Synthesis of Prematurely Terminated Late Transcripts of Polyoma Virus DNA is Resistant to Inhibition by 5,6-Dichloro-1-β-D-ribofuranosylbenzimidazole

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SUMMARY
Exposure of mouse kidney cells to 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) (75 to 300 μM) during the late phase of infection by polyoma virus resulted in nearly complete (90 to 98%) inhibition of virus RNA synthesis. Sedimentation analysis revealed that, although the synthesis of high mol. wt. (> 10S) virus RNA was inhibited in a manner parallel to that of total virus RNA, the synthesis of small (3S to 7S) virus RNA was inhibited by only 40 to 50% in the presence of DRB. As a result, virus RNA synthesized in the presence of DRB contained a peak at 3S to 7S in addition to residual high mol. wt. virus RNA. Small virus RNA from either untreated or DRB-treated cells contained three- to sixfold higher levels of transcripts from the DNA fragment which lies between the BamHI and BglI sites (58.0 to 72.2 map units) than from DNA fragments covering the rest of the virus genome. Furthermore, 80% of the small RNA which hybridized to this fragment was complementary to the L strand of virus DNA. These results suggest that L strand transcripts are initiated within the BglI–BamHI DNA fragment and that a portion of these transcripts is prematurely terminated within several hundred nucleotides of the site(s) of initiation. DRB had little effect on the synthesis of these prematurely terminated RNAs.

INTRODUCTION
The site(s) at which transcription is initiated on the L strand of polyoma virus DNA during productive infection are not yet known with certainty. Analysis of L strand nuclear RNA and mRNA revealed the existence of a number of different capped 5' ends which hybridize to virus DNA between 66 and 69 map units on the virus genome (Flavell et al., 1979, 1980; Treisman, 1980; Treisman & Kamen, 1981). However, there is some indirect evidence that capping may happen elsewhere than at the initial nucleotide in an RNA chain (Schibler & Perry, 1976; Spencer et al., 1978). On the other hand, recent studies using permeabilized cells or isolated nuclei (Contreras & Fiers, 1981; Gidoni et al., 1981) and in vitro transcription systems (Handa et al., 1981) have shown that initiation of transcription can take place at capping sites on the DNA of the related simian virus 40 (SV40). The present study was undertaken to provide independent evidence for the in vivo location of transcriptional initiation sites.

The drug 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB), an analogue of purine ribonucleosides, selectively inhibits RNA synthesis directed by RNA polymerase II in a variety of cell types (Egyhazi, 1974; Granick, 1975; Tamm et al., 1976; Sehgal et al., 1976). The mechanism by which DRB inhibits RNA synthesis has been carefully studied in the case

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of adenovirus transcription (Fraser et al., 1978, 1979; Sehgal et al., 1979). In the presence of DRB, adenovirus RNA chains whose synthesis had begun prior to addition of the drug continue to be elongated, but the initiation of the synthesis of full-length RNA is inhibited. Nevertheless, there is a residual synthesis of short (400 to 800 nucleotides) RNA chains from DNA sequences immediately downstream from adenovirus promoters (Fraser et al., 1978; Sehgal et al., 1979). One interpretation of these results is that DRB causes premature RNA chain termination shortly after initiation of RNA synthesis (Fraser et al., 1978).

These studies have suggested a way to map the sites where transcription initiates in other systems by locating the site of synthesis of DRB-resistant RNA on its template DNA. To this end, we have studied the effect of DRB on the synthesis of polyoma virus RNA during the late phase of infection. In the presence of DRB, the synthesis of high mol. wt. virus RNA is strongly inhibited, but there is residual synthesis of small (3S to 7S) virus RNA. Synthesis of small virus RNA is also apparent in the absence of DRB. A significant fraction of small virus RNA from untreated or from DRB-treated cells hybridizes with the L strand of a fragment of polyoma DNA located just downstream (in the direction of L strand transcription) from the origin of virus DNA replication. This DNA fragment (map units 58.0 to 72.2) contains the sites from which the capped 5' ends of polyoma virus nuclear and cytoplasmic L strand RNAs are transcribed.

METHODS

DRB treatment, labelling and extraction of RNA. Primary cultures of mouse kidney cells (10^7 cells per 100 mm Petri dish) were infected with plaque-purified polyoma virus at an m.o.i. of 20 to 40. Cells were incubated with DRB and labelled with [5-3H]uridine as specified in the text. Labelling was terminated by washing cells with ice-cold 25 mM-tris-HCl pH 7.5, 140 mM-NaCl, 5 mM-KCl, 0.3 mM-Na_2HPO_4 followed by cold 10 mM-triethanolamine-HCl pH 8.5, 10 mM-NaCl, 1.5 mM-MgCl_2 (RSB; Penman, 1966). Cells were then scraped into 0.5 ml of the same buffer per Petri dish. Aliquots of the cell suspension were removed for determination of total cell-associated ^3H radioactivity. After addition of 1 vol. 1% Nonidet P40 in 10% trichloroacetic acid (TCA), these aliquots were spotted on to GF/C filters (Whatman) which were then dried and counted in a toluene-based scintillation mixture. The remainder of the cell suspension was adjusted to 4 ml per Petri dish with RSB containing 10 mM-EDTA instead of 1.5 mM-MgCl_2. Nucleic acids were extracted with 1 vol. phenol–chloroform–isoamyl alcohol (24:24:1) in the presence of 2% sodium tri-isopropyl-naphthalenesulphonate (Serva, Heidelberg, F.R.G.) and subsequently treated with deoxyribonuclease I, as described previously (Acheson & Miéville, 1978).

Preparation of restriction endonuclease fragments of virus DNA. A series of five fragments of polyoma virus DNA which cover nearly the entire virus genome (see Fig. 4) was prepared by digestion of polyoma DNA form I with restriction endonucleases BglI, BamHI, HindIII, EcoRI and XhoI (all from New England Biolabs, Beverly, Mass., U.S.A.). Fragments were separated by electrophoresis on acrylamide–agarose or low-melting agarose gels and eluted by electrophoresis (Acheson & Miéville, 1978) or by phenol extraction from melted agarose (McMaster et al., 1981). Complementary strands of the BamHI–BglI fragment ('fragment 1') were prepared as previously described for HpaII fragments (Acheson & Miéville, 1978). The E and L strands of fragment 1 were identified by hybridization of filter-bound separated strands to late nuclear virus RNA labelled for 20 min with [3H]uridine. Virus RNA so prepared contains mainly L strand transcripts (Acheson & Miéville, 1978). In one experiment, 190 ct/min hybridized with the slowly migrating strand and 23 ct/min with the fast-migrating strand. Thus, the slowly migrating strand of fragment 1 corresponds to the L strand of virus DNA and the fast-migrating strand corresponds to the E strand.

Nucleic acid hybridization. Purified DNA fragments and intact polyoma DNA (further
purified by alkaline sedimentation) were loaded on to Selectron BA-85 nitrocellulose filters after denaturation by boiling in 0.1 × SSC (SSC: 0.15 M-NaCl plus 0.015 M-sodium citrate) and dilution into cold 6 × SSC. Purified separated strands were loaded on to filters without prior denaturation (Acheson & Miéville, 1978). After baking at 80 °C for 2 h in vacuo, filters were cut into 3.5 mm squares, each containing the amount of DNA that would be present in 0.2 µg of intact polyoma DNA.

Hybridizations were performed in such a way that it was possible to measure either the total amount of labelled virus RNA and DNA in a sample, or the amount of (alkali-resistant) virus DNA alone. A 70 µl sample of labelled nucleic acid in 0.01 M-triethanolamine pH 7.4 was adjusted to 0.3 M-NaOH by adding 30 µl 1 M-NaOH. For determination of total labelled virus nucleic acid (RNA and any residual virus DNA) the sample was neutralized by adding 30 µl 1 M-HCl, 14 µl 20 × SSC and 35 µl 1 M-HEPES buffer (Calbiochem) pH 7.45, immediately after NaOH addition. Determination of residual labelled virus DNA was done by incubating the sample in 0.3 M-NaOH at 37 °C overnight before neutralization. In these conditions RNA is degraded while DNA remains intact. Labelled nucleic acids (or residual labelled DNA) were then hybridized with two 3.5 mm square nitrocellulose filters containing polyoma DNA and one blank filter per 180 µl sample. After 40 to 44 h at 65 °C, filters were rinsed with 2 × SSC, treated with ribonuclease A, washed again with 2 × SSC and counted in a toluene-based scintillation mixture as described previously (Acheson & Miéville, 1978). When total ct/min per filter were low (<100 ct/min) counting was carried out for three 10 min periods under conditions where the background counting was less than 3 ct/min.

RESULTS

Effect of DRB on cellular and virus RNA synthesis

DRB inhibits uptake of [3H]uridine into mammalian cells (Sehgal et al., 1976; Tamm et al., 1976). Measurement of the effect of DRB on RNA synthesis by incorporation of exogenous [3H]uridine must therefore be corrected for this inhibition. Baby mouse kidney cells infected with polyoma virus were incubated with 0, 75, 150 or 300 µM-DRB for 30 min beginning 29.5 h after infection. Cells were then labelled for 15 min with [3H]uridine in the presence of the same concentrations of DRB. An aliquot of the washed cells was removed and total cell-associated 3H radioactivity, which is a measure of [3H]uridine uptake, was determined (Fig. 1). RNA was extracted from the remainder of each sample and its specific radioactivity was determined. These values, and those shown in subsequent figures and tables where noted, were corrected for reduced uridine transport as determined in each experiment. Fig. 1 shows that DRB inhibited total (cellular plus virus) RNA synthesis by about 50% at 75 µM, by about 65% at 150 µM and by about 77% at 300 µM.

The mean half-life of late nuclear polyoma virus RNA is about 1 h (Acheson, 1981); thus, incorporation of [3H]uridine into virus RNA during a 15 min labelling period should give an estimate of the rate of virus RNA synthesis. The 3H-labelled virus RNA in each sample was measured by hybridization to an excess of filter-bound polyoma DNA, and the specific radioactivity in virus RNA was determined and corrected for uridine uptake. Fig. 1 shows that virus RNA synthesis was strongly inhibited by DRB in a dose-dependent manner. At a DRB concentration of 300 µM, residual virus RNA synthesis was only 2% of that found in untreated cells. Thus, polyoma virus RNA synthesis was almost completely inhibited by DRB, while cellular RNA synthesis was only partially inhibited. These results are consistent with previous findings (Granick, 1975; Tamm et al., 1976; Tamm, 1977; Fraser et al., 1978) that DRB preferentially inhibits RNA synthesis directed by RNA polymerase II, which is responsible for transcription of polyoma virus DNA (Shmookler et al., 1974).
Fig. 1. Effect of DRB on [3H]uridine uptake, total RNA synthesis, and polyoma virus late RNA synthesis. Cells were treated with DRB and labelled as described in the text. Infected and untreated cells were similarly labelled. Cells were washed and harvested in RSB buffer pH 8.5. An aliquot was removed from each cell suspension to determine total cell-associated [3H] radioactivity. RNA was extracted as described in Methods. Incorporation of radioactivity into total (cellular plus virus) RNA and virus RNA are expressed as 5% TCA-insoluble ct/min per μg RNA and hybrid ct/min per μg RNA respectively. These values have been corrected for the effect of DRB on [3H]uridine uptake by multiplying by 2, 2.7 or 4.4 at DRB concentrations of 75, 150 or 300 μM. Untreated cells incorporated 6600 ct/min per μg into acid-insoluble RNA and 702 ct/min per μg into virus RNA; one Petri dish contained about 200 μg RNA. □, [3H]uridine uptake; ○, 3H ct/min in total RNA; ●, 3H ct/min in virus RNA.

Size of DRB-resistant virus RNA

We carried out the following experiments to determine the size of the residual virus RNA synthesized in the presence of DRB. Infected cells were labelled as before with [3H]uridine for 15 min in the presence of various concentrations of DRB. RNA was extracted and analysed by sedimentation on sucrose gradients, and virus RNA was detected by hybridization of aliquots of each gradient fraction with an excess of filter-bound virus DNA.

Fig. 2(a) shows that virus RNA synthesized in untreated cells sedimented as a heterogeneous collection of molecules mainly in the region of 18S to 60S. These RNAs, some of which are already processed within minutes of their synthesis (N. H. Acheson, unpublished results), probably arise by continuous transcription of the L strand of circular virus DNA for several cycles before termination occurs (Acheson et al., 1971; Acheson, 1978). A small amount of labelled virus RNA sedimented near the top of the gradient in the 4S region. Virus RNA synthesized in the presence of DRB contained progressively less and less high mol. wt. species as the drug concentration was increased from 75 to 300 μM (data shown only for 300 μM-DRB, Fig. 2b). However, virus RNA which sedimented in the 4S region now showed up as a peak. The synthesis of this small virus RNA is thus relatively resistant to DRB. This is more clearly seen by comparing the ratios of ct/min in virus RNA synthesized in the presence versus the absence of the drug (see insert, Fig. 2b). The synthesis of high mol. wt. virus RNA was inhibited by 92 to 98%, while the synthesis of small virus RNA was approx. 50% of that detected in untreated cells.

To examine more closely the slowly sedimenting virus RNA, we analysed RNAs labelled for 1 h in untreated or in DRB-treated cells by sedimentation on sucrose gradients under conditions such that 18S ribosomal RNA sediments nearly to the bottom of the gradient. Hybridization of these gradients (Fig. 3) more clearly revealed the presence of small amounts of labelled virus RNA in the 4S region both in untreated and in DRB-treated cells. Most of the high mol. wt. RNA in both samples had pelleted under these centrifugation conditions. The inset of Fig. 3(b) again shows that the synthesis of virus RNA in the 4S region was much more resistant to DRB than that of high mol. wt. virus RNA. However, there was no indication that the DRB-resistant RNA was of a well-defined molecular size.

The existence of such a small virus RNA is in agreement with the hypothesis that a molar
excess of promoter-proximal RNA is synthesized and that its synthesis is not inhibited in the presence of DRB. In the following sections we describe experiments which localize the site(s) from which this small virus RNA is transcribed.
Fig. 3. Sedimentation profiles of virus RNA smaller than 18S in DRB-treated and untreated cells. Cells were treated with 75 μM-DRB and labelled for 1 h as in previous experiments. Untreated infected cells were similarly labelled. RNA samples were denatured by heating at 80 °C for 2.5 min in 1.5 ml 10 mM-EDTA, 0-1% SDS and layered on to 37 ml 15 to 30% (w/w) sucrose gradients. Sedimentation was for 46 h at 24000 rev/min in a Spinco SW27 rotor at 20 °C. Virus RNA was detected by hybridization of a 70 μl sample of each gradient fraction as described in the legend to Fig. 2. The position of 4S RNA in the gradients was deduced from the profile of total 3H-labelled RNA (not shown) and that of 18S RNA from the absorbance profile at 254 nm (not shown). (a) Virus RNA from untreated cells. The bars show fractions pooled for isolation of 3S to 7S RNA (fractions 7 to 15) and of RNA larger than 19S (fractions 32 to 35 plus the pellet resuspended in 1 ml buffer; a 70 μl sample of the resuspended pellet contained 58000 ct/min virus RNA). ○, 3H ct/min; □, 3H ct/min × 10^{-3}. (b) Virus RNA from cells treated with 75 μM-DRB. The bars show fractions pooled for isolation of 3S to 7S RNA (fractions 7 to 15) and of RNA larger than 19S (fractions 32 and 33 plus the pellet resuspended in 1 ml buffer; a 70 μl sample of the resuspended pellet contained 18000 ct/min virus RNA). Inset shows the ratio of 3H ct/min in virus RNA from DRB-treated cells (multiplied by 1.35 to correct for [3H]uridine transport) to 3H ct/min in virus RNA from untreated cells, calculated for each fraction after alignment of gradients.

Mapping small virus RNAs on polyoma virus DNA

Digestion of polyoma DNA with the restriction endonucleases BglII, BamHI, HindIII, EcoRI and XbaI yielded five DNA fragments which cover nearly the entire virus genome (see Fig. 4) and which can be used to determine from which region of the virus genome small virus RNA is synthesized. These fragments were prepared, bound to nitrocellulose filters and tested for their ability to hybridize with complementary RNA by incubation with late polyoma virus RNA isolated from nuclei of cells labelled for 20 min with [3H]uridine. Greater than 90% of late polyoma virus nuclear RNA is copied from the L strand of virus DNA (Flavell & Kamen, 1977; Bar-Shavit et al., 1978) and all L strand sequences are transcribed (Kamen et al., 1974; Beard et al., 1976; Acheson, 1978). These experiments showed that each DNA fragment hybridized with an amount of late virus RNA roughly proportional to its fractional length of the virus genome (data not shown).

Next, 3H-labelled small virus RNA and (as an internal control) virus RNA larger than 19S were prepared from both untreated and DRB-treated cells (as described in the legend to Fig. 3). Small virus RNA which sediments in the region between 3S and 7S was pooled, since the
Fig. 4. Level of transcription of fragments 1 to 5 in 3S to 7S RNA and RNA larger than 19S from DRB-treated and untreated cells. Samples of 3S to 7S RNA and RNA larger than 19S were prepared from infected cells labelled in the presence or absence of DRB as described in the legend to Fig. 3. RNAs were hybridized with filter-bound DNA fragments 1 to 5 (see text). (a) RNA from untreated cells: 35 µl samples of 3S to 7S RNA (an amount of 3S to 7S RNA found in 0.08 Petri dish) were hybridized; 76 ct/min hybridized to fragment 1. Samples of 8.7 µl of >19S RNA (an amount of >19S RNA found in 0.02 Petri dish) were hybridized; 710 ct/min hybridized to fragment 1. □, 3S to 7S RNA; ○, >19S RNA. (b) RNA from DRB-treated cells: 35 µl samples of 3S to 7S RNA (an amount of 3S to 7S RNA found in 0.2 Petri dish) were hybridized; 83 ct/min hybridized to fragment 1. Samples of 8.7 µl of >19S RNA (an amount of >19S RNA found in 0.05 Petri dish) were hybridized; 220 ct/min hybridized to fragment 1. 3H incorporation in the presence of DRB was corrected for the reduction of [3H]uridine uptake by a factor of 1.35. □, 3S to 7S RNA; ○, >19S RNA.

DRB-resistant RNAs sediment in this region as shown in Fig. 2 and 3. These RNAs were treated with deoxyribonuclease which removed residual virus DNA. They were then hybridized with an excess of filter-bound DNA fragments 1 to 5. Total ribonuclease-resistant hybrid ct/min (corrected for blank values and counter background) were divided by the fractional length of the DNA fragments and the values obtained for fragment 1 were normalized to 1-0. The resulting values are a measure of the abundance of 3H-labelled virus RNA complementary to each of the five DNA fragments. These values are plotted on linearized maps of the polyoma virus genome, at the midpoint of each DNA fragment (Fig. 4). The virus genome is displayed in such a way that L strand transcription proceeds in a rightward direction on the linearized map.

Fig. 4 shows the results of hybridization of small (3S to 7S) or large (>19S) 3H-labelled RNAs from untreated cells to fragments 1 to 5. Large virus RNA contained approx. equal abundances of transcripts from all five DNA fragments. However, small virus RNA contained about fourfold more abundant transcripts from DNA fragment 1 than from fragments 2, 3 and 4; transcripts from fragment 5 were of an intermediate abundance. Similar results were obtained when small or large 3H-labelled RNAs from DRB-treated cells were hybridized to fragments 1 to 5 (Fig. 4b). Large virus RNA contained transcripts from all DNA fragments in approximately equal abundance and small virus RNA contained five- to sixfold more abundant transcripts from fragment 1 than from fragments 2 to 5. Results were similar with RNA labelled for 15 min (not shown) or for 1 h (Fig. 4). Small RNA from untreated or DRB-treated cells was then hybridized with separated strands of fragment 1 DNA. Table 1 shows that about 80% of the RNA which hybridized to fragment 1 was complementary to the L strand.

Thus, both in untreated and in DRB-treated cells there exist small virus RNAs which are preferentially transcribed from the L strand somewhere within a 660 nucleotide-long region situated just downstream (in the direction of L strand transcription) from the origin of DNA replication. The capped 5' termini of polyoma virus nuclear and cytoplasmic L strand RNAs
Table 1. Hybridization of $^3$H-labelled 3S to 7S RNA to separated strands of fragment 1 of polyoma DNA*

<table>
<thead>
<tr>
<th>Source of 3S to 7S RNA</th>
<th>Polyoma DNA</th>
<th>Fragment 1 DNA</th>
<th>Ratio of ct/min hybridized to L strand of fragment 1/ct/min hybridized to E strand</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated cells</td>
<td>321</td>
<td>71</td>
<td>12</td>
</tr>
<tr>
<td>DRB-treated cells</td>
<td>240</td>
<td>78</td>
<td>58</td>
</tr>
</tbody>
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*The 3S to 7S RNA was prepared as described in the legend to Fig. 3 and was hybridized with filter-bound DNA as described in the text.

Discussion

Our results suggest that a major site (or sites) where L strand transcription is initiated in productively infected cells lies within the region of polyoma virus DNA bounded by map positions 58.0 and 72.2. This conclusion rests on the analogy between the present system and those where the effect of DRB has been more fully studied, namely adenovirus 2-infected cells (Fraser et al., 1978, 1979; Sehgal et al., 1979) and uninfected HeLa cells (Tamm, 1977; Tamm & Kikuchi, 1979; Tamm et al., 1980). In the case of adenovirus 2 it has been clearly shown that the major species of DRB-resistant RNA synthesized on the rightward-reading strand originate from a region just downstream from the promoter for the major late transcription unit (Fraser et al., 1978; Sehgal et al., 1979). The mapping of DRB-resistant RNAs has been suggested (Fraser et al., 1978) and exploited (Fraser et al., 1979; Laub et al., 1980) as an experimental approach to mapping promoters of transcription in vivo.

Small RNA species preferentially transcribed from the L strand of fragment 1 of polyoma virus DNA are present both in untreated and in DRB-treated cells (Fig. 2, 3, 4 and Table 1). Synthesis of small RNAs is partially resistant to DRB, whereas synthesis of high mol. wt. virus RNAs is almost completely inhibited by DRB. These findings rule out the possibility that the small virus RNAs arise by degradation from a high mol. wt. precursor.

The relative molar abundance of small and high mol. wt. virus RNAs can be calculated from the data presented in Fig. 2. Since the labelling time (15 min) is long compared with the time required to synthesize RNA chains as long as 20 kb (assuming an elongation rate of 100 nucleotides/s, Greenberg & Penman, 1966) most radioactivity in virus RNA will be in completed chains which are equally labelled throughout their length. Division of ct/min in virus RNA in each fraction by the average chain length in that fraction, therefore, gives an estimate of the relative molar abundances of virus RNAs of different size classes. Such calculations show that there are approx. 0.5 to 1 times as many small (3S to 7S) virus RNA molecules labelled in the presence of DRB as there are large (> 10S) virus RNA molecules labelled in the absence of DRB. If we assume that small and large virus RNAs have similar lifetimes (an untested assumption) this result means that approximately equal numbers of small and large virus RNA molecules are synthesized. By contrast, large virus RNAs are from 10 to 40 times less abundant than small virus RNAs in DRB-treated cells.

Results of other kinds of experiments suggest that initiation of L strand transcription might take place just downstream from the replication origin on polyoma virus DNA. Flavell et al. (1979, 1980), Treisman (1980) and Treisman & Kamen (1981) have determined that the capped 5' ends of polyoma virus nuclear and mRNAs map within the region bounded by 66 to 69 map units. N. H. Acheson (unpublished results) has analysed nascent chains of polyoma virus RNA by labelling infected cells for very brief periods and hybridizing across sucrose gradients with different DNA fragments. His analysis locates the shortest nascent
DRB-resistant polyoma virus RNA

chains (presumably those closest to the initiation site) within the region bounded by 54-0 to 70-5 map units.

A prominent 5' end of the polyoma virus nuclear L strand RNA has been located at 92-6 map units by Treisman & Kamen (1981). It is not known if this 5' end is capped or whether it is created by enzymic cleavage of a continuous RNA molecule initiated elsewhere. The present results do not exclude the possibility that some L strand RNAs may be initiated at that or other sites on the virus DNA.

Is premature termination stimulated after treatment of infected cells with DRB? This effect has been suggested to occur in uninfected HeLa cells (Tamm & Kikuchi, 1979; Tamm et al., 1980) as well as at the major late adenovirus 2 promoter (Fraser et al., 1979) and the SV40 L strand promoter (Laub et al., 1980). However, incorporation of radioactive precursors into RNAs transcribed from early adenovirus transcription units showed that the synthesis of small virus RNAs was never greater in DRB-treated than in DRB-untreated cells (Sehgal et al., 1979). The same is true of the present results; synthesis of small polyoma virus RNAs was partially resistant to DRB inhibition but was not increased in the presence of DRB (see Fig. 3).

This might mean that DRB preferentially inhibits the initiation of RNA chains destined to become full-sized mRNA precursors while not inhibiting the initiation of RNA chains which are normally terminated after incorporation of several hundred nucleotides. If there were two forms of RNA polymerase II, one which gives rise to mRNA precursors and one which synthesizes small promoter proximal chains, DRB might preferentially inhibit the former. Dreyer & Hausen (1978) have demonstrated that RNA polymerase II shows two salt optima for in vitro activity, one at 25 mM-(NH₄)₂SO₄ and one at 300 mM. Preincubation of cells with DRB results in a selective inhibition of the RNA polymerase activity present at 25 mM-(NH₄)₂SO₄. It might be that the RNA polymerase which is active in vitro at 300 mM-(NH₄)₂SO₄ gives rise, in vivo, to prematurely terminated chains. Study of the molar ratios of synthesis of small and high mol. wt. virus RNAs in the presence or absence of DRB, or after treatment of cells at different salt concentrations, might help to distinguish between these models.

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