B. & Darnell, J. E., Jr. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 2571–2575.
15. Sehgal, P. B., Fraser, N. W. & Darnell, J. E., Jr. (1979) *Virology* **94,** 184–191.
16. Fraser, N. W., Sehgal, P. B. & Darnell, J. E., Jr. (1978) *Nature (London)* **272,** 590–593.
17. Tamm, I. & Sehgal, P. B. (1979) in *Alfred Benzon Symposium 13: Specific Eukaryotic Genes,* eds. Engberg, J., Klenow, H. & Leick, V. (Munksgaard, Copenhagen), pp. 424–439.
18. Laub, O. & Aloni, Y. (1975) *J. Virol.* **16,** 1171–1183.
19. Penman, S. (1966). *J. Mol. Biol.* **17,** 117–130.
20. Laub, O. & Aloni, Y. (1976) *Virology* **75,** 346–354.
21. Jakobovits, E. B. & Aloni, Y. (1980) *Virology* **102,** 107–118.
22. Sharp, P. A., Sugden, B. & Sambrook, J. (1973) *Biochemistry* **13,** 3055–3063.
23. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
24. Ferdinand, F. J., Brown, M. & Khoury, G. (1977) *Virology* **78,** 150–161.
25. Shani, M., Birkenmeier, E., May, E. & Salzman, N. P. (1977) *J. Virol.* **23,** 20–28.
26. Tamm, I. & Sehgal, P. B. (1979) *Bristol-Myers Symposium: Effect of Drugs on the Cell Nucleus,* ed. Busch, H. (Academic, New York), in press.
27. Aloni, Y. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39,** 165–178.
28. Acheson, N. H. (1976) *Cell* **8,** 1–12.
29. Tamm, I., Folkers, K., Shunk, C. H. & Horsfall, F. L., Jr. (1954) *J. Exp. Med.* **99,** 227–250.
30. Evans, R., Weber, J., Ziff, E. & Darnell, J. E. (1979) *Nature (London)* **278,** 367–370.
31. Milles, D. R., Dobkin, C. & Kramer, F. R. (1978) *Cell* **15,** 541–550.
32. Lebowitz, P. & Weissman, S. (1979) *Curr. Top. Microbiol. Immunol.* **87,** 43–172.
33. Dreyer, C. & Hausen, P. (1978) *Nucleic Acids Res.* **5,** 3332–3335.
34. Aloni, Y., Dhar, R., Laub, O., Horowitz, M. & Khoury, G. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3686–3690.
35. Hsu, M. & Ford, J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4982–4985.
36. Lavi, S. & Groner, Y. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5323–5327.
37. Bratosin, S., Horowitz, M., Laub, O. & Aloni, Y. (1978) *Cell* **13,** 783–790.
38. Haegeman, G. & Fiers, W. (1978) *Nucleic Acids Res.* **5,** 2359–2371.
39. Jakobovits, E. B., Bratosin, S. & Aloni, Y. (1980) *Nature (London)* **85,** 263–265.
40. Saragosti, S., Moyne, G. & Yaniv, M. (1980) *Cell* **20,** 67–75.
41. Varshavsky, A. J., Sundin, O. & Bohn, M. (1979) *Cell* **16,** 453–466.
42. Scott, W. A. & Wigmore, D. J. (1978) *Cell* **15,** 1511–1518.
43. Waldeck, W., Fohring, B., Chowdhury, K., Gruss, P. & Sauer, G. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5964–5968.