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The RNA polymerase II preinitiation complex formed in the presence of ATP

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ABSTRACT

ATP hydrolysis is required for transcriptional initiation by RNA polymerase II *in vitro*. Reconstituted transcription using purified initiation factors and RNA polymerase II have revealed that the step dependent on ATP hydrolysis occurs at the same time as initiation of RNA synthesis. We report here that ATP hydrolysis is also required for formation of the preinitiation complex in crude extracts. Two distinct preinitiation complexes were identified, one formed in the presence and the other in the absence of ATP. These complexes were isolated by glycerol gradient centrifugation. The preinitiation complex formed in the presence of ATP was able to synthesize transcripts with addition of only ribonucleotide triphosphates, whereas the preinitiation complex formed in the absence of ATP was inactive and required addition of protein fractions and ATP. These results suggest that the inactive preinitiation complex is activated by addition of the protein fractions and ATP hydrolysis. The active preinitiation complex sedimented at ~40 S in glycerol gradient centrifugation, a rate similar to that of RNA polymerase II holoenzyme reported by Maldonado *et al.* [*Nature* (1996), 381, 86–89].

INTRODUCTION

Transcriptional initiation by RNA polymerase II is preceded by assembly of the preinitiation complex, which is composed of RNA polymerase and initiation factors (1,2 and references therein). TFIIB, TFIID, TFIIE, TFIIF and TFIIH are essential initiation factors in formation of the preinitiation complex. Gel retardation, DNA template challenge and restriction site protection experiments have demonstrated intermediates of the preinitiation complex (3–5). TFIID is the TATA box binding factor and forms a complex with the promoter DNA. TFIIB subsequently binds to the complex of TFIID and the promoter DNA. RNA polymerase II and the rest of the initiation factors form the complete preinitiation complex on the promoter DNA in a stepwise manner. Thus, formation of the preinitiation complex is accomplished by the stepwise assembly of the initiation factors and RNA polymerase II.

Discovery of RNA polymerase II holoenzyme has led to another model of preinitiation complex formation (6,7,8–11). RNA polymerase II holoenzyme is a multifunctional complex containing RNA polymerase II. RNA polymerase II holoenzyme in yeast contains the SRB proteins and the SWI/SNF protein complex, in addition to a subset of basal initiation factors (12,13). The SRB proteins were identified as factors capable of suppressing mutations in the C-terminal domain (CTD) of the RNA polymerase II large subunit (10 and references therein). The SWI/SNF complex in the yeast RNA polymerase II holoenzyme appears to remodel chromatin using the energy generated by ATP hydrolysis (13). The mammalian RNA polymerase II holoenzyme, thought to be equivalent to the yeast RNA polymerase II holoenzyme, has been reported to contain the mammalian homolog of SRB7 and also Cdk8 and cyclins C and H (6,9). It contains factors involved in DNA repair, including DNA polymerase ε (6). These reports suggest that the multiple factors which form the RNA polymerase II holoenzyme could form a preinitiation complex under physiological conditions.

The molecular size of the RNA polymerase II holoenzyme has been shown to be equivalent to that of the ribosome (6,7). However, the holoenzymes in crude extracts and in purified fractions showed different sedimentation speeds in sucrose gradient centrifugation and the holoenzyme in crude extracts is heavier than that in purified fractions. These data suggest that the holoenzyme is unstable in purification procedures, including sucrose gradient centrifugation, and that components in the holoenzyme dissociate easily. Despite the fact that it is important to investigate the mechanism of preinitiation complex formation by the holoenzyme, neither a biochemical method to stabilize the holoenzyme nor a reconstituted transcription system with the holoenzyme has been established. Investigation of the transcriptional mechanism of the holoenzyme in crude extracts should yield useful results.

ATP is a substrate for RNA synthesis and has also been shown to be hydrolyzed in transcription. The requirement for ATP hydrolysis in transcription was initially identified using AMP-PNP, which is an unhydrolyzable ATP analog (14). Although AMP-PNP is a potent substrate for RNA chain elongation, transcription requires hydrolyzable adenosine nucleotides, including dATP, in the presence of AMP-PNP (15). Because hydrolysis of the β–γ phosphate bond of ATP (dATP) is not

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directly used for RNA chain elongation, ATP hydrolysis is thought to be required for initiation. Indeed, data from reconstituted transcription reactions have revealed that a step dependent on ATP hydrolysis is involved in initiation (16,17). Luse and Jacob used the method of dinucleotide-primed trinucleotide formation to demonstrate that ATP hydrolysis is required for synthesis of the first phosphodiester bond on a promoter-specific transcript (18). Gralla and co-workers showed that ATP hydrolysis is needed to open the double strands of the DNA template at the transcription start site using $KMnO_4$ as a probe for DNA local melting (19). Among the initiation factors, only TFIIH possesses DNA-dependent ATPase, DNA helicase and CTD kinase activities (20–32). In the reconstituted reaction, however, CTD phosphorylation by TFIIH is dispensable for initiation, suggesting that CTD phosphorylation is not involved in the ATP-dependent step (33–35). Furthermore, recent reports by Conaway, Timmers and co-workers have indicated that ATP hydrolysis is required immediately prior to phosphodiester bond formation of the transcript (36,37). This suggests that ATP hydrolysis could play a role in promoter opening. Taken together, TFIIH could play a role in promoter opening in the ATP-dependent step.

Intermediates of the preinitiation complex formed by purified initiation factors were stable even in the matrix of a polyacrylamide gel in the absence of ATP (3). The step dependent on ATP hydrolysis in initiation is not involved in formation of the preinitiation complex using purified factors. However, data from biochemical and genetic studies suggest that factors involved in ATP hydrolysis, such as the SWI/SNF complex, could be an inherent part of the preinitiation complex (13). It is possible that factors including the SWI/SNF complex could play an important role in the ATP hydrolysis-dependent step.

In this report we focus on the ATP hydrolysis-dependent step in crude transcription reactions. Two transcription conditions reported by Manley, Safer and their co-workers were used (38,39). The primary difference between these methods is that in Manley's method the salt used is KCl, while in Safer's it is $(NH_4)_2SO_4$. To find the better reaction system for analysis of the ATP hydrolysis-dependent step in transcription, we compared these methods in the presence and absence of ATP. We observed a requirement for ATP hydrolysis in Safer's system, but not in Manley's system. In Safer's system an active preinitiation complex was formed on the DNA template in the presence of ATP. In the absence of ATP this complex was inactive. The active complex was capable of synthesizing run-off transcripts with the addition of ribonucleotide triphosphates. The inactive complex was activated by addition of protein fractions and ribonucleotide triphosphates including ATP. These results suggest that ATP hydrolysis is required for formation of the preinitiation complex in crude extracts.

MATERIALS AND METHODS

HeLa cell extracts

HeLa S3 cells were cultured in the presence of 5% serum derived from newborn calves in spinner bottles at 37°C. Extracts were prepared from whole cells and nuclei as described by Manley and Dignam (39,40). The whole cell extract was dialyzed against buffer, as described by Safer *et al.*, for 8 h (38).

Transcription reactions

Except as indicated in the figure legends, reactions (25 µl) contained 30 µg/ml *Sma*I-digested pSmaF (41), 4 mM phosphoenol pyruvate, 200 µg/ml pyruvate kinase and 10 µl whole cell extract dialyzed against Manley's or Safer's buffer. These also contained 50 µM each AMP-PNP, CTP and UTP with 5 µM [α-^{32}P]GTP. Final concentrations of salts in the reactions are as follows: Manley's system (39), 8 mM HEPES–NaOH, pH 7.9, 40 mM KCl, 5 mM $MgCl_2$, 0.04 mM EDTA, 0.8 mM DTT and 8% glycerol; Safer's system (38), 20 mM Tris–HCl, pH 8.0, 6 mM $MgCl_2$, 40 mM $(NH_4)_2SO_4$, 0.2 mM EDTA, 2 mM DTT and 15% glycerol. The reactions were incubated at 30°C for 60 min. After incubation, transcripts were subsequently incubated with proteinase K, tRNA and SDS. The transcripts were extracted by phenol, precipitated with ethanol and analyzed by 6% PAGE with 8 M urea. Gels were exposed to autoradiography. The radioactive bands on the gel were excised and the radioactivity of the bands was measured using a liquid scintillation counter.

Glycerol gradient centrifugation

Reaction mixtures of 200 µl were incubated for 60 min at 30°C with HeLa cell whole cell extract in the absence or the presence of ATP and 30 µg/ml *Sma*I/*Xba*I-digested pSmaF (41) in Safer's buffer (38). The reactions were overlaid on a 15–40% glycerol gradient in SW 50.1 tubes (Beckman) and centrifuged at 4°C and 50 000 r.p.m. for 3 h. Twelve fractions were collected from the bottom of the tubes. Except as indicated in the figure legends, reactions containing 12.5 µl of an aliquot from the fractions were incubated for 30 min at 30°C in Safer's system. The reactions contained 200 µM each ATP, CTP and UTP with 50 µM [α-^{32}P]GTP. Transcripts produced by the reactions were analyzed by the same method as described previously. Transcriptional activity in the fractions of the glycerol gradient was stable after a single cycle of freezing in liquid nitrogen and thawing on ice.

Fractionation of HeLa cell nuclear extract

HeLa cell nuclear extract was fractionated on a phosphocellulose (P11) column as described by Reinberg and Roeder (42).

Protein determination

Protein concentrations were determined using the Bradford method with bovine serum albumin as the standard (43).

RESULTS

The use of Safer's crude transcription reaction indicates a stronger requirement for ATP hydrolysis

To compare Safer's and Manley's transcription systems on the ATP hydrolysis-dependent step in transcription, various concentrations of dATP were added to reactions containing HeLa whole cell extracts and AMP-PNP, GTP, UTP and CTP (38,39). Non-hydrolyzable AMP-PNP was included for RNA chain elongation and dATP for the ATP hydrolysis-dependent step in the presence of AMP-PNP (14). Manley's transcription reactions did not require the addition of dATP (Fig. 1). In the absence of dATP, transcripts from adenovirus major late promoter were detected. About 2-fold activation of transcription was observed in the presence of 100 µM dATP. In contrast, Safer's transcription system required the addition of dATP for transcriptional activity.

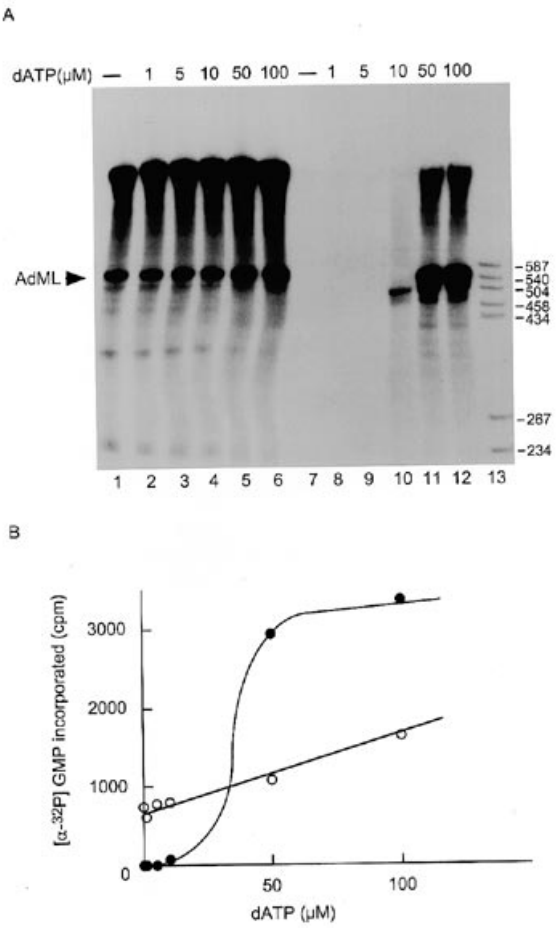

Figure 1. Titrations of dATP in the transcription reactions in the presence of AMP-PNP. (**A**) The transcription reactions were carried out in HeLa cell whole cell extract by Manley's (lane 1–6) and Safer's (lane 7–12) systems as described in Materials and Methods. Various concentrations of dATP were added to the reactions as indicated in the figure. The transcripts were extracted and analyzed by PAGE as described in Materials and Methods. The arrowhead indicates the transcript from the adenovirus major late promoter. Lane 13, *Hap*II-digested pBR322. Portions of this and subsequent figures were prepared from digital replicas of data scanned using a UMAX UC840 Max Vision digital scanner. (**B**) The radioactivity of the bands presented in (A) was measured as described in Materials and Methods. Manley's system, ○; Safer's system, ●.

This was not detected under conditions where the dATP concentration was <5 μM. Strong transcription activity was detected in the presence of 50 μM dATP. These results suggest that addition of dATP is essential in Safer's transcription system in the presence of AMP-PNP. The difference between these transcription systems is the salts. $(NH_4)_2SO_4$ was used in Safer's reaction conditions, whereas KCl was used in Manley's reaction conditions. When reactions from Safer's system were dialyzed against Manley's system, the biphasic titration curve in Safer's reactions changed to the same linear titration as observed in Manley's system (not shown). This suggests that the strength of the ATP hydrolysis requirement in these transcription systems changes upon dialysis. The transcripts produced were sensitive to α-amanitin (1 μg/ml), demonstrating that they were synthesized by RNA polymerase II (data not shown).

Preincubation of ATP or dATP in crude extract activates transcription in Safer's system

The requirement for ATP hydrolysis in Safer's transcription system was extensively analyzed, because the dependency in Safer's system was stronger than that in Manley's (Fig. 1). Safer *et al.* (38) reported that a stable preinitiation complex is formed in the presence of 40 mM $(NH_4)_2SO_4$. When preincubation of the crude extracts and template DNA was carried out in the presence of ATP, transcription activity was unaffected by the addition of 100 mM $(NH_4)_2SO_4$ (Fig. 2A, lanes 1–6). When AMP-PNP was added to the preincubation reaction, transcription was not activated (Fig. 2A, lanes 7–12). These data suggest that the preinitiation complex is formed in the presence of 40 mM $(NH_4)_2SO_4$ and ATP and furthermore that this preinitiation complex is functional under these conditions. These data suggest that ATP hydrolysis is required for formation of the preinitiation complex in the crude extracts.

To determine whether other nucleotide triphosphates are available for formation of the preinitiation complex, we included GTP, UTP and CTP in the preincubation reaction. When ATP or dATP was included, transcription from the adenovirus major late promoter was strongly activated (Fig. 3A). Addition of GTP, UTP, CTP and AMP-PNP did not activate transcription. These data suggest that only ATP and dATP are capable of activating transcription under these conditions.

In order to investigate whether the structure of the template DNA is involved in the requirement for ATP hydrolysis in transcription, the EF1α promoter was used for the transcription reactions (Fig. 3B). DNA sequences surrounding transcription initiation sites of the adenovirus major late and EF1α promoters are 5′-GTCCTC(A+1)CTCTCTTCC-3′ and 5′-AACGTT(C+1)TTT-TTCGCA-3′ respectively (37,44). The first nucleotide of the EF1α transcript is a cytosine residue, whereas that of the adenovirus major late promoter is an adenosine residue. The first adenosine residue in the EF1α transcript appears at residue 10 from the CAP site. Furthermore, the adenovirus major late promoter contains a typical TATA box sequence, whereas the EF1α promoter does not. Figure 3B shows that only addition of ATP activates transcription from the EF1α promoter. These results suggest that the requirement for ATP hydrolysis is unaffected by the first nucleotide of the transcript or by the structure of the template DNA.

Isolation of the preinitiation complex by glycerol gradient centrifugation

The preinitiation complex formed on the adenovirus major late promoter was isolated by glycerol gradient centrifugation (Fig. 4). Preincubation of the crude extracts was carried out in the presence or absence of ATP in Safer's buffer. The reaction mixtures were subjected to glycerol gradient centrifugation and subsequently fractionated. Transcription activity was detected in the fractions on addition of ATP, GTP, CTP and UTP. The active preinitiation complex formed in the presence of ATP sedimented somewhat more slowly than the *Altemia salina* 40S ribosome (Fig. 4, lanes 1–6). The preinitiation complex formed in the absence of ATP was inactive (Fig. 4, lanes 7–12). However, the inactive preinitiation complex could be activated by addition of the 0.1, 0.5 and 1.0 M KCl phosphocellulose fractions derived from HeLa cell nuclear extract and sedimented faster than the active complex (Fig. 4, lanes 13–18). These data strongly suggest that an ATP

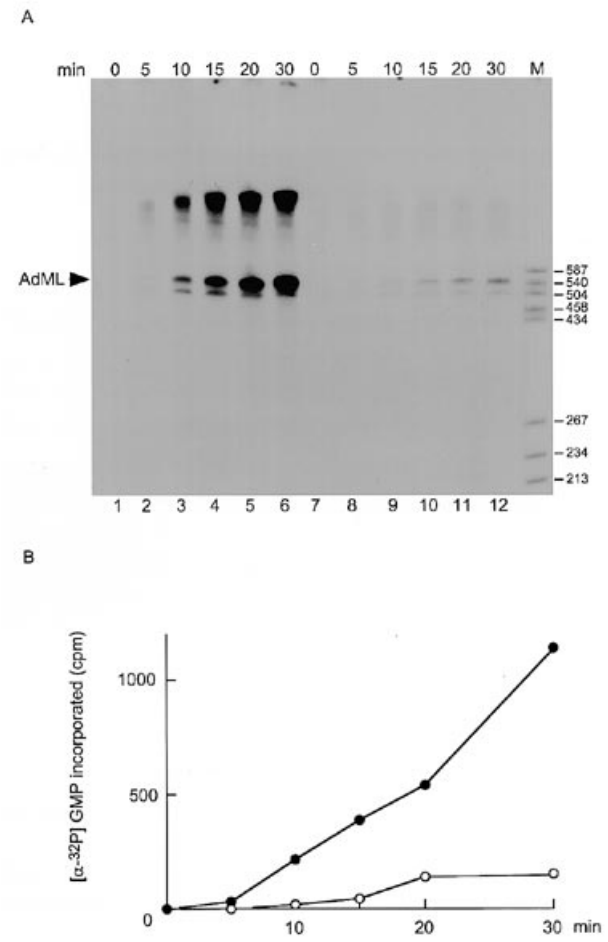

Figure 2. Time course experiments on the preincubation of crude extracts in the presence of ATP or AMP-PNP. (**A**) *Sma*I-digested pSmaF at 20 µg/ml was preincubated with HeLa whole cell extract in the presence of 100 µM ATP (lanes 1–6) and 100 µM AMP-PNP (lanes 7–12). Preincubation of the crude extracts was carried out with 5 mM phosphoenolpyruvate and 250 µg/ml pyruvate kinase under the condition described by Safer and co-workers (38). After preincubation for the times indicated in the figure, 50 µM [α-^{32}P]GTP and 200 µM each ATP, CTP and UTP were added to the reactions at the final concentrations and the reactions were further incubated for 15 min. The transcripts were extracted and analyzed by PAGE, as described in Materials and Methods. The arrowhead indicates the transcript from the adenovirus major late promoter. Lane 13, *Hae*III-digested pBR322. (**B**) Radioactivity of the bands presented in (A) was measured as described in Materials and Methods. Preincubations in the presence of 100 µM AMP-PNP, ○; preincubations in the presence of 100 µM ATP, ●.

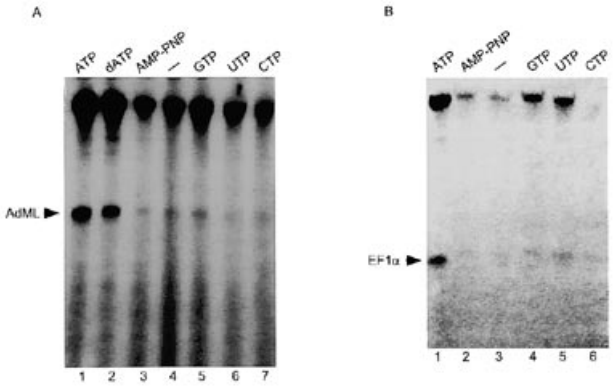

Figure 3. ATP or dATP is required for transcription from the adenovirus major late and EF1α promoters. *Sma*I-digested pSmaF at 20 µg/ml (**A**) and *Apa*I- and *Sph*I-digested pEFgen1-② at 50 µg/ml (44) (**B**) were preincubated with HeLa whole cell extract in the presence of 1 and 1.5% polyvinyl alcohol, respectively. Nucleotide triphosphates were added to the preincubation reactions at a concentration of 100 µM as indicated in the figure. Preincubations were carried out and the transcripts extracted and analyzed by PAGE, as described in the legend to Figure 2. The arrowheads indicate transcripts from the adenovirus major late and EF1α promoters.

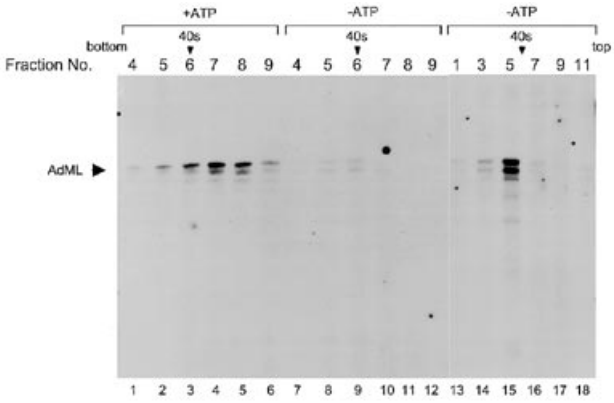

Figure 4. Isolation of the preinitiation complexes by glycerol gradient centrifugation. The preinitiation complexes were formed in the presence (lanes 1–6) or absence (lanes 7–18) of ATP and isolated by glycerol gradient centrifugation as described in Materials and Methods. The fractions of glycerol gradient centrifugation were incubated with ribonucleotide triphosphates in the absence (lanes 1–12) or presence (lanes 13–18) of phosphocellulose 0.1, 0.5 and 1.0 M KCl fractions. Each phosphocellulose fraction contained 1 µg protein. The transcripts were extracted and analyzed by PAGE, as described in Materials and Methods. The arrowheads indicate the position of the *Altemia salina* 40S ribosome in the glycerol gradient centrifugation and the transcript from the adenovirus major late promoter.

hydrolysis-dependent step is involved in formation of the preinitiation complex and also suggest that an inactive preinitiation complex is formed in the absence of ATP. The inactive complex requires addition of the HeLa phosphocellulose fractions for activation. When the preinitiation complex was formed in the presence of AMP-PNP, a similar inactive complex was formed. This complex could also be activated by addition of the phosphocellulose fractions (not shown).

ATP hydrolysis is required to activate the inactive preinitiation complex formed in the absence of ATP

Experiments were carried out to investigate whether ATP hydrolysis is required to activate the inactive complex which forms in the absence of ATP (Fig. 5). The inactive complex isolated by glycerol gradient centrifugation was activated by addition of the 0.1 and 1.0 M KCl phosphocellulose fractions in the presence of ATP (Fig. 5A). The 0.5 M KCl fraction was dispensable. AMP-PNP could not substitute for ATP to activate

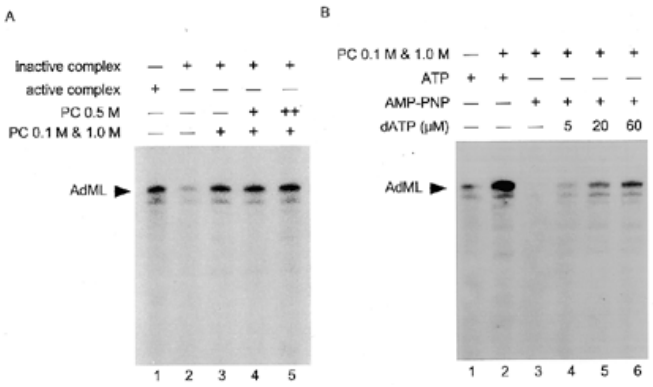

Figure 5. Activation of the inactive preinitiation complex formed in the absence of ATP. (**A**) The active or inactive preinitiation complexes isolated by glycerol gradient centrifugation (Fig. 3, lanes 4 and 15) were incubated with ribonucleotide triphosphates and phosphocellulose 0.1, 0.5 and 1.0 M KCl fractions as indicated in the figure. Each phosphocellulose fraction contained 1 μg protein. The reaction in lane 5 contained 2 μg protein of the phosphocellulose 0.5 M KCl fraction. The transcription reactions were carried out as described in Materials and Methods. The arrowhead indicates transcript from the adenovirus major late promoter. (**B**) The inactive preinitiation complex isolated by glycerol gradient centrifugation (Fig. 3, lane 15) was incubated with ATP, dATP, AMP-PNP or phosphocellulose 0.1 and 1.0 M KCl fractions as indicated in the figure. Each phosphocellulose fraction contained 1 μg protein. ATP and AMP-PNP were added at a concentration of 200 μM. Concentrations of dATP added to the reactions are indicated in the figure. The transcription reactions were carried out as described in Materials and Methods. The arrowhead indicates transcript from the adenovirus major late promoter.

the inactive complex (Fig. 5B). However, when dATP was added to the reactions in the presence of AMP-PNP the inactive complex was activated in the presence of the 0.1 and 1.0 M KCl phosphocellulose fractions (Fig. 5B), suggesting that dATP substituted for hydrolysis of ATP in the presence of unhydrolyzable AMP-PNP. These data suggest that ATP hydrolysis and the phosphocellulose 0.1 and 1.0 M KCl fractions are required to activate the inactive complex.

DISCUSSION

We report here that there is a requirement for ATP hydrolysis in formation of an active preinitiation complex in crude extracts. We conclude that the requirement for ATP hydrolysis is implicated in formation of the preinitiation complex, based on the following results. First, the preinitiation complex formed in the presence of ATP and isolated by glycerol gradient centrifugation was active. The active preinitiation complex was capable of synthesizing a transcript with the addition of ribonucleotide triphosphates. Second, a complex formed in the absence of ATP was inactive. This inactive complex required ATP hydrolysis and the addition of the phosphocellulose 0.1 and 1.0 M KCl fractions for activation. These data suggest that the inactive complex was converted to the active configuration by ATP hydrolysis and the presence of protein fractions.

RNA polymerase II holoenzyme has been reported to be composed of factors involved in transcription, the cell cycle and DNA repair (6,7,9,11–13). The purified holoenzyme sedimented somewhat more slowly than 60 S in sucrose gradient centrifugation, although the holoenzyme in crude extracts sedimented faster than the purified holoenzyme on sucrose gradient centrifugation (6). The difference in these sedimentation speeds suggests that components of the holoenzyme are dissociated by sucrose gradient centrifugation and, furthermore, that the molecular size of the holoenzyme decreases during purification. The instability of the holoenzyme in purification hampers studies of the mechanism of transcription by the holoenzyme and it is not understood which are subunits of the holoenzyme or how a preinitiation complex is formed by the holoenzyme. A reconstituted transcription system using purified general transcription factors and the RNA polymerase II core enzyme has been established to investigate the transcription mechanism in detail. Nevertheless, biochemical studies of a transcription complex formed in crude extracts are still important to understand the transcriptional mechanism of the holoenzyme because it has not been established how the system has been reconstituted.

Sedimentation of the active preinitiation complex formed in the presence of ATP was slightly slower than the 40S marker on glycerol gradient centrifugation, whereas the sedimentation speed of the inactive preinitiation complex formed in the absence of ATP was almost same as that of the 40S maker. It is possible that factors associated with the inactive complex might dissociate when it is activated. The sedimentation speeds of the active and inactive complexes are similar to that of RNA polymerase II holoenzyme purified from HeLa cell nuclear extracts (6,7). It is possible that the active and inactive complexes might contain the holoenzyme. It is also possible that some factors associated with the holoenzyme could be dissociated from the inactive complex by the action of ATP hydrolysis when the holoenzyme forms a preinitiation complex on template DNA. Yeast RNA polymerase II holoenzyme has been reported to contain SRBs, a subset of the initiation factors, and SWI/SNF protein complex (8,12,13). Mammalian RNA polymerase II holoenzyme has been reported to contain a mammalian homolog of SRB7, cyclin dependent kinases and DNA repair factors (6,9). The preinitiation complex formed in the presence of ATP may contain SRBs, cyclin-dependent kinases and SWI/SNF complex.

A strong requirement for ATP hydrolysis was observed in Safer's transcription buffer containing $(NH_4)_2SO_4$ (Fig. 1). Manley's transcription buffer, containing KCl, did not require ATP hydrolysis. ATP hydrolysis has not been reported to be required for formation of the preinitiation complex in reconstituted transcription systems using purified general transcription factors and RNA polymerase II (1,2,11,17). These factors and RNA polymerase II were purified using buffer containing KCl as separate entities and are thought to dissociate from the holoenzyme in crude extracts. Holoenzyme from calf thymus was purified using buffer containing $(NH_4)_2SO_4$ (9). In Manley's transcription buffer the holoenzyme might dissociate to the core RNA polymerase II and the factors in crude extracts and the preinitiation complex formed in Manley's buffer may not contain the factors associated with the holoenzyme. It is possible that Safer's transcription buffer, containing $(NH_4)_2SO_4$, could more easily form a preinitiation complex containing the holoenzyme on template DNA.

To activate the inactive preinitiation complex formed in the absence of ATP, ATP hydrolysis and phosphocellulose 0.1 and 1.0 M KCl fractions derived from HeLa nuclear extracts were required (Figs 4 and 5). The phosphocellulose 0.5 M KCl fraction was not needed to activate the inactive complex (Fig. 5). These phosphocellulose fractions did not contain RNA polymerase II activity, suggesting that proteins supplied to activate the complex

did not include RNA polymerase II (not shown). It is possible that protein components in the phosphocellulose fractions could form the active complex or could change the conformation of the inactive complex by the action of ATP hydrolysis. The DNA helicase activity of TFIIH, a component of the holoenzyme, might change the conformation of the inactive complex (6,9,23,24). It is also possible that an inhibitor might associate with the inactive preinitiation complex and, furthermore, that the inhibitor might dissociate from the inactive complex in the presence of ATP and the phosphocellulose fractions. Factors needed to activate the inactive complex could be components of the RNA polymerase II holoenzyme (6,7). Yeast SWI/SNF complex has been reported to be a component of the holoenzyme (13). A SWI/SNF complex purified from HeLa cells binds a phosphocellulose column and remodels chromatin structure in the presence of ATP (45,46). It is possible that the SWI/SNF complex might be the factor in the phosphocellulose fraction which activated the inactive complex.

Extensive purification of the active and the inactive preinitiation complexes identified in this report will provide information about components associated with these complexes. It will shed light on mechanisms of preinitiation complex formation under physiological conditions.

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