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A DRB (5,6 dichloro-β-D-ribofuranosylbenzimidazole)-resistant adenovirus mRNA

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ABSTRACT

5,6-Dichloro-β-D ribofuranosyl benzimidazole (DRB) inhibitis transcription from the major late adenovirus promoter, located on the r-strand at map position 16.3 on the viral genome. 100-500 nucleotides long RNA chains with capped 5'-termini are transcribed from this promoter in the presence of 70 μM DRB. Synthesis of the mRNA for polypeptide IX which is unspliced and maps on the r-strand between positions 9.7 and 10.9 appears, however, to be DRB-resistant although it is transcribed by polymerase II and capped. Translatable mRNA for polypeptide IX is synthesized in the presence of DRB while the mRNAs for the other viral structural proteins are not synthesized. This differential DRB-inhibition of polymerase II transcription suggests either that short mRNAs may escape inhibition or that unspliced mRNAs are insensitive to the drug.

INTRODUCTION

Initiation of transcription with RNA polymerase II (pol II) in mammalian cells is poorly understood. In vitro systems using chromatin or isolated nuclei fail to initiate (1,2) or do so at a low frequency (3). Recently it was suggested that 5,6 dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) may act by reversibly terminating pol II transcription 400-800 nucleotides after initiation (4,5,6). Adenovirus transcription may be used as a model system to define the mechanism of action for specific inhibitors of transcription. The major late transcription unit on the r-strand (the strand transcribed in the rightwards direction) of the adenovirus type 2 DNA starts at 16.3 map units and terminates beyond 95 map units at the right hand end of the genome (7). Only 400-800 nucleotides long chains are transcribed from this promoter in the presence of DRB. Minor transcription units, active late after infection, are present at the left hand end of the genome. A weak transcription unit which initiates at position 4.5 and terminates at position 10.9 reflects the continued transcription from an early promoter during the late phase of the infectious cycle (6,8). A strong transcription unit, synthesizing the messenger RNA for polypeptide IX, extends between map units 9.7 and

10.9 on the r-strand (9). Polypeptide IX is expressed independently of the late promoter and of DNA replication and its synthesis starts during the intermediate period of the infectious cycle (10).

The present paper demonstrates that DRB has a differential effect on adenovirus transcription. The polypeptide IX transcription unit is insensitive to the drug and the mRNA matures normally in the presence of DRB. In contrast, transcription from the major late promoter is abrogated after 100-500 nucleotides in the presence of the drug. The 5'-terminal cap can be identified in transcripts from both transcription units but poly(A) addition occurs only in polypeptide IX mRNA. Immunoprecipitation of cell extracts from DRB-treated and control cells revealed that polypeptide IX mRNA is translated under DRB-inhibition whereas the major structural proteins which are controlled by the major late promoter are not expressed.

MATERIALS AND METHODS

Virus infection and labeling of cells

HeLa cells, grown in suspension cultures, were infected with adenovirus type 2 and labeled with $^{32}PO_4$ as described (2). DRB was added at a concentration of 70 μM to the growth medium one hour prior to the addition of label.

RNA was isolated from cytoplasmic extracts by phenol extraction at pH 9.0. Nuclear RNA was prepared by phenol extraction in the presence of 7M urea, followed by repeated treatments with DNase I. RNA was sedimented in 15-30% sucrose gradients and poly(A) containing RNA was isolated by chromatography on oligo (dT) cellulose (11).

Restriction endonucleases and cleavage of ad2 DNA

Digestion of ad2 DNA with SmaI was carried out at 30°C in 0.1 M Tris, pH 9.0 with 6 mM β-mercaptoethanol and 6 mM $MgCl_2$. Digestions with the endonucleases EcoRI, BamHI, BglII, BalI and SstI were carried out at 37°C in 6 mM Tris-HCl pH 7.9, 6 mM β-mercaptoethanol and 6 mM $MgCl_2$. The fragments were separated on 1% or 1.4% agarose slab gels in 45 mM tris-borate buffer pH 8.3 with 1.25 mM EDTA. DNA fragments to be used for hybridization were denatured and transferred to nitrocellulose sheets as described by Southern (12).

Acrylamide gel electrophoresis

RNA was size fractionated by electrophoresis in 7% polyacrylamide gels containing 7M urea, 90 mM tris-borate buffer pH 8.3 with 2.5 mM EDTA (13).

Nucleic acid hybridization

Hybridization of RNA with restriction enzyme fragments, immobilized on nitrocellulose strips, was carried out in 6xSSC containing 0.4% SDS.

Quantitative hybridization

Fragment SmaI-H of ad2 DNA or the recombinant plasmid carrying a cDNA copy of IX mRNA was immobilized on nitrocellulose filters. Hybridizations were carried out in 2 ml 6xSSC (SSC = 0.15 M NaCl with 0.015 M sodium citrate), 3 x Denhardt's solution, 0.5% SDS and 20 µg/ml poly(A). After hybridization the filters were washed with repeated changes of 2xSSC with 0.5% SDS. In order to eliminate hybridization to the poly(A)-tract of the recombinant plasmid the filters were incubated at 65°C in 0.1xSSC for 30 min followed by washing in 2xSSC and treatment with 10 µg/ml pancreatic ribonuclease. The filters were counted in a toluene based scintillation fluid after drying.

Nucleotide analysis

RNA was digested with RNase T_1 and T_2 and the resulting oligonucleotides were separated by electrophoresis at pH 3.5 on cellulose acetate in the first dimension followed by homochromatography on PEI-thin layer plates in the second dimension (14).

In vitro transcription

The procedure for the isolation of nuclei late after ad2 infection and the conditions for in vitro transcription have been described previously (2).

^{35}S-methionine labeling of cells

Suspension cultures of HeLa cells were infected with 15,000 ad2 virus particles per cell (10). At 5 hrs post infection the cells were collected by centrifugation and resuspended in Eagles' spinner medium supplemented with 7% calf serum, containing 70 µM of DRB. At 9 hrs post infection the cells were transferred to fresh methionine-free medium containing 70 µM DRB and labeled with ^{35}S-methionine (20 µCi/ml) for 1 hr.

Extracts from cycloheximide-pretreated cells were prepared from cultures treated with cycloheximide (25 µg/ml) from 1 hr post infection. 70 µM DRB was added at 5 hrs post infection. At 9 hrs the cells were washed and resuspended in methionine-free medium containing DRB (70 µM) and labeled with ^{35}S-methionine (20 µCi/ml) for 1 hr.

Cells were infected with 2,000 virus particles/cell and labeled with ^{35}S-methionine between 16 to 17 hrs post infection for preparations of cell extracts late in infection.

Preparation of cell extracts

Cells were harvested by centrifugation after addition of frozen PBS. Cell extracts were prepared in a buffer which contained 0.02 M potassium phosphate, pH 7.5, 0.1 M NaCl, 0.5% sodium deoxycholate, 1% Triton X-100, 0.1% sodium dodecyl sulphate (SDS) and 1 mM phenylmethylsulfonylfluoride (PMSF) (10).

Antisera and immunoprecipitation

Antisera against ad2 hexon (polypeptide II), penton (a virion component consisting of polypeptide III, penton base, and polypeptide IV, fiber), fiber (polypeptide IV), polypeptide VI and polypeptide IX were prepared as previously described (10). Antiserum against ad2 early proteins was prepared as described by Saborio and Öberg (15). Normal serum was obtained from non-immunized rabbits or guinea pigs.

Immunoprecipitation was performed using antibodies and Staphylococcus aureus as adsorbents as described elsewhere (10).

SDS-polyacrylamide gel electrophoresis

Samples were analyzed in 1.5 mm x 140 mm slab gels containing 13% polyacrylamide as described by Maizel (16). The gels were analyzed by fluorography (17).

Cloning procedures

The 9S mRNA was purified by sucrose gradient centrifugation. A double-stranded cDNA copy was synthesized with reverse transcriptase and inserted into the Pst 1 cleavage site of the pBR322 plasmid after dG/dC tailing with terminal transferase (BRL). Details of our cloning procedure for 9S mRNA and the subsequent identification of positive clones are described in a separate communication (18). The recombinant plasmid which was used in the present study contained a complete copy of the IX mRNA except for 50 nucleotides from the 5' end of IX mRNA. Plasmid DNA, to be used in hybridization experiments, was purified by two cycles of CsCl equilibrium centrifugation in the presence of ethidium bromide.

Recombinant DNA safety procedures

The cloning experiments were carried out in a P3 facility at the Pasteur Institute, Paris, France, using an EK2 host-vector system. Permission to use recombinant DNA in hybridization experiments was obtained from the Swedish Committee on Recombinant DNA.

RESULTS

RNA-synthesis in DRB-treated cells

The origin and intracellular distribution of RNA synthesized in the presence of DRB was investigated. Adenovirus infected cells were pretreated with 70 μM DRB for one hour at 11 hours after infection and then labeled with $^{32}PO_4$ for 4 hrs in the continued presence of the drug. Nuclear and cytoplasmic RNA was prepared and sedimented in sucrose gradients (fig. 1A

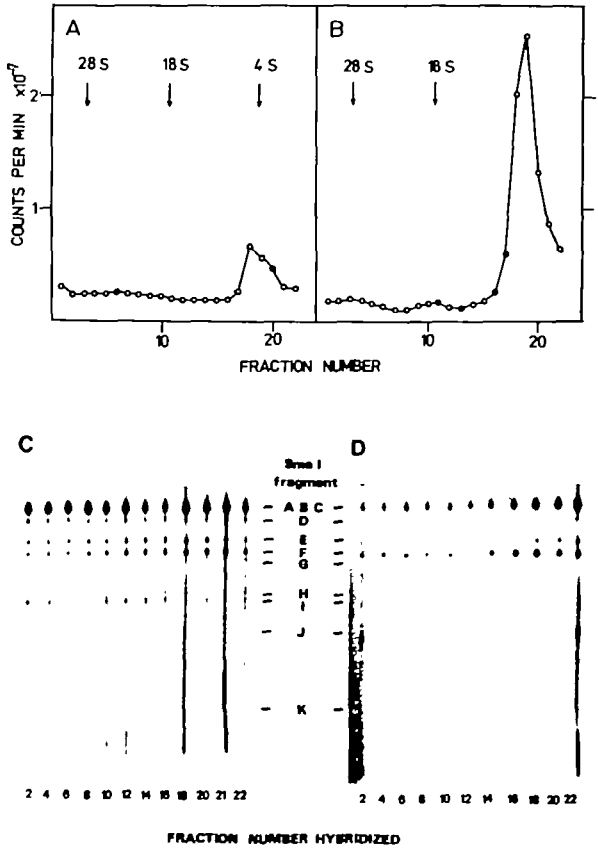

Fig. 1. Sedimentation analysis of viral RNA synthesized in the presence of DRB. Adenovirus-infected cells were labeled with $^{32}PO_4$ 14-20 hrs after infection in the presence of 70 μM DRB. Nuclear and cytoplasmic RNA was purified and sedimented in sucrose gradients. The gradients were fractionated and an aliquot from every second fraction was hybridized with SmaI fragments of ad2 DNA immobilized on nitrocellulose strips. A: sedimentation of nuclear RNA; B: sedimentation of cytoplasmic RNA; C: hybridization of nuclear RNA to SmaI fragments; D: hybridization of cytoplasmic RNA to SmaI fragments.

and 1B). The fractionated RNA was hybridized to the SmaI fragments of ad2 DNA. Both nuclear and cytoplasmic DRB resistant RNA of low molecular weight (4-10S) anneals to SmaI fragments E, F and B, with a preference for fragments F and B (fig. 1C and 1D). The hybridization pattern suggests that the RNA originates from the major late viral promoter at position 16.3 in fragment SmaI-F (19) and from a promoter in fragment SmaI-E, presumably the polypeptide IX promoter (9,18). The hybridization to fragment SmaI-B is due to the synthesis of adenovirus specific 5.5S and 5.2S RNA, whose synthesis appears to be unaffected by DRB (not shown). Low levels of hybridization to SmaI fragments -I and -D were also detected with high molecular weight DRB resistant nuclear RNA but this RNA was not characterized further.

The amount of nuclear and cytoplasmic DRB-resistant RNA was quantitated by hybridizing RNA in the 4-10S size range with an excess of fragments SmaI-E and F immobilized on nitrocellulose filters. Table 1 shows that 65-75% of the RNA transcribed from these fragments accumulates in the cytoplasm in DRB-treated cells.

The size of the RNA transcribed in DRB-treated cells was studied by subjecting $^{32}PO_4$-labeled cytoplasmic low molecular weight RNA, prepared 20 hrs after infection, to polyacrylamide gel electrophoresis. Cytoplasmic RNA from infected cells not treated with DRB was electrophoresed in a parallel slot. The mRNA for polypeptide IX in the control sample, which migrated as a broad band with a length of around 550 nucleotides (fractions 12-13 in fig. 2A) served as a size marker for the DRB-resistant RNA (fig. 2B). RNA from individual gel slices was eluted and hybridized with strips carrying SmaI fragments of ad2 DNA (fig. 2C and 2D). The DRB-resistant RNA hybridizing to SmaI-E comigrates with the mRNA for polypeptide IX, whereas the SmaI-F specific RNA covered a broad size range from 100-500 nucleotides (fig. 2C and 2D). The results indicate that RNA transcribed from the major promoter

Table 1. RNA, hybridizing to fragments SmaI-E and SmaI-F in the nucleus and cytoplasm of adenovirus infected cells treated with DRB.

	Input cpm	cpm in hybrid with SmaI-E	SmaI-F
Nuclear RNA	6.3×10^6	3,700	5,600
Cytoplasmic RNA	26×10^6	10,600	24,300

Fractions 15-21 in Fig. 1 were pooled and hybridized to restriction fragment SmaI-E and SmaI-F (each filter contained DNA equivalents to 20 µg ad2 DNA). The filters were washed, treated with 2 U/ml of RNase T1 for 30 min at room temperature, and the radioactivity was determined.

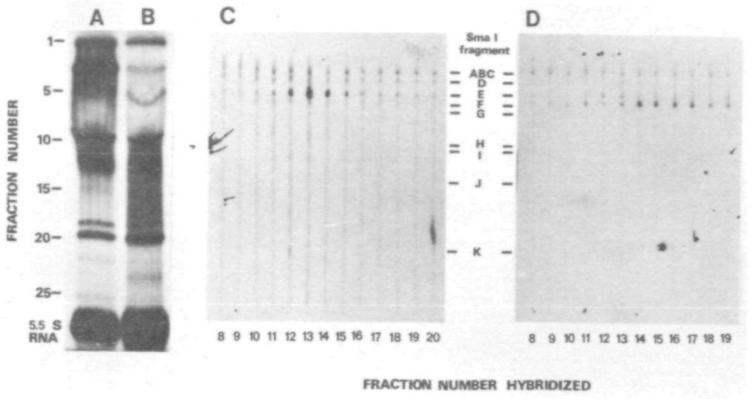

Fig. 2. Polyacrylamide gel electrophoresis of viral RNA synthesized in the presence of DRB. Cytoplasmic low molecular weight RNA from fractions 15-21 in fig. 1B was subjected to polyacrylamide slab gel electrophoresis. Cytoplasmic RNA from untreated cells was prepared from infected cells labeled with $^{32}PO_4$ between 8 and 18 hrs after infection which is 4 hrs longer than for DRB-treated cells. The gel was fractionated into 0.5 cm slices as indicated and the RNA was eluted and hybridized to SmaI fragments of ad2 DNA immobilized on nitrocellulose strips. A: gel electrophoresis of control RNA; B: gel electrophoresis of RNA synthesized in the presence of DRB; C: hybridization pattern of control RNA; D: hybridization pattern of RNA synthesized in the presence of DRB.

in fragment SmaI-F is heterogenously terminated, and its maximum size is around 500 nucleotides. The data also suggest that a transcript is synthesized from the SmaI-E fragment in the presence of DRB. The size of this RNA is similar to that of the mRNA for polypeptide IX which is known to be specified by the SmaI-E fragment. This result suggests that mRNA for polypeptide IX is synthesized in DRB-treated cells. The difference in the amounts of radioactivity from control cells and from DRB-treated cells hybridizing to fragment SmaI-E in Fig. 2C and 2D does not reflect a difference in amounts of RNA since different labeling conditions were used for the two samples.

Mapping of DRB-resistant RNA

To locate the origin of the DRB-resistant RNA on the ad2 genome, $^{32}PO_4$-labeled RNA, isolated after sucrose gradient centrifugation and gel electrophoresis, was hybridized with nitrocellulose strips carrying ad2 restriction enzyme fragments generated by cleaving complete ad2 DNA, or fragments of ad2 DNA, with endonucleases SmaI, EcoRI, HindIII, BamHI, BglII and SstI (fig. 3). The DRB-resistant RNA was found to hybridize to the following fragments:

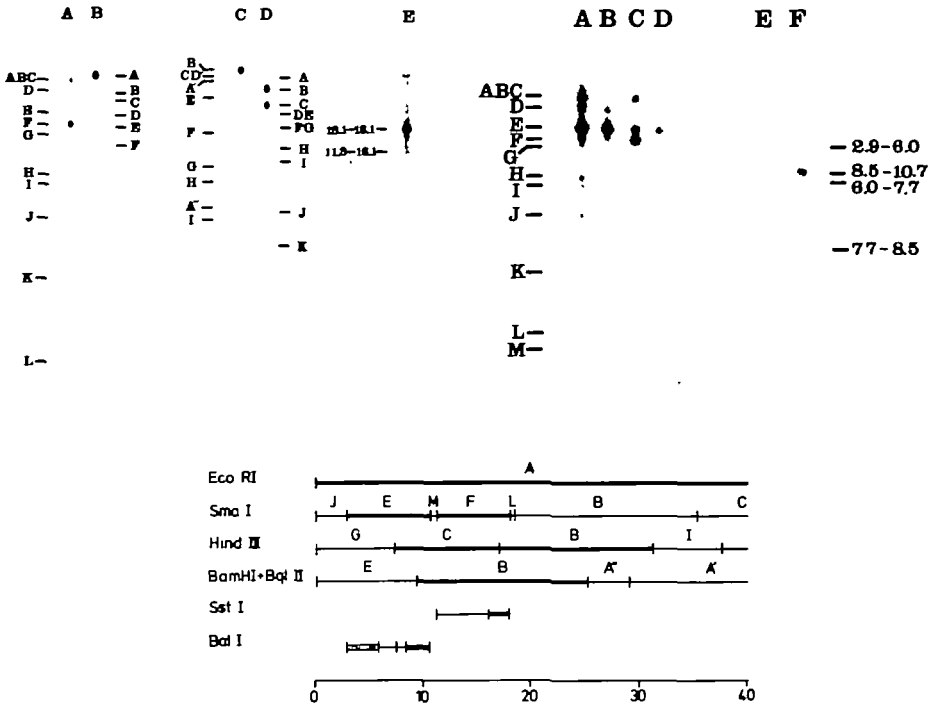

Fig. 3. Mapping of DRB-resistant RNA on the ad2 genome. RNA in the gel slices 12-15 in Fig. 2B was eluted and hybridized to restriction enzyme fragments of ad2 DNA.
I. A: SmaI fragments; B: EcoRI fragments; C: fragments generated by a double digest with BglII and BamHI (only the larger fragments are indicated in the figure); D: HindIII fragments; E: fragment SmaI-F was digested with SstI to produce two fragments, with the map coordinates 11.3-16.1 and 16.1-18.1.
II. RNA in fractions which showed hybridization to SmaI fragments -E and -F was eluted from the gel slices. mRNA for polypeptide IX was partially purified in a similar manner from infected cells labeled with $^{32}PO_4$ 8-18 hrs after infection. The purified RNA from DRB-treated cells and the mRNA for polypeptide IX were subjected to chromatography on oligo (dT) cellulose. The RNA in the flow through (poly(A)$^-$) and the retained RNA (poly(A)$^+$) were hybridized with SmaI fragments of ad2 DNA (panels A-D and with fragments generated by cleaving fragment SmaI-E with BalI (panels E-F). These fragments have coordinates 2.9-6.0, 6.0-7.7, 7.7-8.5 and 8.5-10.7 on the ad2 genome. A: poly(A)$^-$ RNA from mRNA for polypeptide IX; B: poly(A)$^+$ RNA from mRNA for polypeptide IX; C and E: poly(A)$^-$ RNA from DRB-treated cells; D and F: poly(A)$^+$ RNA from DRB-treated cells.
III. The figure illustrates the left 40% of the ad2 genome, and the restriction enzyme fragments used for hybridization. Heavy lines represent the fragments that hybridize with the DRB-resistant RNA. The stippled line indicates the hybridization of RNA from a weak promoter. The restriction enzyme maps were obtained from M. Zabeau, Cold Spring Harbor Laboratory. The cleavage map for SstI was taken from ref. 20. Fragments cleaved by BamHI in double digestions are denoted by A' and A" respectively.

EcoRI-A, HindIII-B and C, BglII-B, SmaI-E, F and B. The map positions of these fragments are also illustrated in the figure. The RNA annealing to fragment SmaI-F was further mapped by hybridization with strips carrying two fragments with the map coordinates 11.3-16.1 and 16.1-18.1, generated by cleaving fragment SmaI-F with SstI. Hybridization was detected only with the fragment located between 16.1 and 18.1 map units. These results suggest that in DRB-treated cells late after ad2 infection, transcription occurs from the major promoter for late transcription. RNA synthesis also takes place between map coordinates 9.4 and 10.7 in the presence of DRB as shown by the hybridization to fragments SmaI-E and BglII-B. This region encodes the gene for polypeptide IX (9). The hybridization to fragments SmaI-B probably reflects hybridization of the viral 5.5S RNA trailing in the polyacrylamide gel. The same hybridization pattern was observed irrespective of whether cytoplasmic or nuclear low molecular weight RNA was analyzed.

The structure of the DRB-resistant RNA was further investigated by chromatography of electrophoretically purified RNA on oligo (dT) cellulose. The poly(A)$^-$ and the poly(A)$^+$ fractions were hybridized to nitrocellulose strips containing SmaI fragments. Fig. 3 shows that RNA originating from the major promoter in fragment SmaI-F is not polyadenylated, whereas the RNA annealing to SmaI-E is polyadenylated.

The RNA was also hybridized to nitrocellulose strips carrying the four fragments generated by cleaving fragment SmaI-E (2.9-10.7 map units) with endonuclease BalI. The $^{32}PO_4$-labeled poly(A)$^-$ RNA hybridized to the two end fragments (2.9-6.0 and 8.5-10.7), while the poly(A)$^+$ RNA hybridized exclusively to the 8.5-10.7 fragment (fig. 3). Similar experiments with RNA from cells not treated with DRB gave identical results for the polypeptide IX mRNA. The results suggest that the polyadenylated mRNA for polypeptide IX is transcribed in DRB-treated cells, and that the promoter for this RNA is located close to the structural gene for polypeptide IX.

In order to examine whether the DRB-resistant transcripts were capped at the 5'-termini, low molecular weight cytoplasmic RNA was isolated from cells treated with 70 μM DRB and selected on filters carrying fragments SmaI-F and SmaI-E. After elution, the RNA was subjected to nucleotide analysis. Both the RNA originating from the late promoter and the polypeptide IX mRNA contain caps as revealed by analysis of the nucleotides in a two dimensional system (data not shown).

Quantitation of the polypeptide IX mRNA synthesis after DRB-treatment

In order to determine to what extent the synthesis of the polypeptide IX

mRNA (9S mRNA) was inhibited as compared to the synthesis of other late RNAs after DRB-treatment, hybridization experiments with specific probes were carried out. To quantitate the IX mRNA synthesis, DNA from a recombinant plasmid carrying a cDNA copy of the IX mRNA was used. To measure the synthesis of mRNAs from the major late promoter, fragment Sma-H was used as a probe. The latter fragment contains sequences which are present in the hexon and the pVI mRNAs, two late mRNA species which are controlled by the late promoter at map position 16.3 (21,22). ^{3}H-uridine labeled RNA, isolated 16 hours post infection from cells maintained in the absence or the presence of DRB, was hybridized to filters carrying either of the two hybridization probes. As is shown in Table 2, DRB inhibitied 94% of the hybridization to fragment SmaI-H whereas the hybridization to the IX cDNA clone was only reduced by around 30%.

Polypeptide IX mRNA is a polymerase II product

Nuclei were isolated 16 hrs post infection with ad2 and incubated with and without 1 µg/ml α-amanitin in a cell-free transcription system (2). Extracted RNA was hybridized to plasmid DNA containing sequences complementary to the polypeptide IX mRNA as described above. Table 3 shows that around

Table 2. DRB inhibition of hexon and polypeptide IX mRNA synthesis[a]

RNA sample[b]	cpm[c] in hybrid with 0.5 µg of fragment Sma-H[d]	cpm[c] in hybrid with 5.5 µg of recombinant plasmid DNA
RNA from control cells	40,137	17,131
RNA from DRB-treated cells	2.556 (93.6%)[e]	11,867 (30.7%)[e]

[a] Adenovirus infected HeLa cells were treated for 1 hr with DRB 16 hrs post infection followed by labeling with 100 µCi/ml of ^{3}H-uridine. RNA was extracted from the cytoplasm and hybridized to nitrocellulose filters carrying fragment SmaI-H (map coordinates 51.1-56.1) and DNA from a recombinant plasmid which contained a cDNA copy of IX mRNA.

[b] Each sample contained total cytoplasmic RNA from the equivalent of $2x10^{6}$ infected cells. The control sample contained $19.5x10^{6}$ cpm and the sample from DRB-treated cells $11.3x10^{6}$ cpm.

[c] Background hybridization to a blank filter (230-320 cpm) was subtracted.

[d] The major RNA species which is transcribed from fragment SmaI-H is the hexon mRNA.

[e] Numbers in parenthesis indicate percent inhibition of RNA synthesis in DRB-treated cells.

Table 3. The effect of α-amanitin on the synthesis of polypeptide IX mRNA in isolated nuclei[a]

	Total incorporation of acid insoluble radioactivity (cpm)	Hybridization to 5.5 μg recombinant plasmid DNA (cpm)
No α-amanitin	1.9×10^6	3 018
1 μg/ml α-amanitin	0.28×10^6	412

[a]The conditions for hybridization to the cloned DNA have been described in the Method section and the legend to Table 2.

85% of the RNA in isolated nuclei late in infection is synthesized by polymerase II since the acid insoluble radioactivity was decreased to this level in the presence of 1 μg/ml of α-amanitin. The synthesis of the polypeptide IX mRNA was also inhibited by 85% at this concentration of α-amanitin as revealed by hybridization to a specific probe. Thus we conclude that polypeptide IX mRNA is a polymerase II product.

Synthesis of polypeptide IX in the presence of DRB

In order to investigate whether the polypeptide IX mRNA which was synthesized in the presence of DRB also was translated, cells were treated with DRB at 5 hrs post infection and labeled in the presence of DRB with ^{35}S-methionine at 9 hrs post infection. Cell extracts were prepared and immunoprecipitated with different antisera. Immunoprecipitation of extracts from DRB-treated cells with an antiserum against polypeptide IX precipitated a 12K polypeptide which comigrated with marker polypeptide IX (Fig. 4, slot 6). A mixture of antisera against structural polypeptides II, III, IV and VI failed to precipitate any polypeptides (Fig. 4, slot 4) while an antiserum against early proteins precipitated several polypeptides (E75K, E45K, E19K, E17.5K, E14K) (Fig. 4, slot 5). These ad2 early polypeptides are synthesized before addition of DRB at 5 hrs post infection (10).

Extracts were also prepared from DRB-treated cells that had been preincubated with cycloheximide. Incubation of adenovirus infected cells with cycloheximide enhances production of viral mRNA during early and intermediate times after infection (23). A 12K polypeptide was specifically immunoprecipitated from these extracts with the antiserum against polypeptide IX (Fig. 4, slot 10). No precipitation was observed with the mixture of antisera against polypeptides II, III, IV and VI (Fig. 4, slot 8). Samples from infected but untreated cells labeled with ^{35}S-methionine between 16-17 hrs post infection were also immunoprecipitated to verify the specificity of the

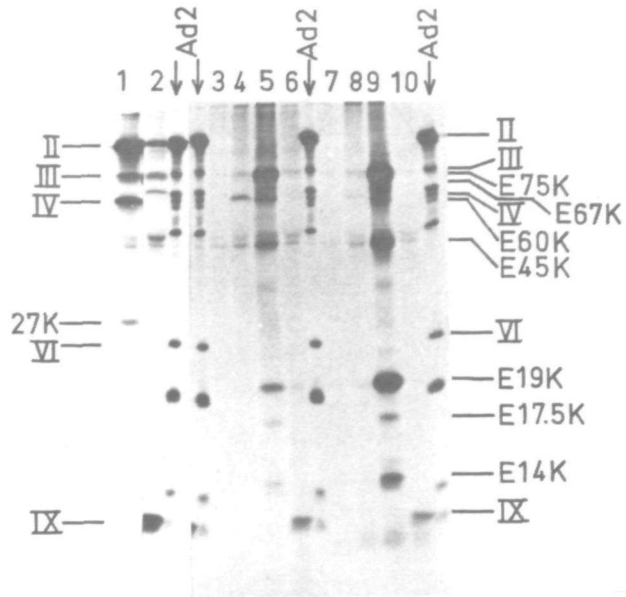

Fig. 4. SDS-polyacrylamide gel electrophoresis of polypeptides immunoprecipitated from DRB-treated cell extracts. Ad2-infected HeLa cells were treated with DRB (70 μM) at 5 hrs post infection. The cells were labeled with ^{35}S-methionine in the presence of DRB for 1 hr at 9 hrs post infection. Another cell culture received cycloheximide (25 μg/ml) at 1 hr, DRB (70 μM) at 5 hrs and ^{35}S-methionine for 1 hr at 9 hrs post infection in the presence of DRB. Cells grown in the absence of drugs were labeled with ^{35}S-methionine for 1 hr at 16 hrs post infection Cell extracts were prepared and immunoprecipitated. The precipitates were analyzed in a 13% SDS-polyacrylamide gel. Extracts from cells grown in the absence of drugs and labeled with ^{35}S-methionine between 16 and 17 hrs post infection were immunoprecipitated with (1) an antiserum against polypeptides II, III, IV and VI and in addition (2) with an antiserum against polypeptide IX. Immunoprecipitation from DRB-treated cells with (3) normal rabbit serum; (4) a mixture of antisera against polypeptides II, III, IV and VI; (5) antiserum against ad2 early proteins (15) and (6) antiserum against polypeptide IX. Immunoprecipitation from DRB- and cycloheximide-treated cells with (7) normal rabbit serum; (8) a mixture of antisera against structural polypeptides II, III, IV and VI; (9) the anti-"early" serum and (10) antiserum against polypeptide IX. (Ad2) ^{35}S-methionine labeled virus marker.

antisera. The antisera against structural polypeptides precipitated polypeptides II, III, IV and pVI (Fig. 4, slot 1). The antiserum against polypeptide IX also precipitated a 12K polypeptide from this extract (Fig. 4, slot 2).

The identity between the immunoprecipitated 12K polypeptide and authentic

virion polypeptide IX has previously been established by tryptic fingerprint analysis (10). These results suggest that a functional mRNA for polypeptide IX is synthesized in the presence of DRB.

DISCUSSION

DRB was originally reported to interfere with initiation of polymerase II transcription in eukaryotic cells (4,24). More recently it has been demonstrated that DRB allows the formation of capped transcripts from the adenovirus major late promoter which are heterogeneous in size and only extends 400-800 nucleotides from the initiation site (5,6). Since similar short transcripts also accumulate in the absence of DRB (25) it appears that the primary action of the drug is to prevent elongation from large transcription units. In accordance with this hypothesis DRB has been used to map the promoter sites of adenovirus transcription at early times in the infectious cycle (6). In confirmation of earlier results we have shown that DRB causes premature termination of transcription after 100-500 nucleotides at the major late transcription unit initiating at 16.3 map units. In contrast, another polII product, the polypeptide IX mRNA, mapping between positions 9.7 and 10.9 to the left of the major late promoter, appears to be resistant to DRB. This mRNA is capped and has a separate promoter 25 nucleotides proximal to the 5' end of the transcript (18). It is 485 nucleotides long and is unspliced (18). In the presence of DRB, about 70 percent of the normal amount of polypeptide IX mRNA is synthesized as compared with less than 10 percent of mRNAs from the major late transcription unit (Table 2). The polypeptide IX mRNA made in the presence of DRB appears also to be functional with regard to translation (Fig. 4). It has previously been demonstrated that interferon mRNA appears to be DRB-resistant since it is synthesized at similar rates in the presence and absence of DRB (26). It was proposed in the latter case that DRB also increased the stability of this mRNA. Three possibilities for the appearance of DRB-resistant polymerase II products may be envisioned. First, short transcripts may escape the elongation block introduced by DRB or secondly DRB may interfere with the organisation of the transcript in the nucleus which is a requirement for the elongation and splicing of nascent chains of RNA. Since polypeptide IX mRNA appears to be the only adenovirus mRNA which does not mature by splicing (18) we cannot discriminate between these two alternatives. A third, more unlikely possibility is that DRB acts via a specific sequence which is absent in the IX mRNA. The characterization of defined DRB-resistant mRNAs from mammalian

cells may help to finally elucidate the mechanism of action of DRB. It appears, however established from the results presented here and those of other investigators (5,6,27) that DRB interferes with a step in chain elongation and not at the initiation of transcription.

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