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Multiple Factors Required for Accurate Initiation of Transcription by Purified RNA Polymerase II*

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A soluble extract prepared from human cells (KB S-100) has been recently shown to direct accurate transcription initiation by purified RNA polymerase II at the major late promoter of adenovirus 2. We have fractionated this extract by chromatography on phosphocellulose, DEAE-cellulose, and DNA-cellulose, and have identified four components that are required for the active and selective initiation of transcription by RNA polymerase II at this promoter. One of these components seems to act by suppressing random, but not selective, transcription by RNA polymerase II. All but one of these components have been shown to be chromatographically distinct from the factors involved in directing selective transcription by RNA polymerase III.

As shown previously by Weil *et al.* (1), a soluble cell-free extract from cultured human KB cells (KB S-100) directs accurate transcription initiation by purified RNA polymerase II at the major late promoter on adenovirus 2 DNA. This same reconstituted system also supports accurate transcription initiation at the mouse β-major globin gene promoter (2). In contrast, the purified RNA polymerase alone transcribes these and other purified DNA templates in a random fashion. Therefore, the S-100 extract must contain other (unidentified) transcription factors not present in the RNA polymerase preparations. Such factors are also active in a crude whole cell HeLa extract which has also been shown to support accurate initiation of transcription by the endogenous RNA polymerase II at the Ad2[1] major late promotor (3).

Soluble extracts from cultured mammalian cells and from *Xenopus* oocytes have also been shown to mediate (via the endogenous RNA polymerase III) the accurate transcription of genes encoding various 5 S and tRNAs and the adenovirus-associated RNAs (see the accompanying paper (4)). Moreover, the fractionation of these extracts has shown that several components are necessary for accurate transcription by RNA polymerase III. One of these factors, specific for 5 S RNA genes, has been purified and shown to interact with a known intragenic control region (5). These studies made it seem highly probable that a similar approach should facilitate the identification of those factors necessary for transcription of the class II genes.

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[1] The abbreviation used is: Ad2, adenovirus 2.

EXPERIMENTAL PROCEDURES

Chromatography of the KB S-100—S-100 extracts, prepared as described in Weil *et al.* (6), were dialyzed for 6 to 8 h against Buffer A (20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (pH 7.9), 20% glycerol, 0.2 mM EDTA, and 0.5 mM dithiothreitol) containing 0.1 M KCl and then stored at −80°C after a 10-min centrifugation at 12,000 × *g*. The S-100 (12 to 16 mg of protein/ml of S-100; 1.2×10^8 cell equivalents/ml of S-100) was applied to a phosphocellulose column (Whatman P11) equilibrated with Buffer A containing 0.1 M KCl (10 mg of protein/ml of bed volume). The column was washed with this buffer and the bound protein was then sequentially step-eluted with Buffer A containing 0.35, 0.6, and 1.0 M KCl. This last buffer also contained 0.2 mg/ml of bovine serum albumin (Pentex). For this and subsequent column chromatography, fractions equivalent to 10% of the bed volume were collected and the appropriate breakthrough and step-eluted fractions (2 to 4 fractions) were pooled on the basis of their absorbance at 280 nm. Pooled fractions which were not used for subsequent chromatography were dialyzed against Buffer A containing 5 mM $MgCl_2$ (Buffer B) and 0.1 M KCl for 4 to 10 h and then stored in aliquots at −80°C. The combined phosphocellulose 0.6 M KCl step fractions were adjusted to 5 mM $MgCl_2$ and dialyzed against Buffer B to a final concentration of 0.1 M KCl. After a low speed centrifugation, this pool was loaded onto a column of DEAE-cellulose (Whatman DE52) equilibrated with Buffer B containing 0.1 M KCl (3 mg of protein/ml of bed volume). The column was washed with this buffer and the bound protein was then step-eluted with Buffer B containing 0.25 M KCl. After the addition of bovine serum albumin to 0.1 mg/ml, the breakthrough pool was applied to a column of single-stranded calf thymus DNA-cellulose (7) (1.05 mg of DNA/ml of bed volume; 5 mg of protein/ml of bed volume) which had been extensively washed with Buffer B containing 0.1 M KCl and 0.1 mg/ml of bovine serum albumin. After washing the column with this buffer, the bound protein was sequentially step-eluted with Buffer B containing 0.1 mg/ml of bovine serum albumin and 0.3, 0.6, and 1.0 M KCl.

Preparation of KB RNA Polymerase II—RNA polymerase II was prepared from the nuclear pellets (P100s) which were recovered in the preparation of the KB S-100s and stored at −80°C in Buffer C (50 mM Tris-HCl (pH 7.9), 25% glycerol, 0.1 mM EDTA, and 0.5 mM dithiothreitol) containing 5 mM $MgCl_2$ (10^8 nuclei/ml). The P-100s were homogenized first in a Dounce homogenizer and then in a Waring Blendor. After dilution with an equal volume of buffer containing 50 mM Tris-HCl (pH 7.9), 0.1 mM EDTA, and 0.5 mM dithiothreitol, the suspension was again homogenized, left on ice for 15 min, and then spun at 13,000 × *g* for 30 min. Polymin-P was added to the supernatant to a final concentration of 0.15% (8). After 30 min of stirring, the precipitated protein and nucleic acids were recovered by centrifugation at 13,000 × *g* for 30 min. The pellet was resuspended by homogenization in a Waring Blendor in a buffer containing 50 mM Tris-HCl (pH 7.9), 10% glycerol, 0.25 M ammonium sulfate, 0.1 mM EDTA, and 0.5 mM dithiothreitol. After centrifugation of the suspension at 16,000 × *g* for 20 min, soluble proteins were precipitated from the supernatant by the addition of solid ammonium sulfate to 0.35 g/

ml. The precipitate was pelleted by centrifugation at 16,000 × *g* for 30 min and dissolved in Buffer C to a final ammonium sulfate concentration of 0.15 M. This fraction was then stirred for 90 min with DEAE-cellulose (Whatman DE52) which had been previously equilibrated with Buffer C containing 0.15 M ammonium sulfate. The unadsorbed proteins (including RNA polymerases I and III) were removed by filtration of the resin in a Buchner funnel. After the resin had been extensively washed with Buffer C containing 0.15 M ammonium sulfate, it was poured into a column. RNA polymerase II activity was step-eluted from the column with Buffer C containing 0.5 M ammonium sulfate. Fractions containing RNA polymerase II activity were pooled and dialyzed against Buffer C to a final concentration of 0.05 M ammonium sulfate. This fraction was then applied to a phosphocellulose column and RNA polymerase II was step-eluted with Buffer C containing 0.25 M ammonium sulfate and 0.5 mg/ml of bovine serum albumin. Fractions containing RNA polymerase II (~300,000 units/ml) were stored at −80°C. The RNA polymerase II prepared in this way was judged to be ~70% pure by sodium dodecyl sulfate-polyacrylamide gel electrophoretic analysis. Approximately 900,000 units of enzyme activity (6) were recovered from the P-100s prepared from 3×10^{11} cells.

Synthesis, Purification, and Analysis of RNA—The conditions for RNA synthesis, the purification of the RNAs and their analysis by polyacrylamide gel electrophoresis and autoradiography were as described in Weil *et al.* (1) except that the template in the transcription reactions was 20 μg/ml of *Sma* I-digested pSmaF DNA (1).

RESULTS

Chromatography of a KB S-100 on Phosphocellulose Separates Three Class II Gene Transcription Components—A soluble extract prepared from human KB cells (KB S-100) (see "Experimental Procedures") was separated into four fractions by chromatography on phosphocellulose as follows. The extract was loaded onto the ion exchange column in a buffer containing 0.1 M KCl and the bound proteins were then sequentially eluted with buffers containing 0.35, 0.6, and 1.0 M KCl (Fig. 1*A*). The breakthrough and step fractions (designated *a*, *b*, *c*, and *d*) were then assayed for their ability to direct selective initiation of transcription by purified RNA polymerase II at the major late promoter of Ad2. The template used in these reactions was the plasmid pSmaF which contains the *Sma* I-F fragment of Ad2 (coordinates 11.6 to 18.2) inserted into the *Sma* I site of pBR313 (1). The DNA was digested with the endonuclease *Sma* I so that accurate initiation of transcription by RNA polymerase II at the Ad2 major late promoter (coordinate 16.45) would lead to the synthesis of a 536-nucleotide run-off transcript (1) (Fig. 1*C*). The gel analysis of RNAs synthesized by RNA polymerase II on this template in the presence of the unfractionated S-100 is shown in *lane 1* of Fig. 1*B*. The *arrow* indicates the 536-nucleotide transcript diagnostic of accurate initiation (see Ref. 1 for a discussion of the high molecular weight RNAs at the top of the gel which result from random transcription). As observed previously (1), synthesis of the 536-nucleotide RNA is almost completely dependent on the addition of exogenous RNA polymerase and is sensitive to a low concentration of α-amanitin (1 μg/ml) (data not shown). None of the individual phosphocellulose fractions (*a*, *b*, *c*, or *d*) alone supported accurate initiation of transcription by RNA polymerase II (Fig. 1*B*, *lanes 2* to *5*). However, when the fractions were assayed in pairwise combinations, the combination of the 0.6 M KCl step (*c*) and 1.0 M KCl step (*d*) fractions (and no other combination) supported a low level of selective transcription (Fig. 1*B*, *lanes 6* to *9*, and data not shown). The addition of the breakthrough (*a*) fraction to a combination of the 0.6 M KCl step (*c*) and 1.0 M KCl step (*d*) fractions led to a dramatic stimulation of transcription (compare *lanes 9* and *10* of Fig. 1*B*). (Occasionally, after fractionation of an S-100 on phosphocellulose, the reconstitution of accurate transcription initiation was absolutely dependent on the presence of the breakthrough fraction. Thus, in these cases and in contrast to the analysis described above, a combination of the 0.6 M KCl step (*c*) and 1.0 M KCl step (*d*) fractions did not by itself appear to support any selective transcription.) Hence, an initial fractionation of the S-100 indicates that at least three components are required for active and selective initiation of transcription by RNA polymerase II at the major late promoter of Ad2. Importantly, the reconstitution of accurate transcription from these components requires a careful adjustment of the relative amount of the various fractions. Selective transcription is generally maximal when the breakthrough (*a*), 0.6 M KCl step (*c*), and 1.0 M KCl step (*d*) fractions are combined in a 1:2.5:2.5 ratio. An increasing amount of the breakthrough fraction leads to an inhibition of transcription.

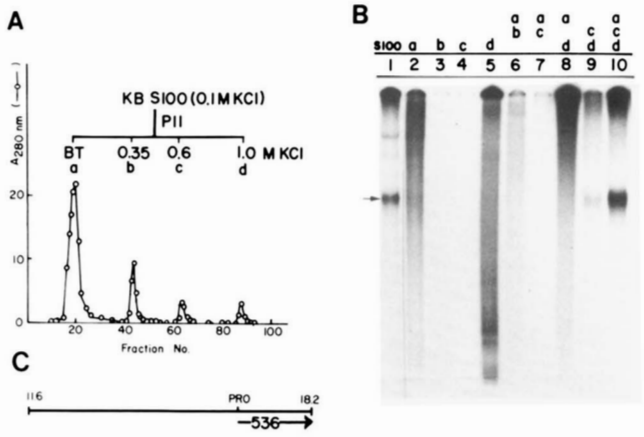

FIG. 1. **Separation of class II gene transcription components on phosphocellulose.** A soluble extract (S-100) prepared from KB cells was chromatographed on phosphocellulose as described under "Experimental Procedures" to give a breakthrough fraction (*a*), 0.35 M KCl (*b*), 0.6 M KCl (*c*), and 1.0 M KCl (*d*) step-eluted fractions. The absorbance profile ($A_{280\ nm}$) of this fractionation scheme is shown in *A*. *B* shows the autoradiogram of the gel analysis of RNAs synthesized in the presence of the S-100 (*lane 1*); the breakthrough fraction (*lane 2*); the 0.35 M KCl step fraction (*lane 3*); 0.6 M KCl step fraction (*lane 4*); the 1.0 M KCl step fraction (*lane 5*); a combination of the breakthrough fraction and the 0.35 M KCl step fraction (*lane 6*), or the 0.6 M KCl step fraction (*lane 7*), or the 1.0 M KCl step fraction (*lane 8*); a combination of the 0.6 M KCl and the 1.0 M KCl step fractions (*lane 9*); a combination of the breakthrough fraction and the 0.6 M KCl and the 1.0 M KCl step fractions (*lane 10*). For the various reactions, 20 μl of the S-100, 5 μl of the phosphocellulose breakthrough fraction, 10 μl of the 0.35 M KCl step fraction, and 12.5 μl of the 0.6 M KCl and 1.0 M KCl step fractions were used. The *arrow* indicates the 536-nucleotide transcript. The Ad2 *Sma* I-F fragment extending from map coordinate 11.6 to 18.2 is depicted in *C*. The major late promoter (*PRO*) at coordinate 16.45 is indicated. The *arrow* depicts the 536 nucleotide run-off transcript diagnostic of accurate initiation (1). (Although this transcript was originally estimated to be 560 nucleotides (1), recent sequencing data[2] indicate that accurate transcription initiation at the major late promoter on the *Sma* I-F fragment of Ad2 DNA would give a 536-nucleotide run-off transcript.)

The Phosphocellulose 0.6 M KCl Step Fraction Contains Two RNA Polymerase II Transcription Factors and an RNA Polymerase III Transcription Factor—The phosphocellulose 0.6 M KCl step (*c*) fraction contains not only a component(s) necessary for selective transcription by RNA polymerase II, but also, as described in the accompanying paper (4), one of the components required for selective transcription by RNA polymerase III. To determine whether common or different components are involved in directing transcription by these two enzymes, the phosphocellulose 0.6 M KCl step (*c*) fraction was chromatographed on DEAE-cellulose to give a breakthrough fraction (*e*) and a 0.25 M KCl step (*f*) fraction (Fig. 2). As shown in the gel analysis of Fig. 3, the DEAE-cellulose

[2] E. Ziff, unpublished observations.

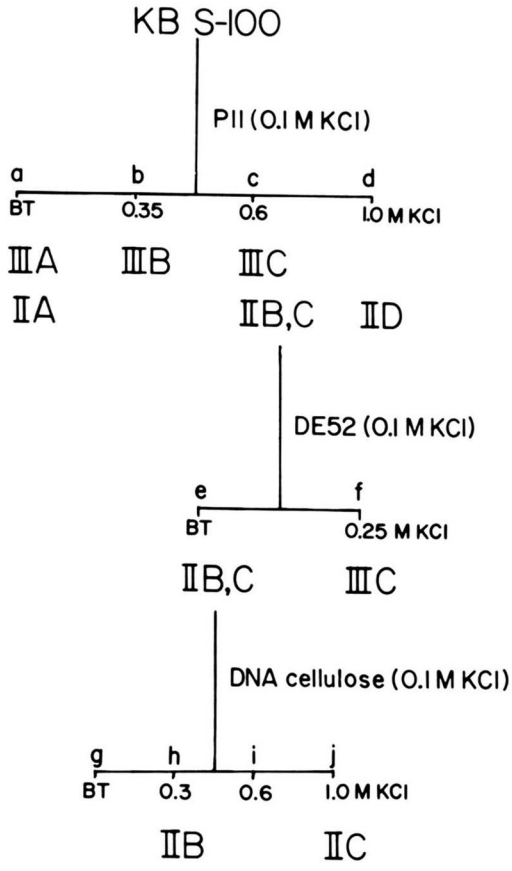

FIG. 2. **Separation of RNA polymerase II and III transcription factors.** The chromatographic steps are described under "Experimental Procedures." The letters *a* to *j* denote the various chromatographic fractions. Accurate initiation of transcription by RNA polymerase II at the major late promotor of Ad2 requires the fractions containing the "factors" *IIA*, *IIB*, *IIC*, and *IID*. The fractions containing the factors *IIIA*, *IIIB*, and *IIIC* are required for selective transcription by RNA polymerase III (see the accompanying paper (4)). BT, P11, and DE52 denote breakthrough, phosphocellulose, and DEAE-cellulose, respectively. The KCl concentrations indicate either the salt concentration at which protein was loaded onto or eluted from a column.

breakthrough (*e*) fraction, but not the step (*f*) fraction, can substitute for the input phosphocellulose 0.6 M KCl step (*c*) fraction in directing accurate transcription by RNA polymerase II in the presence of the phosphocellulose breakthrough (*a*) and the 1.0 M KCl step (*d*) fractions (Fig. 3, *lanes 1*, *3*, and *5*). However, as indicated in the accompanying paper (4), the component(s) in the phosphocellulose 0.6 M KCl step (*c*) fraction which is required for selective transcription by RNA polymerase III fractionates into the DEAE-cellulose step fraction. Hence, the components present in the phosphocellulose 0.6 M KCl step (*c*) fraction required for selective transcription by RNA polymerases II and III are distinct.

A careful inspection of the gel analyses of the transcripts synthesized by RNA polymerase II in the presence of various combinations of the phosphocellulose and DEAE-cellulose fractions (Fig. 1 and 3 and data not shown) suggested the possibility that both the phosphocellulose 0.6 M KCl step (*c*) fraction and the derived DEAE-cellulose breakthrough (*e*) fraction contain a component which inhibits random transcription. For instance, the random transcription by RNA polymerase II in the presence of the phosphocellulose breakthrough (*a*) fraction (which is seen as a smear of radioactivity in *lane 2* of Fig. 1*B*) is greatly reduced by the addition of the phosphocellulose 0.6 M KCl step (*c*) fraction (Fig. 1*B*, *lane 7*). Similarly, the addition of the DEAE-cellulose breakthrough (*e*) fraction, but not the DEAE-cellulose 0.25 M KCl step (*f*) fraction (both derived from the phosphocellulose 0.6 M KCl step fraction), reduces the level of random transcription obtained in the presence of the phosphocellulose breakthrough (*a*) fraction alone (Fig. 3, *lanes 2* and *4*). The chromatographic behavior of this random transcription inhibitory activity (*i.e.* a component present in the phosphocellulose 0.6 M KCl step fraction which subsequently is recovered in the DEAE-cellulose breakthrough fraction) is the same as that described above for a component required, in combination with the phosphocellulose breakthrough and the 1.0 M KCl step fractions, for the accurate initiation of transcription by RNA polymerase II. To determine whether the component inhibiting random transcription differs from the component required

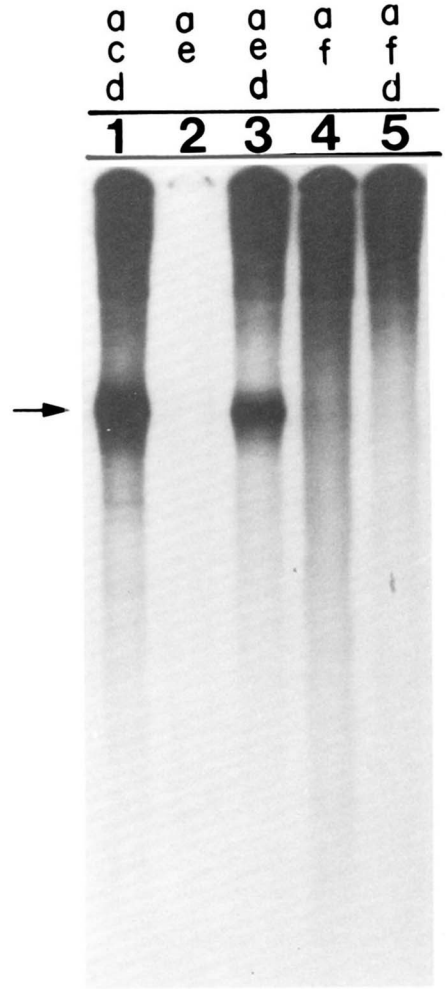

FIG. 3. **DEAE-cellulose chromatography of the phosphocellulose 0.6 M KCl step fraction.** The phosphocellulose 0.6 M KCl step fraction of Fig. 1 was chromatographed on a DEAE-cellulose column as described under "Experimental Procedures." The autoradiogram shows the gel analysis of the RNAs synthesized in the presence of the phosphocellulose breakthrough fraction (*lanes 1* to *5*) in addition to a combination of the phosphocellulose 0.6 and 1.0 M KCl step fractions (*lane 1*); the DEAE-cellulose breakthrough fraction (*lane 2*); a combination of the phosphocellulose 1.0 M KCl step fraction and the DEAE-cellulose breakthrough fraction (*lane 3*); the DEAE-cellulose step fraction (*lane 4*); a combination of the phosphocellulose 1.0 M KCl step fraction and the DEAE-cellulose step fraction (*lane 5*). Two microliters of the phosphocellulose breakthrough fraction and 15 μl of all other fractions were used. The *letters* refer to the chromatographic fractions of the fractionation scheme outlined in Fig. 2. The 536-nucleotide-specific transcript is indicated by the *arrow*.

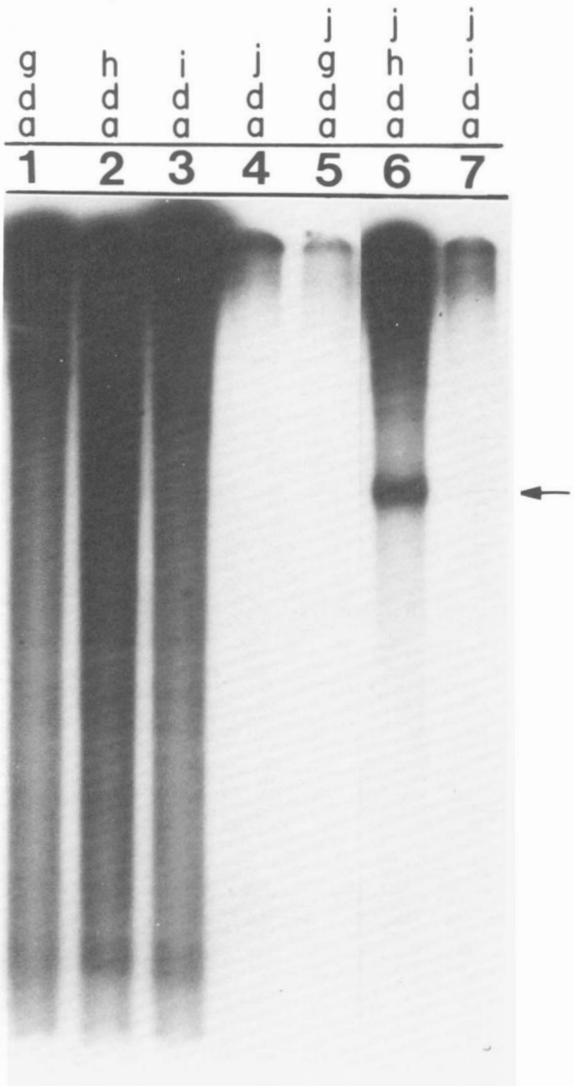

FIG. 4. **DNA-cellulose chromatography of the DEAE-cellulose breakthrough fraction.** The DEAE-cellulose breakthrough fraction (see Fig. 2) was chromatographed on DNA-cellulose as described under "Experimental Procedures." The autoradiogram shows the gel analysis of RNAs synthesized in the presence of the phosphocellulose breakthrough and 1.0 M KCl step fractions (*lanes 1* to *7*) in addition to the DNA-cellulose breakthrough fraction (*lanes 1* and *5*); the DNA-cellulose 0.3 M KCl step fraction (*lanes 2* and *6*); the DNA-cellulose 0.6 M KCl step fraction (*lanes 3* and *7*); and the DNA-cellulose 1.0 M KCl step fraction (*lanes 4* to *7*). Two microliters of the phosphocellulose breakthrough fraction and 10 μl of all other fractions were used. The *letters* refer to the chromatographic fractions depicted in Fig. 2. The 536-nucleotide-specific transcript is indicated by the *arrow*.

for selective initiation of transcription by RNA polymerase II, we have fractionated the DEAE-cellulose breakthrough (*e*) fraction by chromatography on DNA-cellulose. This separation gave a breakthrough fraction (*g*) and 0.3 M KCl step (*h*), 0.6 M KCl step (*i*), and 1.0 M KCl step (*j*) eluted fractions (Fig. 2). As can be seen in the gel analysis of Fig. 4, none of the individual DNA-cellulose fractions can substitute for the input DEAE-cellulose breakthrough (*e*) fraction in directing selective transcription by RNA polymerase II in the presence of a combination of the phosphocellulose breakthrough (*a*) and 1.0 M KCl step (*d*) fractions (Fig. 4, *lanes 1* to *4*). This gel analysis also shows that, in contrast to the high level of random RNA synthesis in the reactions containing either the DNA-cellulose breakthrough (*g*), 0.3 M KCl step (*h*), or 0.6 M KCl step (*i*) fractions (Fig. 4, *lanes 1* to *3*), there is a dramatically reduced level of transcription in the reaction containing the DNA-cellulose 1.0 M KCl step (*j*) fraction (Fig. 4, *lane 4*). The addition of this latter fraction to the former reactions greatly reduced the level of random transcription (Fig. 4, compare *lanes 1* and *5*, *2* and *6*, *3* and *7*). Moreover, selective initiation of transcription at the Ad2 major late promoter is restored in the reaction containing a combination of the DNA-cellulose 0.3 M KCl (*h*) and 1.0 M KCl step (*j*) fractions added to the phosphocellulose breakthrough (*a*) and 1.0 M KCl step (*d*) fractions (Fig. 4, *lane 6*). Hence, the DEAE-cellulose breakthrough fraction contains at least two components, separable by DNA-cellulose chromatography, that are required for selective transcription by RNA polymerase II. One of these components (*i.e.* the factor in the DNA-cellulose 1.0 M KCl step fraction) appears to act by suppressing random transcription. (It can be noted that the phosphocellulose 0.35 M KCl step (*c*) fraction also contains an activity that inhibits random transcription (compare *lanes 2*, *3*, and *6* of Fig. 1*B*). However, since this fraction also inhibited selective transcription (data not shown), this effect was not investigated further.)

DISCUSSION

We have previously shown that a crude cellular extract mediates accurate transcription initiation by a purified RNA polymerase II (1, 2). In the present report, we have shown that, after a multistep chromatographic separation of this extract, four fractions contain components which are necessary for active and selective transcription (Fig. 2). For convenience, these transcription factors have been tentatively designated *IIA*, *IIB*, *IIC*, and *IID* (Fig. 2). These refer in actuality to chromatographic fractions which could contain more than one factor. However, further chromatographic separation of the fractions containing *IIB* and *IID* has failed thus far to resolve any additional transcription components. In addition, the DNA-cellulose fraction containing *IIC* contains only one (>90%) major protein. However, a clear determination of the number of factors required for active and accurate transcription initiation by RNA polymerase II awaits the complete purification of these components. As summarized in Fig. 2, at least three of these RNA polymerase II factors (*IIB*, *IIC*, and *IID*) are distinct from those required for accurate transcription by RNA polymerase III. Although the phosphocellulose breakthrough fraction contains components necessary for active and accurate transcription by both RNA polymerases II and III, it is unlikely, for reasons discussed in the accompanying paper (4), that the same component is involved in transcription by both enzymes.

The factor present in the phosphocellulose breakthrough fraction (*IIA*) appears to act by stimulating a low level of selective transcription which is obtained in the presence of the phosphocellulose 0.6 M KCl step and the 1.0 M KCl step fractions. The stimulatory effect of this factor does not appear to be a general one: this fraction does not stimulate selective transcription of the virus-associated RNA_I gene of Ad2 by RNA polymerase III, as indicated in the accompanying paper (4), nor does it stimulate transcription by RNA polymerase II on calf thymus DNA (data not shown). (We cannot exclude the possibility that *IIA* may be absolutely required for selective transcription; in some instances, the ability to detect any accurate transcription requires not only the phosphocellulose 0.6 M KCl and 1.0 M KCl step fractions, but is also dependent on the presence of the breakthrough fraction. Possibly, the low level of transcription frequently observed in the presence of this latter fraction may reflect contamination of the phos-

phocellulose 0.6 M KCl and/or 1.0 M KCl step fractions with the *IIA* component.)

The chromatographic behavior of the factor (*IIB*) which is eluted from phosphocellulose with 0.6 M KCl and which is subsequently recovered in the DEAE-cellulose breakthrough fraction, is similar to that reported for factors which have been previously purified from eukaryotes on the basis of their ability to stimulate transcription by RNA polymerase II on a variety of DNA templates (9–13). The latter stimulatory factors have never been assayed for a possible role in selective transcription initiation; we do not know whether *IIB* is analogous to one of these factors. However, *IIB* can be completely separated from a contaminating stimulatory activity (data not shown).

Although we have not yet investigated in detail the role or mechanism of action of the transcription factors identified here, *IIC* has been found to dramatically suppress random, but not selective, transcription by RNA polymerase II. This factor has no effect on selective transcription by RNA polymerase III, but will suppress random transcription of calf thymus DNA by RNA polymerases I, II, and III (data not shown). Interestingly, an increasing amount of purified DNA can reverse this inhibitory effect. These results suggest that the factor (*IIC*) acts primarily via interaction with the DNA template and not with the RNA polymerase. It is also possible that this factor is not required directly for accurate initiation but rather that it prevents RNA polymerase from initiating at random (nonpromoter) sites, thus, indirectly making RNA polymerase more available for selective initiation. Nonetheless, these studies have not ruled out a possible direct role of *IIC* in selective transcription since, in its absence, we have not been able to determine whether or not there are any specific transcripts in the overwhelming background of random transcripts. The general transcription suppression properties of *IIC* also raises the possibility that it may be a chromosomal protein(s) such as histone(s). However, *IIC* has been extensively purified and polyacrylamide gel analysis shows no contamination with histone proteins. Furthermore, the addition of histones to the transcription reaction has been shown to inhibit both selective and random transcription (data not shown).

The studies described here have clearly indicated that selective transcription by RNA polymerase II is complex and requires multiple components. However, in addition to the presently identified factors which are necessary for accurate transcription initiation, there may be further components involved in other aspects of transcription. For instance, the assay used here is independent of termination: the template has been truncated close to the promoter (far upstream from the termination site of the transcription unit). Hence, any components which may be required exclusively for site-specific termination would not have been identified. Also, we do not know whether the factors involved in transcription from the Ad2 major late promoter will suffice for the transcription of all class II genes. Possibly different class II genes (or subgroups of these genes) require gene (or group)-specific transcription factors. Additionally, as discussed previously (1, 2), the factors which are active in the cell-free system are most likely general transcription factors which, by themselves, are insufficient for the regulation of gene expression. For instance, transcription is initiated at the Ad2 major late promoter and at the mouse β-major globin promoter in extracts from KB cells, despite the fact that neither adenovirus nor globin genes are expressed in (uninfected) KB cells (1, 2). Hence, there must be additional factors which, directly or indirectly (*e.g.* via template modifications), regulate gene expression. A further investigation of the factors identified here should lead not only to an understanding of their mechanism of action, but should also allow the identification and analysis of regulatory factors.

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