MOLECULAR AND CELLULAR BIOLOGY, Apr. 1989, p. 1465–1475
0270-7306/89/041465-11$02.00/0

Vol. 9, No. 4

Dynamic Interaction between a *Drosophila* Transcription Factor and RNA Polymerase II

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Received 5 October 1988/Accepted 16 December 1988

We have purified factor 5, a *Drosophila* RNA polymerase II transcription factor. Factor 5 was found to be required for accurate initiation of transcription from specific promoters and also had a dramatic effect on the elongation properties of RNA polymerase II. Kinetic studies suggested that factor 5 stimulates the elongation rate of RNA polymerase II on a dC-tailed, double-stranded template by reducing the time spent at the numerous pause sites encountered by the polymerase. The factor was found to be composed of two polypeptides (34 and 86 kilodaltons). Both subunits bound tightly to pure RNA polymerase II but were not bound to polymerase in elongation complexes. Our results suggest that factor 5 interacts briefly with the paused polymerase molecules and catalyzes a conformational change in them such that they adopt an elongation-competent conformation.

The transcriptional machinery responsible for the synthesis of eucaryotic mRNAs is a dynamic complex of RNA polymerase II, accessory factors, DNA, and RNA. A subset of the accessory factors involved have the ability to bind to specific DNA sequences (reviewed in references 11 and 27). These DNA-binding factors bind either to general promoter elements or to specific elements found on one or a few genes and can act to either activate or repress transcription of particular genes (1, 6, 28, 30, 37, 43). Other factors involved in transcription are not sequence-specific DNA-binding proteins, and some of these have been shown to interact directly with RNA polymerase II (3, 33, 42, 47). An accurate description of how RNA polymerase II interacts with the factors as well as how the factors interact with each other, with the template, and with the RNA product must precede an understanding of how transcription is controlled.

Initiation is one of the obvious control points of the transcription cycle. Several different experimental approaches have been designed to study the formation of the preinitiation complex (12, 17) and the early events in the initiation process (4, 5, 25). Numerous factors are required for specific initiation of transcription in vitro (26, 30, 31, 36, 42), and unfortunately a completely defined system has not yet been described. Initiation can be considered to be the transition from a stable preinitiation complex to an elongation complex. To understand more fully the transition, the properties of RNA polymerase II at the endpoint of the transition, i.e., in the elongation complex, must be known.

The elongation properties of RNA polymerase II can be studied without initiation at physiological promoters by using DNA templates extended at the 3′ ends by the addition of poly(dC) (21). RNA polymerase II will initiate transcription on these templates at a specific site without the aid of accessory factors (40). There are several unusual features of the transcription of these templates by RNA polymerase II. First, the nontemplate strand of the double-stranded template is displaced by the advancing polymerase, and the RNA is left as a heteroduplex with the template strand (21). This can be avoided by including RNase H in the reactions to digest the 5′ portion of the nascent heteroduplex, thus allowing renaturation of the template and subsequent displacement of the transcript (22, 38, 40). Second, RNA polymerase II encounters numerous pause sites during the transcription of a dC-tailed template (8, 21). Under physiological salt conditions, pausing is at least partially responsible for the 10- to 20-fold reduction in elongation rate of pure RNA polymerase II on the dC-tailed template compared with the rate seen in vivo (40). Although the mechanism of pausing is unknown, pausing of *Escherichia coli* RNA polymerase at some sites on double-stranded templates has been correlated with G+C-rich regions or potential stem-and-loop structures in the transcript 8 to 10 nucleotides upstream of the nucleotide addition site (24).

A number of factors that modify elongation by *E. coli* RNA polymerase have been identified and purified (7, 20, 46). Two factors that affect elongation by RNA polymerase II have been found. One factor, S-II, stimulates RNA polymerase II in the presence of the nonphysiological divalent cation manganese (39). S-II has also been shown to reduce the tendency of RNA polymerase II to pause during transcription initiated from promoters in vitro (32, 34). Another factor, factor 5, was identified in a fraction obtained from a *Drosophila* K_c cell nuclear extract required for specific initiation of transcription (31). This factor was found to increase the elongation rate of RNA polymerase II on a dC-tailed template (15, 31). We report here that factor 5 is still required for initiation at a physiological promoter after extensive purification. The purified factor also affects transcription of dC-tailed templates by reducing the time that RNA polymerase II spends at pause sites during transcription.

MATERIALS AND METHODS

Materials. Ribonucleoside triphosphates and terminal transferase were obtained from P-L Biochemicals, Inc. (Milwaukee, Wis.); [α-^{32}P]CTP was obtained from Dupont, NEN Research Products (Boston, Mass.) or ICN Pharmaceuticals Inc. (Irvine, Calif.); HEPES (*N*-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid) buffer (free acid) and

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Cleland reagent (dithiothreitol) were obtained from Calbiochem-Behring (La Jolla, Calif.). Other materials were as described by Sluder et al. (40) or reagent grade.

Purification of factor 5. Growth of K_c cells, preparation of nuclear extracts, and general chromatography procedures were as described by Price et al. (31). All columns were run in HGKEDP (25 mM HEPES [pH 7.6], 15% glycerol, indicated molar concentration of KCl, 0.1 mM EDTA, 1 mM dithiothreitol, and 0.1% of a saturated solution of phenylmethylsulfonyl fluoride in isopropyl alcohol). Factor 5 was purified from K_c cell nuclear extracts by two similar procedures. Scheme 1 started with 65 ml of concentrated nuclear extract from about 200 liters of cells. Phosphocellulose (P11; Whatman, Inc., Clifton, N.J.) was used as the first column. The extract was loaded onto a 200-ml P11 column at 125 mM HGKEDP. Material eluting between 0.3 and 0.4 M KCl was dialyzed versus HGEDP until it reached 125 mM KCl and was then loaded onto a 30-ml DEAE-cellulose column (DE52; Whatman). The material bound to DE52 was eluted with a 250 mM KCl step. This fraction was diluted to 200 mM KCl and loaded onto a 1-ml Mono Q column (Pharmacia Fine Chemicals, Piscataway, N.J.). The material bound to Mono Q was eluted with a 200 to 500 mM KCl gradient. A dC-tailed template assay (reactions spotted on DE81 paper) was used to locate the factor 5 activity (described below). Fractions containing factor 5 were pooled, diluted to 150 mM KCl, and loaded onto a 1-ml Mono S column. The material bound to the Mono S column was eluted with a 150 to 400 mM KCl gradient, and factor 5 activity was again located by using the dC-tailed template assay. The fractions containing factor 5 activity were rechromatographed on Mono Q to obtain better resolution than was obtained the first time, when the column was loaded to maximum capacity. All fractions were stored at −80°C.

Purification scheme 2 started with about 75 ml of nuclear extract and was similar to scheme 1. The 0.3 to 0.4 M KCl step from P11 was dialyzed and loaded onto a 30-ml DE52 column at 100 mM KCl. The material bound to the DE52 column was eluted with a gradient (0.1 to 0.5 M KCl). The fractions containing factor 5 activity were pooled and loaded onto a 1-ml Mono S column, and the bound material was eluted with a gradient as described above. The fractions containing factor 5 activity were pooled and loaded onto a 1-ml Mono Q column, and the bound material was eluted with a gradient as described above.

From these fractionation schemes and from other work (31), the KCl concentration at the peak of elution of factor 5 from each of the four resins used is known. Factor 5 elutes from P11 at 380 mM, from DE52 at 175 mM, from Mono Q at 325 mM, and from Mono S at 250 mM. It was not possible to determine quantitatively the amount of factor 5 activity in the crude column fractions because of the presence of other activities that stimulated specific initiation or elongation of transcription. Because of this, it was not possible to determine the fold purification on the basis of recovery of activity achieved during the schemes described above. The final preparation of factor 5 in scheme 2 contained about 20 μg of the 34-86-kilodalton (kDa) complex. Since the purification scheme started with 2 g of nuclear proteins, the overall purification was about 100,000-fold.

In vitro transcription reactions. In vitro transcription reactions used to examine specific initiation at the Act 5C promoter were performed as described by Price et al. (31). A mixture of fractions was used that was dependent on the presence of factor 5 for observation of specific initiation. One of the fractions contained a DNase inhibitor (Mono Q fraction; 40). A fraction containing factor 3 was obtained by chromatography of the DE52 gradient fraction described by Price et al. (31) on Mono Q and elution with a KCl gradient (factor 3 eluted at 275 mM). The factors required along with factor 5 (see above) were present in the P11 0.4 M step, which was applied to a DE52 column as described above. The DE52 flowthrough material, which contained the required factors, was loaded onto Mono S at 0.1 M KCl, and a step elution was made at 0.5 M KCl (Mono S 0.5 M step). The DE52-bound fraction (containing factor 5) was eluted as described above and chromatographed on Mono S. Two activities required for reconstruction of specific transcription were found: factor 5 and another activity that eluted before factor 5 (between 155 and 160 mM). This previously unidentified activity was called factor 8. The factor 5-dependent reconstruction reactions contained DNase inhibitor, factor 3, the Mono S 0.5 M step, factor 8, and purified RNA polymerase II (31). Factor 5 fractions were obtained from the Mono Q column of purification scheme 1. The *Pst*I-digested Act 5C template was present in the reaction at 3 ug/ml.

The dC-tailed templates and reactions involving them were as described by Price et al. (31) or Sluder et al. (40) with modifications as indicated.

Glycerol gradient sedimentation analysis. All glycerol gradients contained 25 mM HEPES (pH 7.6), 0.1 mM EDTA, 1 mM dithiothreitol, 0.1% of a saturated solution of phenylmethylsulfonyl fluoride in isopropyl alcohol, and glycerol concentrations from 15 to 35% (vol/vol). The concentration of KCl in each analysis was as indicated. The samples were layered on top of the 5-ml gradients and centrifuged at 44,000 rpm in an AH-650 rotor (232,000 × *g* maximum; Ivan Sorvall, Inc., Norwalk, Conn.) for the indicated times. Fractions were collected from the bottom of the tube by puncturing the tube with a hollow needle. Marker proteins were obtained from Sigma Chemical Co. (St. Louis, Mo.) and were analyzed in parallel gradients.

The fractions were assayed for ability to stimulate the transcription of RNA polymerase II on the dC–*Bal*I-E template in the presence of RNase H, which further amplified the signal obtained (40). Reactions were for 3 min, which did not allow the polymerase alone enough time to synthesize a sufficient number of transcripts long enough to be detected in the 6% gels used to analyze the products. The ability of factor 5 to stimulate both (i) the number of polymerase molecules that are productively engaged in the synthesis of long transcripts and (ii) the elongation rate resulted in an easily detectable signal when the labeled transcripts were analyzed in 6% RNA gels.

SDS-polyacrylamide gel electrophoresis, blotting, and staining. Protein samples were analyzed on sodium dodecyl sulfate (SDS) protein gels and blotted to nitrocellulose as described by Weeks et al. (45). India ink staining of proteins bound to nitrocellulose was carried out as described by Hancock and Tsang (16). Silver staining of protein gels was done as described by Morrissey (29).

Elongation complex formation and analysis. Elongation complexes were established by using low concentrations of UTP to limit the elongation rate as described by Sluder et al. (40). Under these conditions, transcripts under 100 nucleotides in length are synthesized and the RNA polymerase II remains in a ternary complex with the template and nascent transcript. The elongation complexes were chromatographed on a 2.4-ml, 20-cm-long Sephacryl S400 column (Pharmacia) equilibrated in 0.06 M HGKEDP (see above) at

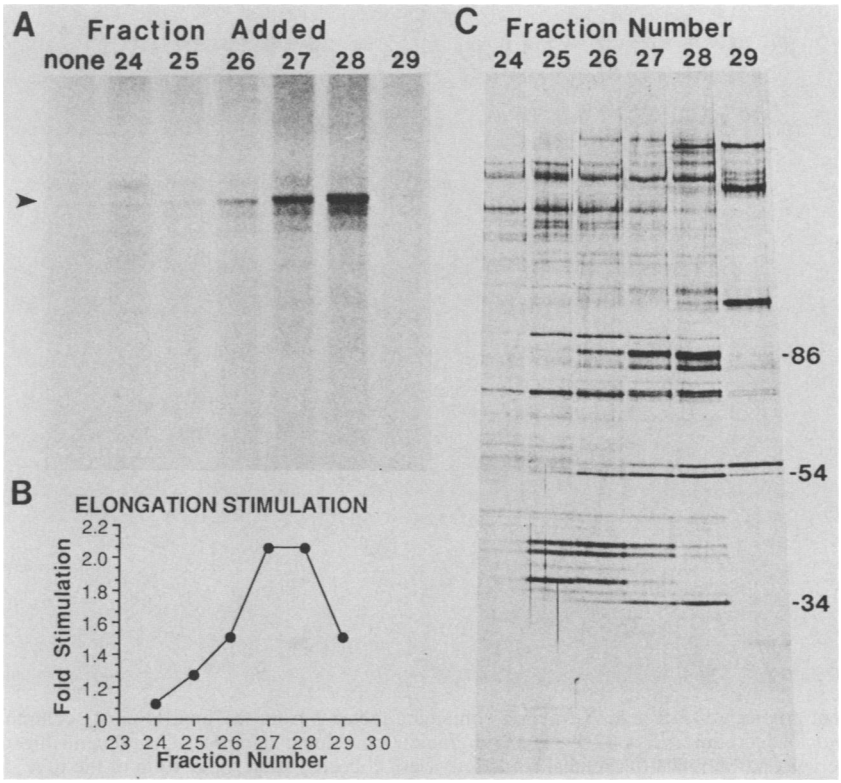

FIG. 1. Requirement for factor 5 in initiation of transcription in vitro. (A) The final Mono Q column fractions (purification scheme 1 in Materials and Methods) were tested for ability to support specific transcription by using the *Pst*I-digested Act 5C actin template (450-base runoff; arrow). All reactions contained RNA polymerase II, purified DNase inhibitor, partially purified factor 3, and fractions derived from the 0.3 to 0.4 M step from the phosphocellulose column of K_c cell nuclear extracts (see Materials and Methods). Individual reactions had either no addition (lane marked none) or 1 μl of the indicated fractions. RNA was synthesized in the presence of [α-^{32}P]CTP, and the reactions were phenol extracted and analyzed directly on a 6% polyacrylamide gel run in 6 M urea and TBE (89 mM Tris [pH 8.0], 89 mM boric acid, 2 mM EDTA). (B) Portions (1 μl) of the same column fractions were assayed for ability to stimulate RNA polymerase II in a dC-tailed template assay (see Materials and Methods for details). Fold stimulation was calculated by dividing the total counts incorporated in each reaction by the amount incorporated by RNA polymerase II alone. (C) A 6 to 15% gradient polyacrylamide protein gel was used to analyze the same column fractions. The bands were visualized by silver staining. The sizes of selected proteins are indicated in kilodaltons on the right.

24°C. The complexes eluted from the column close to the void volume within 15 min after loading.

Elongation complexes were established for the dual-template experiment in the same way (30 μM [α-^{32}P]CTP, 600 μM ATP, 600 μM GTP, and very low levels of UTP found as a contaminant in the other nucleotide triphosphates). dC-3025–RNA polymerase II complexes were mixed with dC-*Bal*I-E–RNA polymerase II complexes that had been established with the indicated increasing concentrations of factor 5. UTP was added to 600 μM after 1 min, and elongation was allowed to proceed under labeling conditions for 3 min. This step was followed by a 5-min chase with 1.2 mM CTP.

RESULTS

Purification of factor 5. *Drosophila* K_c cell nuclear extracts are capable of carrying out efficient transcription of exogenous genes (30). When these extracts are subjected to chromatography on phosphocellulose, multiple fractions are required to obtain efficient reconstruction of specific transcription (31). One of the fractions, the phosphocellulose 0.4 M step, was found to contain activities that affected the elongation properties of RNA polymerase II (31). These activities were fractionated by chromatography on DEAE-cellulose (DE52). One of the fractions that bound to DE52 contained an activity that was required for initiation of specific transcription from the four promoters tested. This same fraction also contained an activity, factor 5, that was able to increase the elongation rate of RNA polymerase II on double-stranded DNA. Using the stimulation of RNA polymerase II elongation as an assay, we have now purified factor 5 and find that it is also required for specific initiation of transcription. Two purification schemes were developed, using both conventional and fast-protein ion-exchange chromatography (see Materials and Methods).

Dependence on factor 5 for reconstruction of specific transcription was tested by using a runoff assay with the *Drosophila* Act 5C actin promoter (Fig. 1). RNA polymerase II and all other required fractions besides factor 5 (see Materials and Methods) did not support specific transcription (Fig. 1A, lane marked none). When individual fractions of the final Mono Q gradient elution (purification scheme 1) were added to this factor mix, specific transcription peaked in fractions 27 and 28 (Fig. 1A). The same fractions were also tested for ability to stimulate elongation by RNA polymerase II, using the dC-tailed template assay (see Materials and Methods). The activity that stimulated elongation by RNA polymerase II also peaked in fractions 27 and 28 (Fig. 1B). The absolute values in this elongation stimulation assay are somewhat imprecise, since the counts incorporated by the polymerase in the presence of factor 5 are divided by the

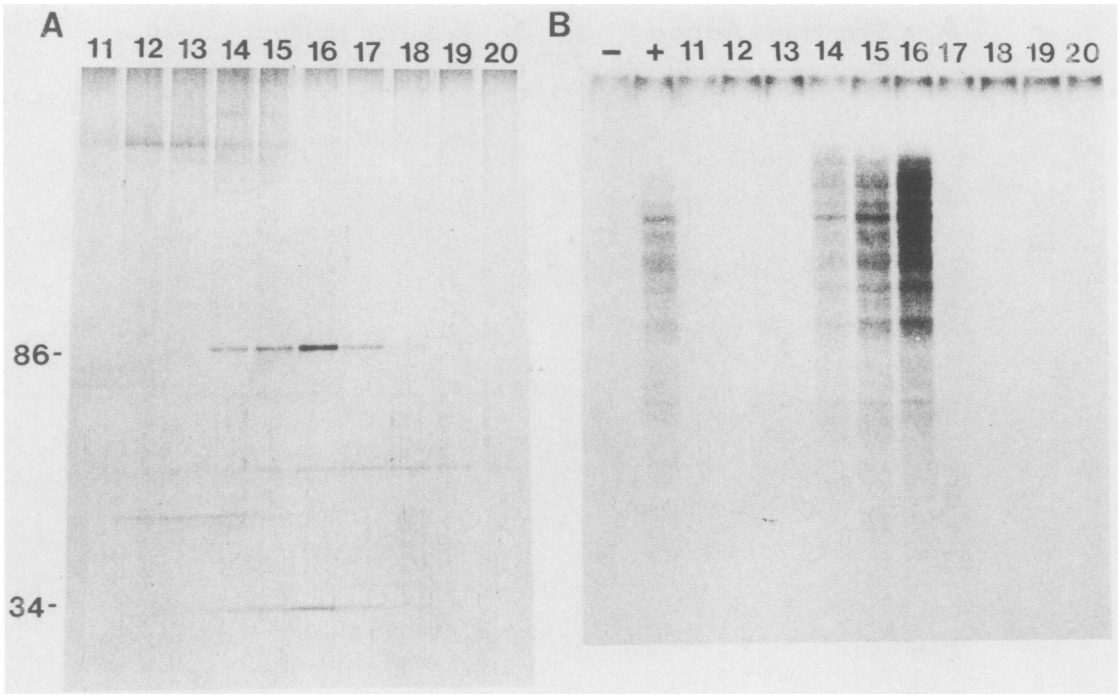

FIG. 2. Glycerol gradient analysis of factor 5. A fraction containing factor 5 from the final Mono Q column (purification scheme 2 in Materials and Methods) was loaded onto a 15 to 32.5% glycerol gradient containing 200 mM KCl and centrifuged for 22 h at 44,000 rpm in an AH-650 rotor (see Materials and Methods for details). Fractions were collected from the bottom of the tube, and the indicated fractions were analyzed on an SDS protein gel (A) as in Fig. 1 (B). The numbers on the left indicate the sizes (in kilodaltons) of selected proteins. Portions (2.5 μl) of the same fractions were also assayed for the ability to stimulate RNA polymerase II in the presence of RNase H during transcription of the dC–*Bal*I-E template (see Materials and Methods for details). The labeled RNA products from the reactions were analyzed on a 6% acrylamide RNA gel (see legend to Fig. 1). Products were analyzed from reactions with either no addition (−) or 1 μl of the Mono Q fraction that was subjected to gradient analysis (+). The length of the RNA detected ranged from about 150 to 1,500 nucleotides.

counts incorporated by RNA polymerase II alone, which are relatively high. This could account for the slight difference in the relative ability of fractions 27 and 28 to stimulate elongation compared with their ability to support the appearance of specific runoff transcriptions. A silver-stained SDS protein gel of the assayed fractions (Fig. 1C) showed that the abundance of four proteins (86, 84, 54, and 34 kDa) paralleled the factor 5 activity. On the preceding column (Mono S), the 86- and 34-kDa proteins paralleled reconstruction, but the 84- and 54-kDa proteins clearly peaked much earlier in the gradient elution profile (data not shown). This result suggested that factor 5 was one or both of the remaining peptides (86 and 34 kDa).

Factor 5 has two subunits. By using the elongation stimulation assay, another scheme was followed to purify factor 5 (see Materials and Methods, purification scheme 2). This scheme was similar to purification scheme 1 except that a gradient on DE52 was used instead of a step elution. When the proteins were analyzed across the peak of activity of the final column, both the 34-kDa and the 86-kDa proteins exactly correlated with the stimulatory activity (data not shown). A side fraction containing less factor 5 and more contaminating proteins than the peak fraction was subjected to glycerol gradient sedimentation (see Materials and Methods). The 34- and 86-kDa proteins cosedimented (Fig. 2A) with an apparent molecular size of about 120 kDa. The elongation-stimulating activity also was found in the same fractions (Fig. 2B). These results strongly suggest that factor 5 is a heterodimer composed of one 34-kDa polypeptide and one 86-kDa polypeptide. This 1:1 ratio of the two peptides was found in all analyses.

The subunit compositions of RNA polymerase II and factor 5 were compared (Fig. 3). A portion of the peak fraction of factor 5 from the Mono Q column (scheme 2) was analyzed on a gradient SDS protein gel beside the preparation of RNA polymerase II that was used for these studies. The proteins were blotted to nitrocellulose and stained with India ink (see Materials and Methods). Factor 5 and RNA polymerase II both contained a subunit that ran with an apparent molecular size of 34 kDa. To examine the relationship, if any, between these two proteins, this blot and others like it were probed with antisera to RNA polymerase II (45) that contained antibodies to the 34-kDa polymerase subunit. Although there was a strong reaction with the polymerase subunit, no detectable signal was seen with the factor 5 subunit (data not shown). Evidently, the similarity in size of these two polypeptides is merely coincidental.

Effect of factor 5 on the elongation properties of RNA polymerase II. To study the elongation phase of transcription, we have used a double-stranded template that has dC tails (21). Purified RNA polymerase II will initiate transcription of these templates exactly 3 nucleotides upstream of the single strand–double strand junction (40). Our initial observations indicated that factor 5 was able to increase the number of polymerase molecules productively engaged in transcribing the dC-tailed template (31). This prompted us to examine the production of short transcripts that would have run off the bottom of the gels used previously to analyze longer species. It was found that the polymerase encountered a block to elongation after 14 nucleotides had been incorporated. After 10 min of synthesis, 95% of the polymer-

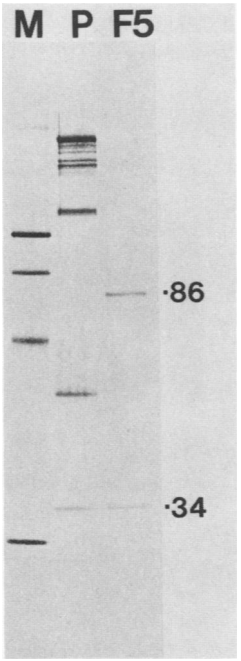

FIG. 3. Comparison of the subunits of RNA polymerase II and factor 5. A 2-μl amount of the preparation of RNA polymerase II that was used for most of these studies and 5 μl of the peak fraction from the final column (Mono Q) of factor 5 purification scheme 2 (see Materials and Methods) were electrophoresed on a 6 to 15% gradient polyacrylamide-SDS protein gel. The proteins in the gel were electrophoretically transferred to nitrocellulose and stained with India ink (see Materials and Methods). Lanes: M, marker proteins (205 [weakly stained], 116, 97, 66, 45 [very weakly stained], and 29 kDa); P, RNA polymerase II; F5, factor 5.

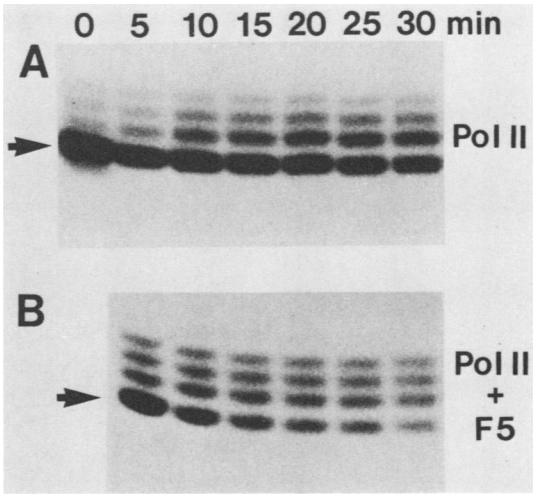

FIG. 4. Time course of the effect of factor 5 on pausing at the 14-mer. RNA polymerase II was incubated with dC-3025 template under pulse-labeling conditions for 3 min (10 μm [α-^{32}P]CTP). The reaction was split into two tubes containing cold CTP to make a final concentration of 1.2 mM. One reaction contained only RNA polymerase II (A); the other also contained factor 5 (B). Time points were taken from the reactions at the indicated times. RNA was extracted and run in an 18% polyacrylamide gel with 6 M urea and TBE. Only the portion of the autoradiograph with small RNA species is shown. Arrows indicate positions of the 14-mer. The three bands above the 14-mer are 15, 16, and 17 nucleotides in length.

ase molecules are paused at this site and therefore are not productively engaged in transcription of the template (40).

We have now examined the effect of factor 5 on the paused polymerase by using a pulse-chase protocol. RNA polymerase II was incubated with a dC-tailed template under labeling conditions that resulted in the 14-mer found in the paused complex being the predominant species (Fig. 4A, lane 0). Unlabeled CTP was added to dilute the labeled precursor, and the reaction was split into two tubes, one of which contained factor 5. Equal portions of the two reaction mixtures were removed, and the reaction was stopped at the indicated chase times (Fig. 4). RNA polymerase II alone was able to leave the 14-mer pause site only slowly (Fig. 4A). Factor 5 caused about a threefold increase in the rate at which RNA polymerase II left the 14-mer pause site (Fig. 4B). The bands above the 14-mer are transcripts resulting from pausing after the addition of nucleotides 15, 16, and 17. Factor 5 also reduced the time that RNA polymerase II spent at these pause sites. The overall effect of factor 5 was to increase the number of polymerase molecules that were producing long transcripts.

Another of the effects of factor 5 that was initially observed (31) was a stimulation of the elongation rate of RNA polymerase II on the dC-tailed template. The elongation rate of RNA polymerase II on a dC-tailed template is much lower than the in vivo rate (40). Transcription of a dC-tailed template by RNA polymerase II yields transcripts of discrete lengths, reflecting the presence of numerous sequence-dependent pause sites along the template. It is very likely that these pause sites are responsible for the decreased elongation rate on the dC-tailed template. Since factor 5 increases the elongation rate, it may act to decrease the length of time that the polymerase spends at the pause sites. Supporting this idea, the results presented above demonstrated that factor 5 reduced the time that RNA polymerase II spent at the 14-mer pause site (Fig. 4). Although the 14-mer site does not seem to be a sequence-dependent pause site (40), the main difference that we have been able to detect between the sequence-dependent pauses and the 14-mer pause are quantitative, with the 14-mer pause being about an order of magnitude longer than the strongest sequence-dependent pause that we have seen (data not shown).

We compared the effects of purified factor 5 (purification scheme 1) on the RNA polymerase II molecules paused at the 14-mer site and on the elongation rate of the polymerases that were further into the template. In both experiments, a pulse-chase protocol was used in which RNA polymerase II was allowed to bind to a dC-tailed template and synthesize short labeled transcripts (less than 14 nucleotides) in a master reaction. Equal portions of this master reaction were mixed with either water or increasing amounts of factor 5, and transcription was allowed to continue under nonlabeling chase conditions for 10 min (for examination of the effect on the 14-mer) or 3 min (for examination of the elongation rate of the productively engaged polymerase molecules). Increasing factor 5 reduced the number of RNA polymerase II molecules that remained paused at the 14-mer site at the end of the 10-min chase (Fig. 5A). When the amount of 14-mer was quantitated by scintillation counting of bands excised from the dried gel, the effect of factor 5 on the 14-mer was found to be saturated at 2 μl (Fig. 5C). The elongation rate of RNA polymerase II also increased with increasing amounts of factor 5 (Fig. 5B). When the maximum elongation rate was calculated and the percentage of the maximum increase in rate was plotted, the factor 5 effect again saturated at 2 μl (Fig. 5C). The difference between the two dose-response curves will be discussed later.

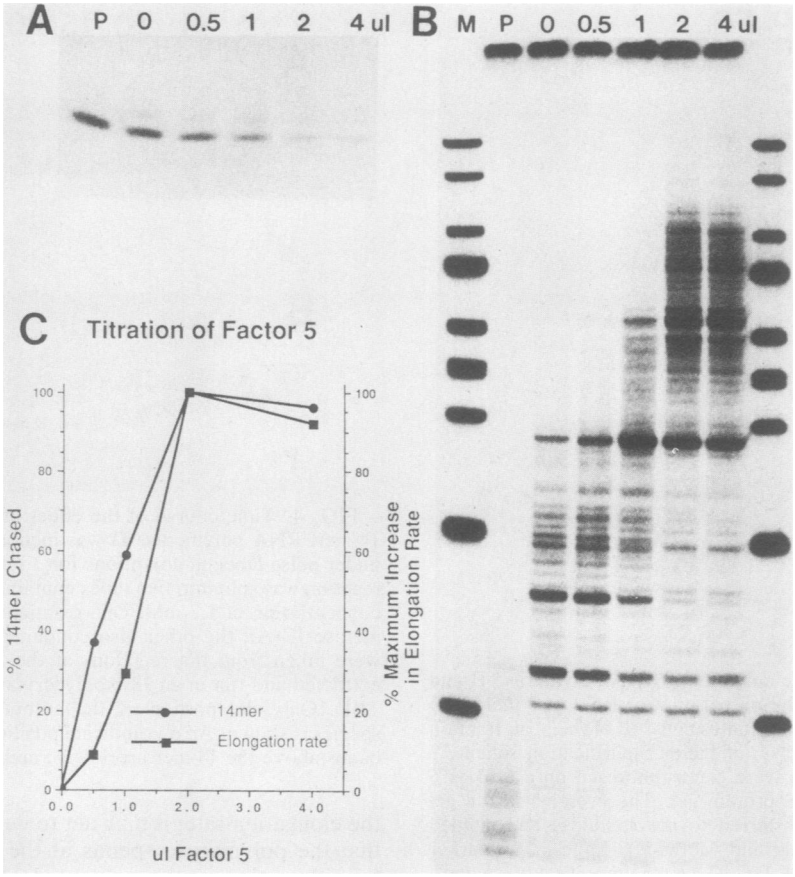

FIG. 5. Titration of the effect of factor 5 on 14-mer and on elongation rate. (A) Conditions similar to those in Fig. 4 were used to generate pulse-labeled 14-mer. The reaction mixture was apportioned into tubes containing cold CTP and the indicated increasing levels of factor 5 (purification scheme 2). After 10 min of chase, the reactions were stopped; samples were extracted and analyzed on an 18% RNA gel. Lanes: P, pulse-labeled RNA at beginning of chase; all other lanes were chased for 10 min with the indicated levels of factor 5. (B) A similar pulse chase protocol was carried out by using dC–*Bal*I-E. After 3 min of chase, the reaction was stopped; samples were extracted and analyzed on a 6% RNA gel. Lanes; M, pBR322 cut with *Hin*FI, kinase labeled, and then cut with *Eco*RI (sizes are 1,631, 998, 633, 517/506, 396, 344, 298, 221/220, and 154 nucleotides); P, pulse-labeled RNA at beginning of chase; all other lanes were chased for 3 min with the indicated amounts of factor 5. (C) Dose-response curves. The 14-mer was quantitated from the gel shown in panel A by scintillation counting of excised gel slices. The amount chased was calculated as the difference between the amount of the 14-mer remaining at the end of the chase without factor 5 (0) and that remaining in the factor 5 chase reactions. The amount of 14-mer chased at saturating levels of factor 5 was designated 100%, and the other values were normalized to this. The elongation rates in panel B were estimated from the difference in maximum RNA length between the end of the pulse reaction (P) and the end of the chase reactions. The autoradiograph in B was scanned with a laser densitometer; starting from the bottom of the gel, the point at which 95% of the total transcript density had been reached was used as the maximum RNA length. The increase in elongation rate was calculated as the difference between the elongation rate in the absence of factor 5 and that in its presence. The increase observed at saturating levels of factor 5 was defined as the maximum increase, and the other values were normalized to this.

Association of factor 5 with RNA polymerase II. Since factor 5 has an effect on the elongation properties of RNA polymerase II, we wondered whether it could physically associate with the polymerase. Such association has been demonstrated for a procaryotic elongation factor, NusA (14), and for an RNA polymerase II stimulatory factor, S-II (19). Purified factor 5 (purification scheme 2) was incubated with purified RNA polymerase II, and the mixture was subjected to glycerol gradient sedimentation (see Materials and Methods). Analysis of the proteins found in the gradient (Fig. 6) indicated that in this experiment some of the 86-kDa subunit cosedimented with RNA polymerase II. It was not possible to determine whether the 34-kDa subunit of factor 5 was bound because the polymerase has a subunit of the same size (Fig. 3). The ratio between the amount of the 86-kDa subunit that was not bound to the polymerase (i.e., sedimented in the upper portion of the gradient) and the amount that was bound was estimated to be about 3:1. The analysis was performed again except that four times the amount of RNA polymerase II was used with the same amount of factor 5. In this experiment, all of the 86-kDa subunit was found to cosediment with the RNA polymerase II (data not shown). In addition, the 34-kDa subunit was not found in the upper portion of the gradient, which indicated that both subunits of factor 5 were associated with the RNA polymerase II. The RNA polymerase II-factor 5 complex from the gradient was assayed by using the dC-tailed template and was found to have a slightly greater elongation rate than did RNA polymerase II taken from a parallel gradient to which no factor 5 had been added (data not shown). The usual dramatic effect of factor 5 was not seen because the conditions of the glycerol gradient caused dilution of the pure factor 5 and RNA polymerase II, which resulted in a reduction of the elongation rate stimulation effect (Fig. 5).

The ability of factor 5 to bind to RNA polymerase II during transcription was also addressed. Elongation com-

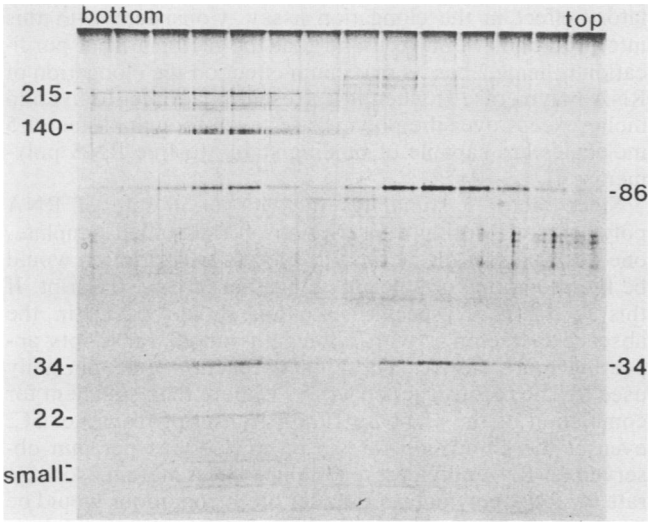

FIG. 6. Association of factor 5 with RNA polymerase II. A 50-μl amount of factor 5 (peak fraction of purification scheme 2) was incubated with 1,360 U of RNA polymerase II for 10 min. The mixture was layered onto a 5-ml, 15 to 35% glycerol gradient containing 60 mM KCl. Centrifugation was for 7 h at 44,000 rpm in an AH-650 rotor. The gradient was collected from the bottom, and the fractions containing factor 5 and RNA polymerase II were analyzed on a 6 to 15% gradient SDS protein gel which was silver stained. The sizes (in kilodaltons) of the RNA polymerase II subunits (left) and the factor 5 subunits (right) are indicated.

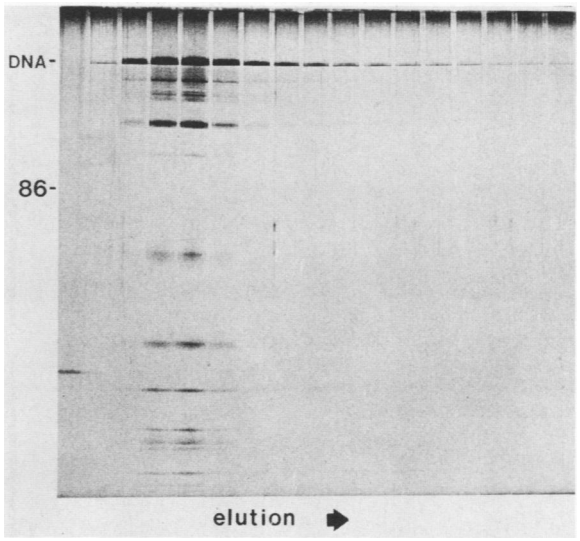

FIG. 7. Analysis of elongation complexes. Elongation complexes were established on dC–*Bal*I-E (7,000 bp) with RNA polymerase II and factor 5 (purification scheme 2). The 50-μl reaction mixture was chromatographed at 24°C on a 2.4-ml column of Sephacryl S400. Equal portions of each fraction eluting from the column were analyzed on a 6 to 15% gradient SDS protein gel which was silver stained. Details of the reactions and column conditions are presented in Materials and Methods. The mobilities of the template (DNA) and the 86-kDa subunit of factor 5 are indicated on the left.

plexes were established in a dC-tailed template reaction containing RNA polymerase II and factor 5, using low levels of nucleoside triphosphates (see Materials and Methods). These complexes were quickly chromatographed on Sephacryl S400, a method that we have found will separate the large ternary complexes of DNA, RNA polymerase II, and nascent RNA from free polymerase and other unbound proteins. SDS protein gel analysis of the column fractions indicated, surprisingly, that there was no detectable 86-kDa subunit of factor 5 present in the tertiary complexes that were found to elute close to the void volume (Fig. 7). When portions of the complex-containing fractions were phenol extracted and analyzed in an RNA gel, the pattern of labeled nascent RNA was the expected for factor 5-stimulated polymerase (data not shown). Elongation complexes were analyzed by Sephacryl S400 chromatography a number of times, with identical results. Since factor 5 associated stably with pure RNA polymerase II under similar conditions (Fig. 6), the results indicated that transcription of the dC-tailed template caused release of at least the 86-kDa subunit of factor 5. Since the included fractions from the sizing column significantly diluted the free factor 5, it was not possible to detect the individual subunits in the gel; therefore, it was not clear whether the 34-kDa protein was also released during elongation. The extensive dilution also prevented any direct assay of factor 5 activity.

An experiment using two different dC-tailed templates was designed to determine whether factor 5 activity was released from elongating RNA polymerase II. For this experiment, elongation complexes with only RNA polymerase II were established on a short template (250 base pairs [bp]); in a separate reaction, complexes with RNA polymerase II and increasing levels of factor 5 were established on a long dC-tailed template (7 kbp). On the basis of glycerol gradient analysis results, the maximum level of factor 5 was chosen to be less than equimolar with the RNA polymerase II. Elongation in the initial reactions was under pulse conditions, generating short transcripts of less than 50 bases. The reactions were then mixed, and elongation under chase conditions produced long transcripts whose abundance would depend on the level of factor 5 activity. The results are shown in the first four lanes of Fig. 8. Transcripts from the two templates were for the most part separated into the upper (long) and lower (short) sections of the gel. As expected, increasing levels of factor 5 caused an increase in the amount of transcripts seen from the long template. The RNA polymerase II on the short template was also stimulated by factor 5, which suggested that factor 5 activity was released from the polymerase on the long template and was able to stimulate polymerase on the short template. In a similar experiment performed as a control, RNA polymerase II was left out of the short template reaction (Fig. 8, lanes 5 through 8). The lack of efficient transcription of the short template indicated that the RNA polymerase II in the long template reaction was bound stably and the polymerase was not in excess over the long template. When RNA polymerase II was left out of the long-template reaction, the effect of factor 5 on the short template (Fig. 8, lanes 9 through 12) was similar to that seen in the first lanes.

DISCUSSION

Factor 5, a transcription factor for *Drosophila* RNA polymerase II, has been purified from K_c cell nuclear extracts. We show here that the factor is required for specific initiation of transcription from the Act 5C actin promoter in vitro. In addition, factor 5 affects the elongation properties of RNA polymerase II on a double-stranded template in vitro. Both activities correlated with two polypeptides (34 and 86 kDa) that form a 120-kDa complex.

Factor 5 as an initiation factor. We have shown that factor 5 subjected to four successive purification steps (scheme 1)

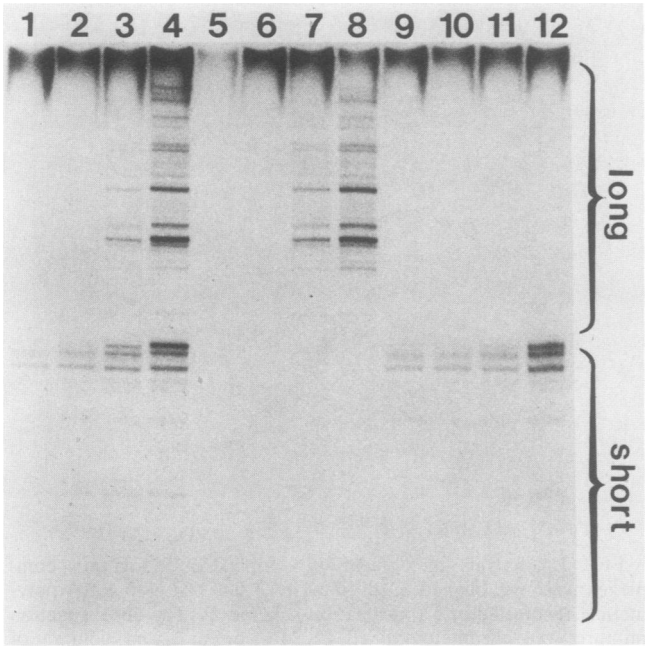

FIG. 8. Factor 5 exchange between two elongation complexes. Elongation complexes were established under low nucleoside triphosphate conditions on two templates. In one reaction, complexes with RNA polymerase II only were set up on dC-3025 (250 bp, short template). In other reactions, complexes were set up with RNA polymerase II and increasing levels of factor 5 (purification scheme 2) on dC–*Bal*I-E (7 kbp, long template). The reactions were mixed together, and elongation continued for 5 min. The reactions were stopped, and the mixture was analyzed on a 6% RNA gel. Lanes 1 through 4 contained 0, 0.5, 1.0, and 1.5 μl, respectively, of factor 5; similar titrations were done for lanes 5 through 8 and 9 through 12. Reactions in lanes 5 through 8 were similar to those in lanes 1 through 4 except that the RNA polymerase II was left out of the dC-3025 complex. Reactions in lanes 9 through 12 were similar to those in lanes 1 through 4 except that RNA polymerase II was left out of the long-template reaction. Details of the reactions and conditions are presented in Materials and Methods.

is still required for observation a specific runoff transcript from the Act 5C actin promoter. During the purification scheme, we identified a new activity, factor 8, that was also required for specific initiation. In preliminary schemes for factor 5 purification, the ability of factor 5 to support specific transcription was lost as purification proceeded. This result was due in part to the fact that factor 8 had not been identified and was being separated from the factor 5 activity. In addition, we have noticed that the elongation-stimulating activity of factor 5 seems to decrease as purification proceeds and that, in general, whenever the elongation-stimulating activity decreases, so does the ability to support specific initiation. We do not know whether this decrease in activity is due to the loss of another factor, besides factor 8, which initially cochromatographs with factor 5 or whether factor 5 is unstable. Some preparations of factor 5 (scheme 2, for example) have a detectable elongation-stimulating activity but have almost no ability to support initiation of transcription from a promoter. These fractions, which had been frozen and thawed a number of times before we discovered factor 8, had also lost much of their ability to stimulate elongation. It is possible that inactive factor 5 is able to bind to RNA polymerase II and inhibit specific initiation of transcription; however, since it is released during elongation, the remaining active factor 5 could still exert a stimulatory effect in the elongation assay. Consistent with this interpretation, we have found that the factor 5 from purification scheme 2 has its maximum effect on the elongation of RNA polymerase II when it is present in a three- to fivefold molar excess over the polymerase, even though all factor 5 molecules are capable of binding stably to free RNA polymerase II.

Since factor 5 stimulates the elongation rate of RNA polymerase II during transcription of a dC-tailed template, one possible role for factor 5 in specific transcription would be the promotion of efficient elongation of the transcript. If this is the case, specific initiation should occur in the absence of factor 5, with full-length runoff transcripts appearing more slowly. The 20-min reaction time normally used for the reconstruction assays is more than sufficient for completion of the 450-base runoff transcript from Act 5C, even at the elongation rate of 65 nucleotides per min observed for RNA polymerase II alone (40). A more reasonable rate for RNA polymerase II under these conditions would be greater than 200 nucleotides per min, since the template is renaturing behind the polymerase (40) and another elongation factor, DmS-II, is present in the reconstruction reactions. However, the production of runoff transcript in the reconstruction assays was absolutely dependent on the presence of factor 5, and no shortened transcripts were observed in its absence (Fig. 1); this finding indicates that factor 5 is required for the initiation of specific transcription. It is possible that factor 5 also has an effect on the elongation rate of the RNA polymerase II after it has specifically initiated, but this effect would not be detected under the conditions used in the runoff assays performed here.

The view that factor 5 acts during initiation is strengthened by the similarity of the factor to mammalian transcription factors which have been demonstrated to be involved in initiation. Greenblatt and co-workers (2, 3, 41) have isolated three mammalian proteins from crude extracts by affinity chromatography on columns containing immobilized RNA polymerase II. These RNA polymerase II-associating proteins (RAPs) have molecular sizes of 30, 38, and 72 (mouse) or 74 (human) kDa (41). RAP30 and RAP74 interact with each other and are required for specific transcription from a variety of promoters (2) and thus appear to constitute a general transcription factor. RAP30-74 has been shown to be functionally, chromatographically, and antigenically (2, 3) similar, if not identical, to the general transcription factor TFIIE isolated from HeLa cell extracts by Roeder and co-workers (10, 33). TFIIE activity was also found to associate with purified RNA polymerase II (33). On the basis of the ability of factor 5 to associate with RNA polymerase II, the molecular weights of its subunits, and the similarity of its chromatographic properties to those of TFIIE (10, 33), factor 5 appears to be analogous to RAP30-74 and TFIIE. Both TFIIE (33) and RAP30-74 (2, 3) have been found to be required for the establishment of a complete transcription complex capable of initiating transcription but not for the formation of the preinitiation complex. TFIIE has recently been resolved into two activities, TFIIE and TFIIF, and immunological studied indicate that TFIIF contains RAP30 (13).

Factor 5 as an elongation factor. In both of the purification schemes presented here, factor 5 was purified on the basis of its ability to stimulate RNA polymerase II in the dC-tailed template assay. We previously noted that factor 5 had two effects in this elongation assay, increasing both the elongation rate of RNA polymerase II and the number of polymerase molecules productively engaged in the synthesis of long

transcripts (31), and we have further characterized these effects here. Factor 5 stimulates the rate of elongation by RNA polymerase II about three- to fivefold (Fig. 5; unpublished results). The pattern of pause sites seen with pure RNA polymerase II on the dC-tailed template is not changed by factor 5. Since the polymerase spends much time at specific pause sites and relatively little time between pause sites, it is reasonable to assume that the overall elongation rate is determined by the strength of the pause sites (23). In support of this idea, we have found that the measured average elongation rate determined as the polymerase passes through regions of the template that contain strong pause sites is significantly lower than on regions of the template that do not contain strong pause sites (data not shown). Factor 5 could act either by increasing the rate of addition of each nucleotide or by affecting only the paused polymerase molecules. The ability of factor 5 to increase the number of polymerase molecules that are productively engaged in transcription is accounted for by the effect that factor 5 has on polymerase paused at base 14 of the dC-tailed template (Fig. 4 and 5). Factor 5 causes about a threefold increase in the rate of exit from the 14-mer pause site.

At saturating stimulatory amounts, the quantitative effects of factor 5 on reducing pause time at the 14-mer and increasing the elongation rate are the same (three- to fivefold). However, the early portions of the dose-response curves are different (Fig. 5). The curve for elongation rate stimulation exhibits a somewhat sigmoidal shape, with the response of the elongation rate to factor 5 lagging the response at the 14-mer. This difference can be explained by postulating that although factor 5 must act at only one site in the 14-mer assay, it must act at multiple points to achieve maximum stimulation of elongation rate. Logical points of interaction would be the major pause sites. A reduction in residence time at only one or two of the many pause sites on a template would not significantly change the overall elongation rate, and in order to see the maximum increase in elongation rate, the polymerase would need to be acted on by factor 5 at each of these points. A reduction in pause time by factor 5 at each site would cause the overall elongation rate to increase. Under our assay conditions, the molar amount of factor 5 that is required to achieve maximum elongation rate stimulation is about three to five times the amount of RNA polymerase II. This could be because maintainenance the concentration of factor 5 at a high level is the important element in elongation rate stimulation, since the factor must be able to quickly locate the paused polymerase molecules. It should be possible to test the relative importance to elongation stimulation of concentration versus molar ratio. The RNA polymerase II-factor 5 complexes isolated from glycerol gradients (Fig. 6) exhibited only a low level of rate stimulation compared with the level found with RNA polymerase II alone, possibly because such complexes were assayed under conditions that were much more dilute than those normally used and the concentration of factor 5 was not optimal.

Role of factor 5 during transcription. Factor 5 binds tightly to free RNA polymerase II but is not stably associated with the polymerase during elongation on a dC-tailed template. In this respect it resembles the bacterial sigma factor, which associates with core RNA polymerase before and during initiation but is released as the polymerase becomes stably committed to elongation (46 and references therein). Although most sigma factors in procaryotes have single subunits, it appears that several of the smaller factors may require additional protein components to form a functional

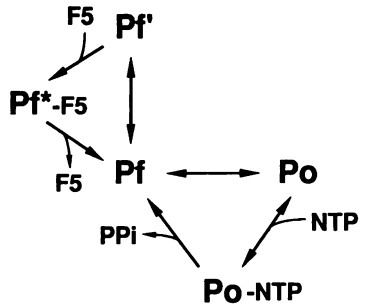

FIG. 9. Model of the role of factor 5 during elongation on a dC-tailed template. F5, Factor 5, Pf, polymerase with filled nucleotide addition site; Pf', paused polymerase; Po, polymerase with open nucleotide addition site; Po-NTP, polymerase with nucleotide bound; Pf*-F5, intermediate with factor 5 bound to polymerase. The conversion of paused RNA polymerase II into elongation-competent polymerase is stimulated by factor 5. See text for explanation.

sigma factor (reviewed in reference 18). Unlike sigma, factor 5 is not sufficient for promoter recognition by RNA polymerase, though it could be involved in the interaction of RNA polymerase II with the preinitiation complex of template and promoter-specific binding proteins.

There are three major steps in the elongation reaction carried out by RNA polymerase II (Fig. 9). After a nucleotide has been added to the existing 3' end of the nascent RNA and the nucleotide addition site has been filled (Pf), the polymerase must translocate to allow an open site (Po) for the next nucleotide to bind (Po-NTP) and eventually be added. During elongation, the three steps (translocation, nucleotide binding, and nucleotide addition) are repeated by the polymerase in a processive manner. The polymerase can be considered to be in elongation mode during this cycle. On dC-tailed templates, the polymerase frequently pauses for extended periods of time, which accounts for the low rate of elongation by purified enzyme on these templates. For the following discussion, we assume that the paused polymerase is unable to carry out the translocation step (represented in Fig. 9 at Pf'). Although the polymerase could theoretically be trapped at another part of the cycle, this would not change the following arguments. The important aspect of the model is that the polymerase is in one of two states, elongation competent or paused. The effect of factor 5 on the elongation properties of RNA polymerase II as it transcribes a dC-tailed template can be explained if factor 5 stimulates the paused polymerase to reenter elongation mode. This stimulation would have to involve only brief interaction of factor 5 with RNA polymerase II (Pf*-F5 in Fig. 9), since factor 5 is not associated stably with the elongation complex (Fig. 7 and 8).

The cause of RNA polymerase II pausing on the dC-tailed template is not known. The first pause at base 14 is found on all dC-tailed templates and seems to be due to the general structure of the template, although the sequence of the tail could also be involved (40). The pause sites occurring further downstream are sequence dependent, different templates having different sets of pause sites (8, 9, 21, 35, 40). RNA polymerase from *E. coli* also pauses during the transcription of double-stranded DNA in a sequence-dependent manner (reviewed in references 7 and 46). Pause sites occur downstream of some but not all potential stem-and-loop structures, downstream of some but not all G+C-rich regions, and in other regions that have neither stem-and-loop structures nor G+C-rich regions (24). We propose that at pause sites, RNA polymerase II falls into a conformation that

precludes elongation. Furthermore, we propose that the effect of factor 5 could be to cause a conformational change in the paused polymerase, shifting it into elongation mode. The various features of the template and RNA product that might cause the polymerase to exit elongation mode (i.e., to pause) could all be overcome by affecting the conformational state of paused polymerase.

The presence of factor 5 during the transcription of a dC-tailed template by RNA polymerase II has no effect on the pattern of RNA resulting from sequence-specific pausing except to speed up its appearance (Fig. 5; data not shown). This suggests that factor 5 does not affect the potential for the polymerase to pause but affects the polymerase only after it has paused. This effect is different from that of S-II, an RNA polymerase II-stimulatory factor (39) that seems to reduce the tendency for the polymerase to pause at certain sites (32, 34; unpublished data).

The stable complex between factor 5 and RNA polymerase II that forms in the absence of DNA could have a conformation similar to that of the intermediate (Pf*-F5) proposed in Fig. 9. In this way, the role of factor 5 in specific initiation may be similar to its postulated role during elongation on the dC-tailed template, namely, to convert the polymerase into an elongation-competent mode. The conversion of paused RNA polymerase II to its elongation-competent state during transcription of the dC-tailed template could then be considered reinitiation. This would explain the apparent paradox of an initiation factor stimulating the elongation phase of transcription. If the effect of factor 5 on paused polymerase is in fact similar to an initiation event, then the dC-tailed template assay may be useful in developing an understanding of a general pathway for the establishment of productive elongation.

ACKNOWLEDGMENTS

We thank Meei Liu for technical assistance and Paul Modrich for helpful discussions.

This work was supported by Public Health Service grants GM35500 and GM28078 from the National Institutes of Health.

LITERATURE CITED

1. **Briggs, M. R., J. T. Kadonaga, S. P. Bell, and R. Tjian.** 1986. Purification and biochemical characterization of the promoter-specific transcription factor, Sp1. Science **234**:47–52.
2. **Burton, Z. F., M. Killeen, M. Sopota, L. G. Ortolan, and J. Greenblatt.** 1988. RAP30/74: a general initiation factor that binds to RNA polymerase II. Mol. Cell. Biol. **8**:1602–1613.
3. **Burton, Z. F., L. G. Ortolan, and J. Greenblatt.** 1986. Proteins that bind to RNA polymerase II are required for accurate initiation of transcription at the adenovirus 2 major late promoter. EMBO J. **5**:2923–2930.
4. **Cai, H., and D. S. Luse.** 1987. Transcription initiation by RNA polymerase II *in vitro*: properties of preinitiation, initiation, and elongation complexes. J. Biol. Chem. **262**:298–304.
5. **Cai, H., and D. S. Luse.** 1987. Variations in template protection by the RNA polymerase II transcription complex during the initiation process. Mol. Cell. Biol. **7**:3371–3379.
6. **Carthew, R. W., L. A. Chodosh, and P. A. Sharp.** 1985. An RNA polymerase II transcription factor binds to an upstream element in the adenovirus major late promoter. Cell **43**:439–448.
7. **Chamberlin, M. J., J.-F. Briat, R. L. Dedrick, M. M. Hanna, C. M. Kane, J. R. Levin, R. L. Reynolds, and M. C. Schmidt.** 1985. Factors involved in elongation and termination of bacterial and mammalian transcription, p. 47–73. *In* P. Marks (ed.), Genetics, cell differentiation and cancer. Academic Press, Inc., New York.
8. **Coulter, D. E., and A. L. Greenleaf.** 1985. A mutation in the largest subunit of RNA polymerase II alters RNA chain elongation *in vitro*. J. Biol. Chem. **260**:13190–13198.
9. **Dedrick, R. L., C. M. Kane, and M. J. Chamberlin.** 1987. Purified RNA polymerase II recognizes specific termination sites during transcription *in vitro*. J. Biol. Chem. **262**:9098–9108.
10. **Dignam, J. D., P. L. Martin, B. S. Shastry, and R. G. Roeder.** 1983. Eukaryotic gene transcription with purified components. Methods Enzymol. **101**:582–598.
11. **Dynan, W. S., and R. Tjian.** 1985. Control of eukaryotic RNA synthesis by sequence-specific DNA-binding proteins. Nature (London) **316**:774–778.
12. **Fire, A., M. Samuels, and P. A. Sharp.** 1984. Interactions between RNA polymerase II, factors, and template leading to accurate transcription. J. Biol. Chem. **259**:2509–2516.
13. **Flores, O., E. Maldonado, Z. Burton, J. Greenblatt, and D. Reinberg.** 1988. Factors involved in specific transcription by mammalian RNA polymerase II: RNA polymerase II-associating protein 30 is an essential component of transcription factor IIF. J. Biol. Chem. **263**:10812–10816.
14. **Greenblatt, J., and J. Li.** 1981. Interaction of the sigma factor and the nusA gene protein of E. coli with RNA polymerase in the initiation-termination cycle of transcription. Cell **24**:421–428.
15. **Greenleaf, A. L., R. S. Jokerst, W. A. Zehring, B. J. Hamilton, J. R. Weeks, A. E. Sluder, and D. H. Price.** 1987. *Drosophila* RNA polymerase II: genetics and in vitro transcription, p. 459–463. *In* W. S. Reznikoff et al. (ed.), RNA polymerase and the regulation of transcription. Elsevier/North-Holland Publishing Co., New York.
16. **Hancock, K., and V. C. W. Tsang.** 1983. India ink staining of proteins on nitrocellulose paper. Anal. Biochem. **133**:157–162.
17. **Hawley, D. K., and R. G. Roeder.** 1985. Separation and partial characterization of three functional steps in transcription initiation by human RNA polymerase II. J. Biol. Chem. **260**: 8163–8172.
18. **Helmann, J. D., and M. J. Chamberlin.** 1988. Structure and function of bacterial sigma factors. Annu. Rev. Biochem. **57**: 839–872.
19. **Horikoshi, M., K. Sekimizu, and S. Natori.** 1984. Analysis of the stimulatory factor of RNA polymerase II in the initiation and elongation complex. J. Biol. Chem. **259**:608–611.
20. **Horwitz, R. J., J. Li, and J. Greenblatt.** 1987. An elongation control particle containing the N gene transcriptional antitermination protein of bacteriophage lambda. Cell **51**:631–641.
21. **Kadesch, T. R., and M. J. Chamberlin.** 1982. Studies of *in vitro* transcription by calf thymus RNA polymerase II using a novel duplex DNA template. J. Biol. Chem. **257**:5286–5295.
22. **Kane, C. M.** 1988. Renaturase and ribonuclease H: a novel mechanism that influences transcript displacement by RNA polymerase II *in vitro*. Biochemistry **27**:3187–3196.
23. **Kassavetis, G. A., and M. J. Chamberlin.** 1981. Pausing and termination of transcription within the early region of bacteriophage T7 DNA *in vitro*. J. Biol. Chem. **256**:2777–2786.
24. **Levin, J. R., and M. J. Chamberlin.** 1987. Mapping and characterization of transcriptional pause sites in early genetic region of bacteriophage T7. J. Mol. Biol. **196**:61–84.
25. **Luse, D. S., T. Kochel, E. D. Keumpel, J. A. Coppola, and H. Cai.** 1987. Transcription initiation by RNA polymerase II *in vitro*: at least two nucleotides must be added to form a stable ternary complex. J. Biol. Chem. **262**:289–297.
26. **Matsui, J., J. Segall, P. A. Weil, and R. G. Roeder.** 1980. Multiple factors required for accurate initiation of transcription by purified RNA polymerase II. J. Biol. Chem. **255**:11992–11996.
27. **McKnight, S., and R. Tjian.** 1986. Transcriptional selectivity of viral genes in mammalian cells. Cell **46**:795–805.
28. **Mitchell, P. J., C. Wang, and R. Tjian.** 1987. Positive and negative regulation of transcription *in vitro*: enhancer-binding protein AP-2 is inhibited by SV40 T antigen. Cell **50**:847–861.
29. **Morrissey, J. H.** 1981. Silver stain for proteins in polyacrylamide gels: a modified procedure with enhanced uniform sensitivity. Anal. Biochem. **117**:307–310.
30. **Parker, C. S., and J. Topol.** 1984. A *Drosophila* RNA polymerase II transcription factor contains a promoter-region-specific

DNA-binding activity. Cell **36**:357–369.
31. **Price, D. H., A. E. Sluder, and A. L. Greenleaf.** 1987. Fractionation of transcription factors for RNA polymerase II from *Drosophila* K_c cell nuclear extracts. J. Biol. Chem. **262**:3244–3255.
32. **Rappaport, J., D. Reinberg, R. Zandomen, and R. Weinmann.** 1987. Purification and functional characterization of transcription factor SII from calf thymus: role in RNA polymerase II elongation. J. Biol. Chem. **262**:5227–5232.
33. **Reinberg, D., and R. G. Roeder.** 1987. Factors involved in specific transcription by mammalian RNA polymerase II: purification and functional analysis of initiation factors IIB and IIE. J. Biol. Chem. **262**:3310–3321.
34. **Reinberg, D., and R. G. Roeder.** 1987. Factors involved in specific transcription by mammalian RNA polymerase II: transcription factor IIS stimulates elongation of RNA chains. J. Biol. Chem. **262**:3331–3337.
35. **Reines, D., D. Wells, M. J. Chamberlin, and C. M. Kane.** 1987. Identification of intrinsic termination sites *in vitro* for RNA polymerase II within eukaryotic gene sequences. J. Mol. Biol. **196**:299–312.
36. **Samuels, M., A. Fire, and P. A. Sharp.** 1982. Separation and characterization of factors mediating accurate transcription by RNA polymerase II. J. Biol. Chem. **257**:14419–14427.
37. **Sawadogo, M., and R. G. Roeder.** 1985. Interaction of a gene-specific transcription factor with the adenovirus major late promoter upstream of the TATA box region. Cell **43**:165–175.
38. **Sekeris, C. E., W. Schmid, and W. Roewekamp.** 1972. Stimulation of *in vitro* transcription by ribonuclease H (hybridase). FEBS Lett. **24**:27–31.
39. **Sekimizu, K., N. Kobayashi, D. Mizuno, and S. Natori.** 1976. Purification of a factor from Ehrlich ascites tumor cells specifically stimulating RNA polymerase II. Biochemistry **15**:5064–5070.
40. **Sluder, A. E., D. H. Price, and A. L. Greenleaf.** 1988. Elongation by *Drosophila* RNA polymerase II: transcription of 3′-extended templates. J. Biol. Chem. **263**:9917–9925.
41. **Sopta, M., R. W. Carthew, and J. Greenblatt.** 1985. Isolation of three proteins that bind to mammalian RNA polymerase II. J. Biol. Chem. **260**:10353–10360.
42. **Tsai, S. Y., P. Dicker, P. Fang, M.-J. Tsai, and B. W. O'Malley.** 1984. Generation of monoclonal antibodies to RNA polymerase II for the identification of transcriptional factors. J. Biol. Chem. **259**:11587–11593.
43. **Tsai, S. Y., I. Sagami, H. Wang, M.-J. Tsai, and B. W. O'Malley.** 1987. Interactions between a DNA-binding transcription factor (COUP) and a non-DNA binding factor (S300-II). Cell **50**:701–709.
44. **Tsai, S. Y., M.-J. Tsai, L. E. Kops, P. P. Minghetti, and B. W. O'Malley.** 1981. Transcription factors from oviduct and HeLa cells are similar. J. Biol. Chem. **256**:13055–13059.
45. **Weeks, J. R., D. E. Coulter, and A. L. Greenleaf.** 1982. Immunological studies of RNA polymerase II using antibodies to subunits of *Drosophila* and wheat germ enzyme. J. Biol. Chem. **257**:5884–5891.
46. **Yager, T. D., and P. H. von Hippel.** 1987. Transcript elongation and termination in *Escherichia coli*, p. 1241–1275. *In* F. C. Neidhardt, J. L. Ingraham, K. B. Low, B. Magasanik, and H. Schaechter (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology, vol. 2. American Society for Microbiology, Washington, D.C.
47. **Zheng, X.-M., V. Moncollin, J.-M. Egly, and P. Chambon.** 1987. A general transcription factor forms a stable complex with RNA polymerase B (II). Cell **50**:361–368.