Inhibition of Transcription by the Trimeric Cyclin-dependent Kinase 7 Complex*

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Cyclin-dependent kinase 7 (CDK7) can be isolated as a subunit of a trimeric kinase complex functional in activation of the mitotic promoting factor. In this study, we demonstrate that the trimeric cdk-activating kinase (CAK) acts as a transcriptional repressor of class II promoters and show that repression results from CAK impeding the entry of RNA polymerase II and basal transcription factor IIF into a competent preinitiation complex. This repression is independent of CDK7 kinase activity. We find that the p36/MAT1 subunit of CAK is required for transcriptional repression and the repression is independent of the promoter used. Our results demonstrate a central role for CAK in regulation of messenger RNA synthesis by either inhibition of RNA polymerase II-catalyzed transcription or stimulation of transcription through association with basal transcription repair factor IIIH.

Cyclin-dependent kinase 7 (CDK7)1 was originally isolated as the catalytic subunit of the trimeric cdk-activating kinase (CAK) complex. This complex, consisting of CDK7, cyclin H, and MAT1, is responsible for activation of the mitotic promoting factor in vitro (1–3). The discovery that CDK7 was also a component of the basal transcription repair factor IIIH (TFIIH) implicated a dual role for CDK7 in transcription as part of TFIIH and in the control of the cell cycle as the trimeric CAK complex (4–7). TFIIH is a multisubunit protein complex identified as a factor required for RNA polymerase II (RNAPII)-catalyzed transcription (8–11), and subsequently this complex was found to play a key role in nucleotide excision repair (12–14). At least nine polypeptides with molecular masses of 89, 80, 62, 52, 44, 40, 37, 36, and 34 kDa co-purify with mammalian TFIIH. The cDNAs encoding all of these subunits have been identified as the gene product of ERCC3 (XPB) and ERCC2 (XPD), respectively (13–15). p62 and p44 are now been cloned. p89 and p80 are the gene products of ERCC3, which plays a key role in nucleotide excision repair factor IIH.

The trimeric CAK complex (1–3) was essentially pure and could be isolated from 3 g of HeLa nuclear extract (Fig. 1A). Nuclear extract was loaded on a 1-liter column of phosphocellulose (Whatman) and fractionated stepwise by the indicated KCl concentrations in buffer A (20 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 10 mM 2-mercaptoethanol, 20% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 1 mM leupeptin, and 1 μg/ml pepstatin. The phosphocellulose 0.3 M KCl fraction (400 mg) was dialyzed to 0.1 M KCl in buffer A and loaded on a 100-ml DEAE-Sephacel column (Amersham Pharmacia Biotech). The column was eluted with 0.5 M KCl in buffer A. The 0.5 M KCl elution (260 mg) was dialyzed to 100 mM KCl in buffer A and loaded on a 100-ml Q-Sepharose column (Sigma). The column was resolved using a linear 10-column gradient of 100–600 mM KCl. Fractions containing CDK7 (~200 mM

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1 The abbreviations used are: CDK7, cyclin-dependent kinase 7; CAK, cdk-activating kinase; TF, transcription repair factor; RNAPII, RNA polymerase II; holo, holoenzyme; CTD, carboxy-terminal domain; HTLV, human T-cell leukemia virus; TBP, TATA-binding protein; TB, TBP-TFIIIB.

Experimental Procedures

Purification of CAK from HeLa Nuclear Extract—Trimeric CAK was purified from 3 g of HeLa nuclear extract (Fig. 1A). Nuclear extract was loaded on a 1-liter column of phosphocellulose (Whatman) and fractionated stepwise by the indicated KCl concentrations in buffer A (20 mM Tris-HCl, pH 7.9, 0.2 mM EDTA, 10 mM 2-mercaptoethanol, 20% glycerol, 0.2 mM phenylmethylsulfonyl fluoride, 1 μg/ml leupeptin, and 1 μg/ml pepstatin. The phosphocellulose 0.3 M KCl fraction (400 mg) was dialyzed to 0.1 M KCl in buffer A and loaded on a 100-ml DEAE-Sephacel column (Amersham Pharmacia Biotech). The column was eluted with 0.5 M KCl in buffer A. The 0.5 M KCl elution (260 mg) was dialyzed to 100 mM KCl in buffer A and loaded on a 100-ml Q-Sepharose column (Sigma). The column was resolved using a linear 10-column gradient of 100–600 mM KCl. Fractions containing CDK7 (~200 mM

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KCl, 37 mg) were dialyzed to 10 mM potassium phosphate in buffer B (5 mM Hepes, pH 7.5, 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 10 μM CaCl2, 10% glycerol, 1 μg/ml leupeptin, and 1 μg/ml pepstatin) and loaded on a 20-ml hydroxyapatite column (American International Chemical). The column was resolved using a linear 10-column volume gradient of 10–600 mM potassium phosphate in buffer B. CDK7-containing fractions (100 mM potassium phosphate, 8.6 mg) were dialyzed to 1 M ammonium sulfate in buffer C (20 mM Hepes, pH 7.9, 4 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 0.5 mM EDTA, 10% glycerol) and fractionated on a phenyl-Superose HR 5/5 column (Amersham Pharmacia Biotech). The phenyl-Superose column was resolved using a linear 10-column volume gradient of 1M to 0M ammonium sulfate in buffer B. CDK7-containing fractions (~0.4 mM ammonium sulfate, 0.5 mg) were precipitated with 60% ammonium sulfate and fractionated on a Superose 6 HR 10/30 column (Amersham Pharmacia Biotech) equilibrated in 1M KCl in buffer A. 

RESULTS

The Trimeric CAK Complex Purified from HeLa Cells Is a Transcriptional Repressor—The trimeric CAK complex from HeLa nuclear extract was purified as described under “Experimental Procedures” (Fig. 1A) using the CTD-kinase activity and Western blot analysis with antibodies against CDK7 and cyclin H. Nuclear extract was initially fractionated by phosphocellulose chromatography. The 0.3M KCl step elution contained approximately 40% of CDK7 immunoreactivity and was devoid of TFIIH activity or ERCC3 immunoreactivity, which fractionated into the 0.5M KCl step elution (data not shown). The 0.3 mM KCl fraction was therefore further purified through five additional steps (Fig. 1A). Analysis of the CTD kinase activity of CAK on the sizing column (Superose 6) revealed the peak of activity eluting at ~120 kDa, consistent with the previously reported size for the trimeric CAK complex (21). We estimate that our purification resulted in ~2000-fold enrichment in CAK. Analysis of column fractions in a transcription system reconstituted with recombinant TBP, TFIIIB, TFIIE, TFIIF, highly purified HeLa or rat holo-TFIIH, and highly purified HeLa core RNAPII revealed an inhibitory activity copurifying with the CDK7 complex throughout the purification (Fig. 1B). The transcriptional inhibitory activity was also observed in the
last two steps of the purification (phenyl-Superose and Superose 6) (Fig. 1, C and D). Highest concentrations of the CAK complex tested resulted in about 95% inhibition in basal transcription.

**Recombinant Trimeric CAK Displays Transcriptional Inhibitory Activity**—To determine if the inhibitory activity associated with the HeLa fractions is the CAK complex, we expressed either the trimeric (CDK7-cyclin H-MAT1) or the dimeric CAK complex (CDK7-cyclin H) in insect SF9 cells. These complexes were purified as previously reported (21) (Fig. 2 A). Moreover, as the highly purified holo-TFIIH complex also contains CAK, we sought to determine the action of the trimeric CAK complex in an assay free of TFIIH. Similar to a previous report (31), reconstituted transcription using the rat supercoiled somatostatin promoter is independent of TFIIH or TFIIE but is highly stimulated by these factors (Fig. 2 B). Analysis of recombinant trimeric CAK complex in reconstituted transcription assay either in the presence (Fig. 2 C, lanes 1 and 2) or the absence (lanes 3–5) of TFIIH revealed that this complex is a potent inhibitor of transcription. The inhibitory effect of CAK is achieved at roughly a 1:1 stoichiometry (0.25 pmol) with other basal factors.

The p36/MAT1 Subunit of the Trimeric CAK Complex Is Required for the Inhibitory Activity—To further ascertain the role of the p36/MAT1 subunit of the CDK7 complex in transcriptional repression, we compared the recombinant dimeric and trimeric CAK complexes. Although the two CAK complexes displayed similar kinase activity as ascertained by phosphorylation of a CTD peptide (Fig. 3 A), analysis of the dimeric CAK in transcription revealed that p36/MAT1 is required for the inhibitory activity of the CAK complex (Fig. 3 B). In contrast to the trimeric CAK, which inhibited transcription driven from either the supercoiled somatostatin (lanes 2 and 3) or supercoiled HTLV-1 (lanes 8 and 9) promoters, the dimeric CAK was devoid of any inhibitory activity with either promoter (lanes 4–6 or 10 and 11). These data indicate that the p36/MAT1 subunit of the CAK complex is required for inhibition of transcription and that the CAK-mediated inhibition is independent...
of the promoter used.

p36/MAT1 Is Not Sufficient for Transcriptional Inhibition—To determine whether the p36/MAT1 subunit of the CAK complex is sufficient for transcriptional repression, the p36/MAT1 subunit was expressed in Escherichia coli, and the purified p36/MAT1 was analyzed for its activity in transcription. In contrast to trimeric CAK (Fig. 3B, lane 2), the addition of p36/MAT1 not only failed to inhibit transcription (lanes 3–5) but also displayed a small stimulatory activity (compare lanes 1 and 3). These results demonstrate that although p36/MAT1 is required for the inhibitory activity of the CAK complex, it is not sufficient for inhibition.

Addition of p36/MAT1 to Dimeric CAK Can Reconstitute Transcriptional Repression—The p36/MAT1 subunit of CAK was produced in SF9 cells, and purified protein was tested for its ability to confer repression when added to the dimeric CAK complex. As Fig. 3C indicates, neither the dimeric CAK (lane 2) nor the p36/MAT1 protein alone were sufficient to mediate repression. However, the addition of the p36/MAT1 subunit to dimeric CAK reconstitutes the transcriptional repression observed with trimeric CAK (lane 4).

The Trimeric CAK Inhibits Transcription by Precluding RNAPII and TFIIF from the Preinitiation Complex—To analyze which step during the formation of the preinitiation complex the trimeric CAK may target to repress transcription, we incubated the basal transcription factors in a stepwise fashion with the DNA for 30 min before adding the trimeric CAK complex (Fig. 4A). Preincubation of DNA with either TBP (lane 2) or TBP and TFIIH (lane 3) could not overcome the inhibitory effect of CAK, indicating that the TBP-TFIIH complex (TB) formation is not the target of the CAK complex. However, the addition of TFIIF to the preinitiation complex, which results in the formation of the TBF complex, could partially relieve the CAK repression (lane 4). Formation of the TBPolF or TBPolFE complex by further preincubation with RNAPII or RNAPII and TFIIIE resulted in a complete recovery of transcription (lanes 5 and 6). These results indicate that trimeric CAK precludes the entry of RNAPII and TFIIF into a competent preinitiation complex, and a preformed preinitiation complex is refractory to the action of trimeric CAK. This contention is further substantiated when we analyzed whether the addition of excess TFIIF or RNAPII can overcome the inhibitory activity of the trimeric CAK complex. As shown in Fig. 4B, the addition of increasing amounts of TFIIF partially overcomes the CAK-mediated repression (lanes 3 and 4), whereas the addition of excess RNAPII could completely restore transcription (lane 5). We conclude that trimeric CAKs repress transcription by precluding RNA-P II and TFII F entry into the preinitiation complex.

The Mechanism of CAK-mediated Repression Is Independent of Its Kinase Activity—To address whether the kinase activity of CDK7 plays a role in CAK-mediated inhibition, we analyzed a kinase-deficient mutant of CDK7, in which lysine 41 was

![Fig. 4](image-url)
replaced by alanine (Fig. 3A, lane 3). The purified dimeric CAK/K41A, produced in insect cells, was mixed with the p36/MAT1 subunit, produced in insect cells, and analyzed in a reconstituted transcription system. As Fig. 4C indicates, the addition of kinase-deficient CAK resulted in a potent inhibition of transcription (compare lane 1 to lanes 2 and 3). These results indicate that CAK-mediated inhibition is not because of the kinase activity of CAK and may result from CAK physically destabilizing the preinitiation complex.

**Excess TFIIH Can Overcome the Inhibitory Effect of CAK—** Because TFIIH displays a stimulatory activity in transcription by stabilizing the preinitiation complex, we analyzed whether increasing concentrations of TFIIH can relieve the CAK-mediated repression. As Fig. 4D reveals, the addition of excess TFIIH can overcome the inhibitory activity of CAK (compare lanes 2–4 to 6–8). These results indicate that TFIIH and CAK are in a competition for the preinitiation complex. Whereas TFIIH stimulates transcription by stabilizing the preinitiation complex formation, CAK exerts an inhibitory effect by disrupting its formation.

**DISCUSSION**

The novelty of this work lies in the following. First, it demonstrates transcriptional inhibitory activity for trimeric CAK in a fully defined system, comprised of essentially homogeneous basal factors and RNA polymerase II. Second, it shows that CAK inhibits transcription by preventing RNA polymerase II and TFIIF entry into the preinitiation complex. Third, it presents evidence for the requirement of p36/MAT1 in the CAK-mediated inhibitory effect. Finally, it demonstrates that the inhibitory activity is independent of the kinase activity of CDK7.

The trimeric CAK complex was initially identified as the kinase complex responsible for phosphorylation and consequent activation of other cyclin-dependent kinases from mammalian cells (1–3). It was later discovered that CDK7, cyclin H, and MAT1 were also components of the basal transcription factor TFIIH (4–7). Furthermore, it was observed that TFIIH can be dissociated into two subcomplexes, one containing the core TFIIH subunit (XPB, p62, p51, p44, and p34) and the other containing XPD and the three CAK subunits (18, 19). We found that the trimeric CAK purified from HeLa cells contained a transcriptional inhibitory activity in a highly purified reconstituted transcription system. The inhibitory activity associated with the HeLa fractions was demonstrated to be mediated by the trimeric CAK complex, because the recombinant trimeric CAK produced in insect cells inhibited transcription. Interestingly, the p36/MAT1 subunit of the trimeric CAK was required for the transcriptional inhibition. This observation lends further support to the physiological relevance of CAK-mediated inhibition, as the predominant form of CAK in mammalian extracts not associated with TFIIH contains the p36/MAT1 subunit (19, 21). The transcriptional inhibition by the CAK complex did not result from CDK7 kinase activity, because the kinase-deficient mutant of CAK is also a potent inhibitor of transcription. Our studies revealed that CAK inhibited transcription by preventing the formation of the TBPolF complex. Therefore, either preforming the TBPolF complex or the addition of excess TFIIH, RNAPII, or TFIIH was able to stabilize the complex and to overcome the inhibitory effect of CAK.

A number of studies have concluded that the trimeric CAK complex represents the form of CAK involved in cell cycle control (1–3, 21). Here we present evidence indicating that the trimeric CAK complex displays a novel role in transcription distinct from that of its function when associated with TFIIH. Our findings are a further support for CAK as a regulator of transcription in addition to the function of CAK in cell cycle control.

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