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# DNA-dependent transcription of adenovirus genes in a soluble whole-cell extract

(RNA polymerase II/transcription initiation/recombinant DNA/mRNA promoter/gene regulation)

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ABSTRACT **We have developed a cell-free system for studying the synthesis of mRNA in mammalian cells. The system consists of a dialyzed and concentrated whole-cell extract derived from HeLa cells, small molecules and cofactors needed for transcription, and exogenously added DNA. Accurate transcription by RNA polymerase II is entirely dependent upon addition of promoter-containing eukaryotic DNA. At optimal DNA and extract concentrations, transcription initiation from the adenovirus serotype 2 late promoter is readily detectable, and specific transcripts over 4000 nucleotides in length are observed. The RNA synthesized *in vitro* contains the same 5′ capped RNase T1 undecanucleotide as does the *in vivo* transcript. RNA synthesis also initiates accurately at both an early and an intermediate adenovirus promoter site.**

The availability of *in vitro* systems for the study of transcription has been of prime importance in elucidating mechanisms of gene regulation in prokaryotes (1, 2). The lack of similar eukaryotic systems has left many important problems unresolved. For example, virtually nothing is known about the signals and factors required for accurate initiation by RNA polymerase II (3). Control of initiation of transcription plays a major role in gene regulation in mammalian cells. At the moment, it is not clear that prokaryotic models of operators, repressors, and activators can be extended to transcription control in animal cells. We have been working to develop eukaryotic *in vitro* systems, and have recently shown that an *in vitro* system consisting of isolated nuclei is capable of carrying out many of the reactions involved in mRNA synthesis, including accurate initiation of transcription by RNA polymerase II (4, 5). Recently, Weil *et al.* (6) demonstrated that accurate initiation of transcription occurs on exogenously added DNA at a known viral transcription start site when purified RNA polymerase II is mixed with a cytoplasmic extract. Here we describe a simple system consisting primarily of a soluble whole-cell extract that accurately initiates transcription on exogenously added DNA templates. We feel that this system will prove useful for studying the biochemical mechanisms involved in the control of transcription and gene expression in mammalian cells.

## MATERIALS AND METHODS

**Preparation of DNA.** Adenovirus serotype 2 (Ad2) virus and DNA were prepared as described (7). Restriction endonuclease DNA fragments *Bal* I-D [21.5–28.3 map units (m.u.)] and *Bal* I-E (14.7–21.5 m.u.) were inserted into plasmid pBR322 with *Bam*HI linkers and cloned (8). *Sma* I-F fragment (2.9–11.3 m.u.) was similarly inserted into pBR322 but with *Eco*RI linkers (gift of Kathleen Berkner and Frank Laski). Plasmid DNA was prepared by standard protocols (9).

**Preparation of HeLa Cell Extracts.** Cell lysates were prepared according to the method of Sugden and Keller (10). HeLa cells were grown in suspension culture in Eagle's minimal essential medium supplemented with 5% horse serum to a density of approximately $8 \times 10^5$ cells per ml. All further operations were done between 0 and 4°C. Cells were washed in phosphate-buffered saline containing $MgCl_2$ and the cell pellet was resuspended in four packed-cell volumes of 0.01 M Tris·HCl, pH 7.9/0.001 M EDTA/0.005 M dithiothreitol. After 20 min, the cells were lysed by homogenization in a Dounce homogenizer, using eight strokes with a "B" pestle. Four packed-cell volumes of 0.05 M Tris·HCl, pH 7.9/0.01 M $MgCl_2$/0.002 M dithiothreitol/25% sucrose/50% (vol/vol) glycerol were then added and the mixture was stirred gently. To this suspension one packed-cell volume of saturated $(NH_4)_2SO_4$ was added dropwise; the lysate was then gently stirred for an additional 20 min. The extract was then centrifuged at 50,000 rpm for three hours in a Beckman 60 Ti rotor. The supernatant was decanted so as not to disturb the pellet and precipitated by addition of solid $(NH_4)_2SO_4$ (0.33 g/ml of suspension). After the $(NH_4)_2SO_4$ was dissolved, 0.01 ml of 1 M NaOH per 10 g of $(NH_4)_2SO_4$ was added, and the suspension was stirred for an additional 30 min. The precipitate was collected by centrifugation at $15,000 \times g$ for 20 min and resuspended with $1/10$ the volume of the high-speed supernatant into a buffer containing 50 mM Tris·HCl, pH 7.9, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$, 0.2 mM EDTA, 1 mM dithiothreitol, and 15% glycerol. This suspension was dialyzed for 4–8 hrs against each of two changes of 100 vol of the same buffer. The dialysate was centrifuged at $10,000 \times g$ for 10 min and the supernatant was quick frozen in small samples in liquid $N_2$. Samples stored at −80°C retained full activity for at least 2 months. In some later experiments (e.g., the one shown in Fig. 5) the dialysis buffer was 20 mM Hepes, pH 7.9/100 mM KCl/12.5 mM $MgCl_2$/0.1 mM EDTA/2 mM dithiothreitol/17% glycerol.

***In Vitro* Incubations and Purification of RNA.** Standard 50-μl reaction mixtures contained 15 mM Tris·HCl, pH 7.9, 7 mM $MgCl_2$, 32 mM $(NH_4)_2SO_4$ 0.2 mM EDTA, 1.3 mM dithiothreitol, 10% glycerol, 500 μM ATP, CTP, and GTP, 50 μM UTP containing 10 μCi of [$\alpha$-$^{32}$P]UTP, 15 μl of extract, and 2.5 μg of DNA (1 Ci = $3.7 \times 10^{10}$ becquerels). More recently, such as in the experiment shown in Fig. 5, we have altered the reaction mixtures so that the Tris was replaced with 12 mM Hepes, pH 7.9, the $(NH_4)_2SO_4$ was replaced with 60 mM KCl, and the amount of extract was increased to 30 μl. Reaction mixtures were incubated at 30°C for 60 min. RNA was extracted from the reaction mixture as described (4) and precipitated with ethanol. The pellet was redissolved in 0.2% NaDodSO$_4$. After addition of $NH_4OAc$ to 1 M (a total volume of 400 ml), RNA was again precipitated with 2 vol of ethanol, redissolved in 0.2% NaDodSO$_4$/0.3 M NaOAc, pH 5.2, and precipitated with ethanol. This pellet, free of unincorporated

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Abbreviations: Ad2, adenovirus 2; m.u., map unit(s); DBAE-cellulose, dihydroxylborylcellulose.

nucleotide triphosphates, was dissolved in 0.2% Sarkosyl/2 mM EDTA and stored at −20°C. RNA was analyzed by glyoxalation and agarose gel electrophoresis as described by McMaster and Carmichael (11). For cap analysis, RNA was digested with RNase T1, bound to columns of dihydroxylborylcellulose (DBAE-cellulose), eluted, and fingerprinted as described (4, 12, 13).

## RESULTS

**Extract and DNA Optima for Accurate Initiation.** The initiation site for late Ad2 transcription has been positioned at 16.5 m.u. (Fig. 1) (14–16). We have shown that transcription initiates here in nuclei isolated from Ad2-infected HeLa cells (4), and Weil *et al.* (6) recently demonstrated that purified RNA polymerase will initiate at this site on exogenously added viral DNA in the presence of a cytoplasmic extract obtained from uninfected HeLa cells. To test the transcription potential of whole cell extracts (prepared as described in *Materials and Methods*) *Bam*HI-digested pBR322-*Bal* I-E DNA mixture was used as exogenously added template. Initiation at 16.5 m.u. and elongation to the end of *Bal* I-E would produce a 1750-nucleotide RNA (Fig. 1).

The effect of varying the concentration of the whole cell extract on transcription from the late promoter of Ad2 is shown in Fig. 2*A*. Increasing concentrations of extract (protein 1–7.5 mg/ml) were added to reaction mixtures containing either pBR322-*Bal* I-E or pBR322-*Bal* I-D DNA (unseparated, restriction endonuclease-digested DNAs). A quarter of the transcription products were treated with glyoxal and resolved by electrophoresis in an agarose gel. When low concentrations of extract were added to a reaction mixture containing pBR322-*Bal* I-E, a heterogeneous mixture of RNA product was observed (Fig. 2*A*, lane 1). In striking contrast, if the amount of extract in the reaction mixture was doubled, synthesis of RNA was essentially abolished (Fig. 2*A*, lane 2). Addition of still higher concentrations of extract to reaction mixtures containing the pBR322-*Bal* I-E DNA specifically stimulated synthesis of a 1750-nucleotide RNA derived from the late Ad2 promoter (Fig. 2*A*, lanes 3 and 4). At the highest concentration of extract tested, this RNA species represents approximately 40% of the applied sample. (Three examples of analysis of *in vitro* transcription of the *Bal* I-E fragment are shown in Figs. 2*A*, lane 4, 2*B*, lane 4, and 3*A*, lane 3. The specificity of transcription seen in these samples is typical of our total results.) A comparison of lanes 1 and 4 in Fig. 2*A* suggests that, in addition to enhancing specific transcription from the late promoter of Ad2, higher extract concentration also suppresses nonspecific transcription. At either high or low extract concentration, the pBR322-*Bal* I-D fragments are less effective in stimulating RNA synthesis. In particular, a high concentration of extract showed no detectable bands when pBR322-*Bal* I-D DNA was used as template (Fig. 2*A*, lane 6). This is not due to inhibition of RNA synthesis by *Bal* I-D DNA because a mixture of pBR322-*Bal* I-E and pBR322-*Bal* I-D DNAs still resulted in the synthesis of the 1750-nucleotide species (data not shown).

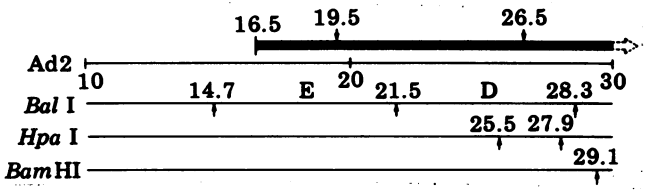


FIG. 1. The 5′ end of the Ad2 late transcription unit. Transcription begins at approximately 16.5 m.u. and continues rightward to almost the end of the Ad2 genome (100 m.u.). One m.u. is roughly 350 base pairs. The coordinates of the three leader segments on late mRNAs are indicated by the numbers above the solid arrow. The sites at which three restriction endonucleases used in these experiments cleave this region of the Ad2 genome are indicated.

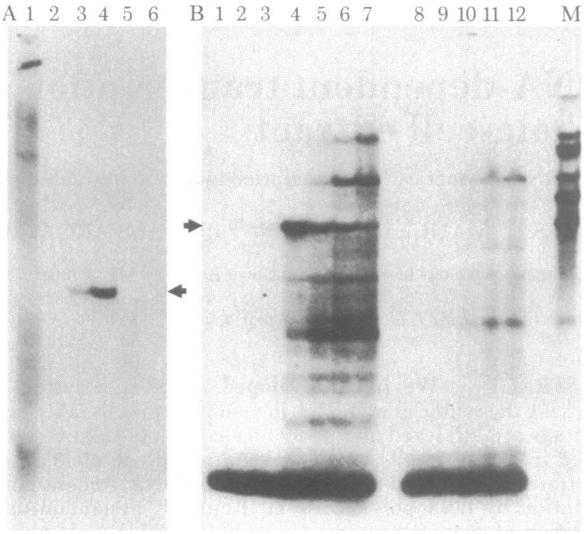


FIG. 2. Extract and DNA concentration optima for accurate transcription. RNA was synthesized in standard reaction mixtures except that either the extract (*A*) or DNA (*B*) concentration was varied, as follows. (*A*) Lanes 1–4, *Bal* I-E-pBR322 DNA template: 2.5, 5, 10, or 15 μl of extract; lanes 5 and 6, *Bal* I-D-pBR322 DNA template: 2.5 or 15 μl of extract. (*B*) Lanes 1–7, 0, 12.5, 25, 50, 75, 100, or 125 μg of *Bal* I-E-pBR322 DNA template per ml; lanes 8–12, 25, 50, 75, 100, or 125 μg of *Bal* I-D-pBR322 DNA template. After extraction and glyoxalation, 25% of each sample was resolved in a 1.4% agarose gel. The number of cpm (Cerenkov radiation) loaded in each slot was as follows: (*A*) 1, 9700; 2, 3400; 3, 2900; 4, 2500; 5, 2600; 6, 1100; (*B*) 1, 2700; 2, 3100; 3, 3400; 4, 4900; 5, 5800; 6, 5900; 7, 6700; 8, 3700; 9, 4000; 10, 4400; 11, 4700; 12, 4900. (The cpm in *B* were approximately 2 times higher than usually obtained. This may have resulted from incomplete removal of nucleotide triphosphates during ethanol precipitation.) The arrows indicate the position of the 1750-nucleotide transcript that originates from the Ad2 late promoter. M is an *Eco*RI digest of Ad2 DNA labeled *in vitro* by nick translation.

The results of varying the concentration of DNA present in standard reaction mixtures are shown in Fig. 2*B*. Concentrations of template DNA less than 50 μg/ml produced virtually no transcription (Fig. 2*B*, lanes 1–3). (We believe the small labeled RNA resulted from labeling of endogenous tRNA present in the lysate.) Reaction mixtures containing DNA at 50 μg/ml (Fig. 2*B*, lane 4) yielded the same high levels of specific transcription shown in Fig. 2*A*, lane 4. At higher DNA concentrations, 75 μg/ml (Fig. 2*B*, lane 5) and 100 μg/ml (Fig. 2*B*, lane 6), the amount of the 1750-base-long transcript synthesized decreased and other discrete transcripts appeared. S1-nuclease mapping suggests they arise from "end-to-end" transcription of the *Bal* I-E DNA fragment and the pBR322 DNA molecule (data not shown). We have not determined which class of polymerase is responsible for this end-to-end transcription. The origin of the lower molecular weight species seen in Fig. 2*B*, lanes 5 and 6, is not clear. Fig. 2, lanes 8–12, shows the effects of increasing concentrations of pBR322-*Bal* I-D DNA as template. The 1750-nucleotide transcript was not detected. At the higher DNA concentrations, we again observed the transcription of lower and higher molecular weight RNAs. The amount of this synthesis was lower with pBR322-*Bal* I-D DNA than with the pBR322-*Bal* I-E DNA.

**Additional Properties of *in Vitro* Transcripts Originating from the Ad2 Major Late Promoter.** Fig. 3*A* shows that synthesis of the 1750-nucleotide transcript was sensitive to α-

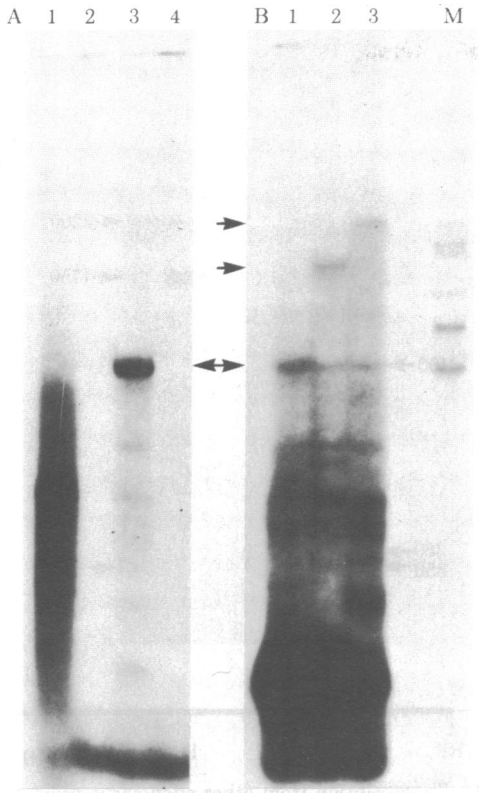


FIG. 3. (*A*) α-Amanitin sensitivity of transcription from the Ad2 late promoter. RNA was synthesized in 100-μl reaction mixtures, each containing 400 μCi of [α-$^{32}$P]UTP. Lanes 1–3 contained *Bal* I-E-pBR322 DNA as template and lane 4, *Bal* I-D-pBR322 DNA. Lane 1 contained 5 μl of extract; lane 2, 30 μl of extract + α-amanitin at 0.5 μg/ml; lanes 3 and 4 each contained 30 μl of extract. One percent of the RNA extracted from each reaction mixture was glyoxalated and analyzed by gel electrophoresis. The number of cpm loaded in each slot was: 1, 7100; 2, 8100; 3, 3300; 4, 1900. (*B*) *In vitro* transcription of total Ad2 DNA. RNA was extracted from standard reaction mixtures that had contained 50 μCi of [α-$^{32}$P]UTP. The Ad2 DNA template had been previously digested with *Bal* I (lane 1), *Hpa* I (lane 2), or *Bam*HI (lane 3). Twenty-five percent of each sample was glyoxalated and resolved in a 1.4% agarose gel; 34,000 cpm was loaded in slot 1, 28,600 in slot 2, and 14,500 in slot 3. The arrows indicate the positions of runoff transcripts from the Ad2 late promoter.

amanitin at 0.5 μg/ml (compare lanes 2 and 3 of Fig. 3*A*). This implicates RNA polymerase II as the enzyme responsible for the specific synthesis. Fig. 3*B*, lanes 1–3, shows that virion DNA is also active in these extracts. Here the added viral DNA had been cleaved with *Bal* I, *Hpa* I, or *Bam*HI restriction endonuclease, and these fragments stimulated synthesis of 1750-, 3150-, and 4400-nucleotide RNA chains, respectively (arrows). These are the expected RNA products for initiation occurring at the late promoter site at position 16.5 m.u. and elongation continuing to the end of each fragment (see Fig. 1). Note that the results in Fig. 3*B* show that the whole cell extract system will elongate RNA chains over 4.4 kilobases.

The origins of most of the lower molecular weight species in Fig. 3*B* have not been established. Because several adenovirus promoters other than the major late promoter are active in this system (see below), at least some of these transcripts likely result from initiation at these other sites. Some may also result from processing of one of these primary transcripts. Note that lanes 2 and 3, as well as 1, contain an RNA species of approximately 1750 nucleotides. The presence of these bands in lanes 2 and 3 may have resulted from an occasionally observed actinomycin D-insensitive DNA-independent reaction, which we believe is due to end-labeling of endogenous 18S ribosomal RNA (unpublished results). The heavily overexposed band at the bottom of each track represents one or both of the two small virus-associated (VA) RNAs, which are RNA polymerase III products (17).

**Fingerprint Analysis of 5′ "Cap" Structure.** The structure of the 5′ end of the Ad2 late mRNAs has been well studied (12, 13). We therefore decided to examine the structure of the 5′ end of the Ad2 RNA synthesized *in vitro*. For this, RNAs extracted from the reaction mixtures analyzed in Fig. 3*A* were digested with RNase T1. About 5% of samples made with *Bal* I-E pBR322 and *Bal* I-D-pBR322 DNA as template were directly analyzed by two-dimensional fingerprint analysis (Fig. 4 *A* and *B*, respectively). The remainders were resolved by selection of "capped" oligonucleotides on columns of DBAE-cellulose and then fingerprinted. When we analyzed the RNA from the *Bal* I-E reaction after selection on DBAE-cellulose, only two large oligonucleotides were detected (Fig. 4*C*, arrows). To identify these oligonucleotides, we eluted them, as well as several of the

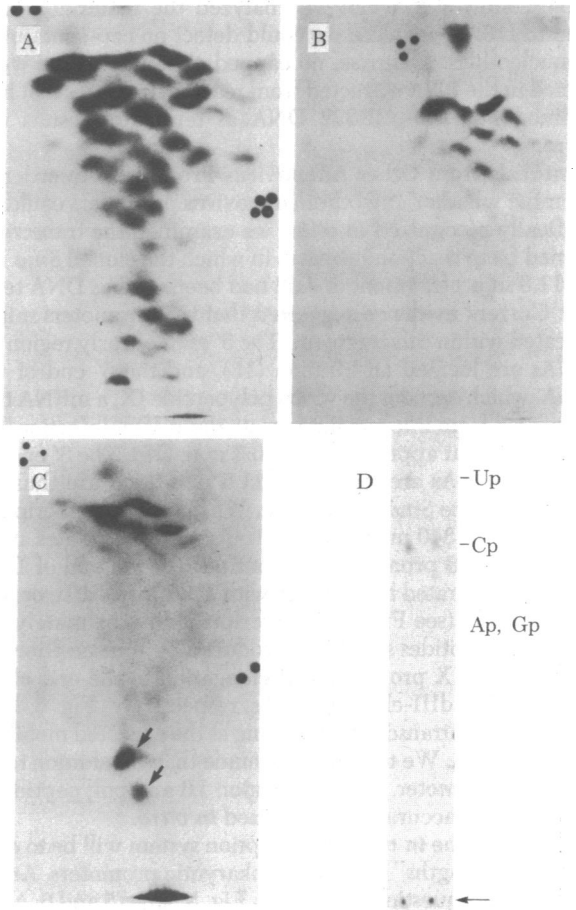


FIG. 4. Fingerprint analysis of RNA synthesized *in vitro*. The remainder of the RNA from each of the samples that had been analyzed by gel electrophoresis in Fig. 3 was digested with RNase T1. Five percent of each sample was analyzed directly by two-dimensional fingerprinting. (*A* and *B*) Fingerprints of RNA synthesized with *Bal* I-E-pBR322 DNA and *Bal* I-D-pBR322 DNA respectively, as templates. Electrophoresis was from left to right, and homochromatography from bottom to top. (*C*) Fingerprint of the oligonucleotides selected by DBAE-cellulose chromatography from the reaction mixture that had contained *Bal* I-E-pBR322 DNA. The arrows indicate the position of the Ad2 capped T1 oligonucleotide (see text). (*D*) Products obtained when aliquots of the major capped oligonucleotide in *C* were digested with RNase A (left) or RNase T2 (right) and analyzed by electrophoresis at pH 3.5 on DEAE-paper. Electrophoresis was from bottom to top. The arrow shows the position of the T2-resistant cap structure.

smaller oligonucleotides at the top of the fingerprint, and digested them with either RNase A or RNase T2. The only oligonucleotides that showed the partial resistance to RNase T2 expected of a 5′ cap structure (Fig. 4*D*) were the two indicated by the arrows in Fig. 4*C*. Furthermore, these two oligonucleotides gave identical nearest-neighbor analyses when digested with either of the two nucleases. The existence of two forms of the late capped oligonucleotide $m^7$GpppAmpCpUpCpUpCpUpUpCpCpGp(C) (14) has been routinely observed (e.g., ref. 12). Further results (not shown) demonstrated that the cap oligonucleotide was completely resistant to RNase U2 digestion, thereby showing the absence of non-2′-*O*-methylated A residues. Similar analysis of RNA made in the presence of a low concentration of $\alpha$-amanitin showed that not only does the synthesis of the entire 1750-nucleotide transcript require RNA polymerase II, but also synthesis of the very first 11 nucleotides is dependent on this enzyme. The fingerprint of the RNA synthesized in reaction mixtures containing a low concentration of extract (see Fig. 3*A*, lane 1) was extremely complex. However, when we analyzed the material which bound to DBAE-cellulose we could detect no cap-containing oligonucleotides. Likewise, no capped oligonucleotides were detected in the RNA extracted from reaction mixtures that had contained *Bal* I-D-pBR322 DNA as template (results not shown).

**Synthesis from Other Adenovirus Promoters.** In order to determine whether any other adenovirus promoters could be specifically recognized *in vitro*, we examined the transcripts obtained from reaction mixtures in which the cloned *Sma* I-F (2.9–11.3 m.u.) fragment of Ad2 had been used as DNA template. Current evidence suggested that two promoters might be located within this fragment. The 5′ ends of early region 1B mRNAs are located at 4.9 m.u. (18), and the 5′ end of the mRNA, which encodes the virion polypeptide IX, a mRNA that is expressed from intermediate to late times after infection, has been mapped at approximately 10.3 m.u. (19). The 3′ ends of all these mRNAs are located at 11.5 m.u., and are thus not contained on the *Sma* I–fragment. We expected transcripts of 2200, 1100, or 340 nucleotides if transcription initiates at the early region 1B promoter and continues to the end of DNA templates generated by cleavage with *Eco*RI, *Hin*dIII, or *Kpn* I, respectively (see Fig. 5). Transcripts of approximately 350 and 380 nucleotides should be produced by transcription initiation at the IX promoter and elongation to the end of the *Eco*RI- or *Hin*dIII-cleaved DNAs, respectively. Fig. 5, lanes 2–4, shows that transcripts migrating at the expected mobilities are synthesized. We therefore conclude that, in addition to the major late promoter, the early region 1B and polypeptide IX promoters are accurately recognized *in vitro*.

One use of the *in vitro* transcription system will be to compare the "strengths" of various eukaryotic promoters. An approach to this question is shown in Fig. 5, lanes 5 and 6. An aliquot of *Eco*RI-cut *Sma* I-F-pBR322 DNA was mixed with a given amount of either *Bal* I-E-pBR322 or *Bal* I-D-pBR322 DNA and transcribed *in vitro*. The results show that both the IB and late promoters functioned when added in the same reaction mixture. The late promoter appears to be the stronger of the two.

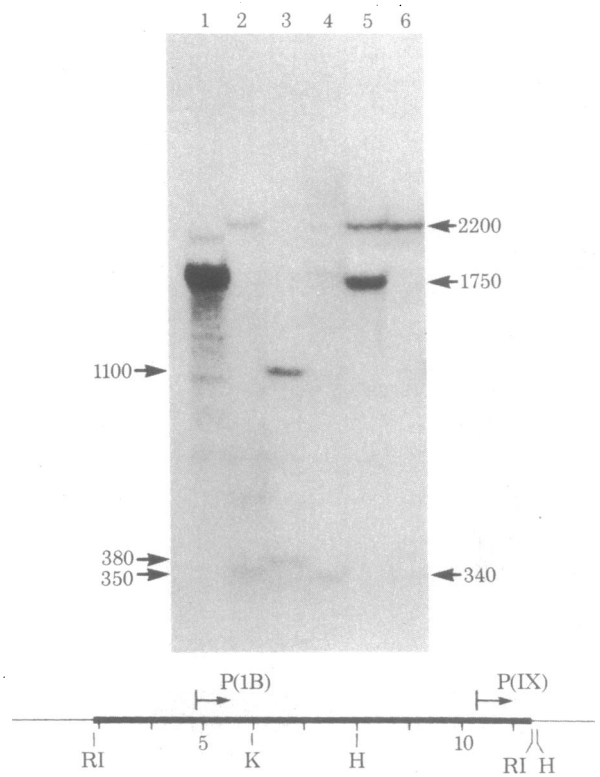


FIG. 5. Transcription from other adenovirus promoters. RNA was synthesized in standard reaction mixtures. DNA templates were: 1, *Bal* I-E-pBR322; 2, adenovirus 5 *Sma* I-F-pBR322; 3, *Sma* I-F-pBR322 digested with *Hin*dIII; 4, *Sma* I-F-pBR322 digested with *Kpn* I; 5, 1 $\mu$g of *Bal* I-E-pBR322 + 1 $\mu$g of *Sma* I-F-pBR322 + 0.5 $\mu$g of *Bal* I-D-pBR322; 6, 1 $\mu$g of *Sma* I-F-pBR322 + 1.5 $\mu$g of *Bal* I-D-pBR322 DNA. The drawing shows the adenovirus 5 map coordinates and the sites at which the restriction enzymes *Eco*RI (RI), *Kpn* I (K), and *Hin*dIII (H) cleave the adenovirus 5 DNA (thick line) or the pBR322 DNA (thin line). (*Eco*RI sites in the recombinant plasmid correspond to *Sma* I sites in adenovirus 5.) The inferred locations of the two adenovirus promoters are indicated by the arrows above the line. The arrows indicate the position in the gel to which RNA molecules of the indicated sizes (nucleotides) would migrate, as determined by comparison with the mobilities of glyoxalated DNA fragments obtained from a *Sma* I digest of $^{32}$P-labeled Ad2 DNA and a *Hph* I digest of $^{32}$P-labeled pBR322 DNA (not shown).

## DISCUSSION

The development of a DNA-dependent soluble *in vitro* transcription system for mammalian RNA polymerase II has been a long-standing objective in the study of eukaryotic gene regulation. Therefore, it was unexpected when we found that concentrated whole cell extracts are proficient in initiating transcription at suspected promoter sites on exogenously added DNA. We have shown that a whole cell extract initiates transcription at the late promoter site of Ad2, and we have evidence suggesting that two other viral promoters are utilized by the system as well. In collaboration with T. Maniatis and H. Handa, respectively, we have obtained preliminary evidence that the human $\beta$-globin gene and the early and late simian virus 40 genes are accurately transcribed *in vitro* (unpublished results).

Two types of evidence suggest that the whole cell extract initiates transcription at the late promoter site of Ad2. First, addition to *in vitro* reaction mixtures of viral DNA fragments cleaved by restriction endonucleases at sites 530 (*Sma* I, data not shown), 1750, 3150, or 4400 base pairs from the late promoter site at 16.5 m.u. stimulates synthesis by RNA polymerase II of RNA chains of these lengths. Second, RNA transcribed *in vitro* from late promoter is modified to form one predominant capped T1-resistant oligonucleotide, which is identical to the *in vivo*-synthesized undecanucleotide analyzed previously (12, 4). This *in vitro*-synthesized cap is completely modified by methylation at the 2′ position of the ribose on the penultimate adenosine. RNA synthesized *in vitro* from exogenously added DNA in the system of Weil *et al.* (6) was also shown to be capped with methylation of the 2′ position of ribose. On the

basis of sizing of runoff products, the whole cell extract also appears to initiate transcription from at least two other adenovirus promoter sites, early region 1B (4.9 m.u.) and the region coding for IX polypeptide (10.3 m.u.). During the lytic cycle of Ad2, the early region 1B is expressed from 4–6 hr after infection (20), whereas synthesis of the mRNA for polypeptide IX is prominent slightly later at an intermediate to late stage of infection (20). It is interesting that three viral promoters that appear to be utilized at three different stages of infection *in vivo* are all recognized *in vitro* by an extract prepared from uninfected cells.

Specific initiation by RNA polymerase II at the late adenovirus promoter site has been previously reported by Weil *et al.* (6). In this case, saturating amounts of purified RNA polymerase II were added to reaction mixtures containing a S-100 cytoplasmic extract prepared from uninfected cells. Under optimal salt, divalent cation, RNA polymerase II, and DNA concentration we calculate that these authors obtained approximately $5.2 \times 10^{-4}$ pmol of a specific transcript from the late adenovirus promoter site per 50-$\mu$l reaction mixture. The whole cell extract system, described here, yields $3.0 \times 10^{-3}$ pmol from the same promoter site in an identical volume. Thus the concentrated whole cell extract is at least as active as the RNA polymerase II-supplemented system. It is interesting that 100–150 units of purified RNA polymerase II is required to saturate the cytoplasmic extract system, while assays of the amount of RNA polymerase II activity present in the whole cell extract, by the method of Schwartz *et al.* (21), suggest that only 6–10 units of active RNA polymerase II are present in our comparable reaction mixture (results not shown). Perhaps the whole cell extract contains factors lost in the cytoplasmic extract system that enhance the efficiency of initiation by RNA polymerase II. The major advantages of the whole cell extract transcription system are its ease of preparation, flexibility, and possible retention of factors disgarded in other protocols. The ability to follow specific transcription at such an early stage in the fractionation of the extract will permit reconstitution of the system with purified factors.

The availability of a DNA-dependent soluble transcription system will also permit the investigation of regulatory factors that control the rate of initiation at different promoter sites. That these studies will be interesting is suggested by the fact that the late promoter of adenovirus is efficiently recognized by a whole cell extract from uninfected HeLa cells. As mentioned previously, Weil *et al.* (6) have also shown that RNA polymerase II and cytoplasmic extracts from uninfected HeLa cells will recognize the late promoter of adenovirus. Introduction *in vivo* of this same segment of DNA as part of the virus genome into the nuclei of HeLa cells does not result in detectable levels of transcription from this promoter until the onset of viral DNA replication (22). However, early promoters from the same viral genome are efficiently transcribed before viral DNA replication. One of these early promoters is the 1B region promoter, which is transcribed, but less efficiently than the late promoter in the whole cell extract from uninfected HeLa cells (see Fig. 5, lane 5). It is possible that recognition of a promoter site in the *in vitro* reaction by RNA polymerase II might reflect a nonphysiological interaction between the protein complex and the initiation site. Hence, within the cell, additional factors would be required for promoting initiation. Alternatively, early in the lytic cycle the late promoter site of Ad2 could be sequestered by proteins or modified so that RNA polymerase II does not recognize it. Another possibility is that the late promoter actually does function at early times after infection, but RNA has not been detected because it is degraded, or prematurely terminated, *in vivo*. This model would suggest that control of adenovirus gene expression might occur, at least in part, at a step other than initiation.

The availability of *in vitro* systems for transcription of mammalian genes will provide a wealth of information on the biochemistry of RNA processing and modification. Highly labeled substrates can now be synthesized that are similar, if not identical, to intracellular nuclear intermediates and that can be used to assay for specific processing enzymes. It is intriguing that several smaller specific size RNA species are resolved after electrophoresis of RNA synthesized *in vitro* from the late Ad2 promoter (e.g., Fig. 2*B*). Some of these RNAs are of the correct size to be intermediates in the processing of late viral RNA by RNA splicing. The purification and characterization of all those hypothetical activities that cleave, splice, and modify RNA in the nucleus of a mammalian cell, as well as identification of the signals on nucleic acids that bring about these reactions, should be facilitated by this *in vitro* system.

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