Journal of Cellular Biochemistry 93:2–10 (2004)

FAST TRACK

Effects of Phosphorylation by Protein Kinase CK2 on the Human Basal Components of the RNA Polymerase II Transcription Machinery

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Abstract We have investigated the role of phosphorylation by vertebrate protein kinase CK2 on the activity of the General Transcription Factors TFIIA, TFIIE, TFIIF, and RNAPII. The largest subunits of TFIIA, TFIIE, and TFIIF were phosphorylated by CK2 holoenzyme. Also, RNA polymerase II was phosphorylated by CK2 in the 214,000 and 20,500 daltons subunits. Our results show that phosphorylation of TFIIA, TFIIF, and RNAPII increase the formation of complexes on the TATA box of the Ad-MLP promoter. Also, phosphorylation of TFIIF increases the formation of transcripts, where as phosphorylation of RNA polymerase II dramatically inhibits transcript formation. Furthermore, we demonstrate that CK2β directly interacts with RNA polymerase II, TFIIA, TFIIF, and TBP. These results strongly suggest that CK2 may play a role in regulating transcription of protein coding genes. J. Cell. Biochem. 93: 2–10, 2004.

Key words: protein kinase CK2; RNA polymerase II; general transcription factors; phosphorylation; binding

RNA polymerase II (RNAPII) is a multisubunit enzyme that requires additional factors to initiate transcription at the promoters [Roeder, 1976; Weil et al., 1979; Matsui et al., 1980]. These factors are named the General Transcription Factors (GTFs) and include TFIIA, TFIIB, TFIID, TFIIE, TFIIF, and TFIIH. The first step in the initiation of transcription is the binding of TATA-binding protein (TBP), a subunit of TFIID, to the TATA box of the promoters, followed by the binding of TFIIB [Lee et al., 1991; Starr and Hawley, 1991]. After TFIIB follows the recruitment to the promoter of the complex TFIIF–RNAPII. Once incorporated, the binding of TFIIE and TFIIH follows to form a preinitiation complex which is ready to initiate transcription upon the addition of ribonucleotide triphosphates [Hampsey, 1998].

Protein kinase CK2 is ubiquitous serine and threonine protein kinase that phosphorylates a large number of proteins and it is present in all eukaryotic cells [Pinna, 1990; Issinger, 1993; Allende and Allende, 1995]. More than 300 protein substrates are known to be phosphorylated by CK2 including metabolic enzymes, signal transduction factors, transcription factors, tumor suppressors, and cytoskeletal proteins. CK2 is a tetrameric enzyme composed for two catalytic subunits α and/or α′ and two β subunits [Chester et al., 1995; Valero et al., 1995]. The β subunit can stimulate the phosphorylation of the most of the substrates, however, in some cases it inhibits phosphorylation as is the case with MDM-2 and calmodulin [Bidwai et al., 1993; Meggio et al., 1994].

Several years ago, it was reported that protein kinase CK2 phosphorylates two subunits of RNAPII (214,000 and 20,500 daltons) and it was proposed that this phosphorylation regulates transcriptional activity [Dahmus, 1981]. Also, studies with RNAPIII from *Saccharomyces cerevisiae* indicated that phosphorylation by CK2 was required for transcriptional activity. CK2 phosphorylates the TBP, a subunit of TFIIIB [Ghavidel and Schultz, 1977]. It

Grant sponsor: FONDECYT-CHILE; Grant number: 2000081.

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Received 14 May 2004; Accepted 17 May 2004

DOI 10.1002/jcb.20209

Published online 26 July 2004 in Wiley InterScience (www.interscience.wiley.com).

has been reported that CK2 copurifies with epitope-tagged RNAPI from mammalian cells [Hannan et al., 1998]. Furthermore, it has recently been reported that endogenous human CK2 associates stably with TFIIIB and phosphorylates the BRF component of TFIIIB [Lei et al., 1998]. These data suggest that CK2 may play a fundamental role in promoting transcription complex assembly. It is know that protein kinase CK2 phosphorylates RNAPII and GTFs such as TFIIF [Dahmus, 1981; Rossignol et al., 1999]. Dephosphorylated TFIIF is less competent to form preinitiation complexes (PIC) which may suggest that phosphorylation could be a key event in the regulation of transcription by RNAPII [Kitajima et al., 1994]. However, whether phosphorylation of TFIIF by CK2 has any functional consequence on transcription still remains an open question.

In this work we demonstrate that human TFIIA, TFIIE, TFIIF, and RNAPII are phosphorylated by vertebrate CK2. The phosphorylation of TFIIF and RNAPII has functional consequences on the formation of a preinitiation complex. Phosphorylation of TFIIF increases both the formation of a PIC and the transcription. However, phosphorylation of RNAPII increases the formation of a PIC, but decreases basal transcription. Furthermore, we demonstrate that CK2β is able to interact with TBP, TFIIA, TFIIF, and RNAPII.

MATERIALS AND METHODS

Purification of Recombinant Human GTFs, RNAPII, and CK2

Recombinant human TBP, TFIIB, TFIIE, and TFIIF were expressed and purified according to Maldonado et al. [1996]. Native bovine RNAPII was purified as published [Maldonado et al., 1996]. TFIIA was expressed and purified according to Sun et al. [1994]. Recombinant *Xenopus laevis* CK2α and catalytically inactive subunit CK2αA^{156} were expressed and purified by Ni-NTA-agarose according to published procedures [Hinrichs et al., 1993; Cosmelli et al., 1997]. Glutathione-S-transferase (GST) and fusion proteins GST–CK2α and GST–CK2β were purified by glutathione-agarose chromatography according to the manufacturer instructions [Smith and Johnson, 1988]. GST–CK2β was further treated with thrombin and free GST was bound to glutathione-agarose. All the proteins were at least 90% pure as judged by SDS–PAGE followed by Coomassie blue staining. CK2 holoenzyme was reconstituted by mixing equal amounts of CK2α and thrombin treated CK2β at 4°C for 30 min.

Phosphorylation Assays

The phosphorylation assays were performed in 25 μl reaction volume containing 1 pmol each of CK2α and CK2β and 10 pmol of each protein substrate. The reactions were carried out for 30 min in 50 mM HEPES pH 7.8, 75 mM KCl, 7 mM $MgCl_2$, 0.5 mM DTT, and 100 μM [γ-^{32}P]ATP (NEN, 500–1,000 cpm/pmol). The reaction was stopped by the addition of 5 μl of 5× Laemmli sample buffer [Laemmli, 1970] and analyzed in a 10% or a 4–16% gradient SDS–PAGE gel. The gels were dried and exposed 30 min to a X-ray film (KODAK MS).

Protein–Protein Interactions Analysis

Recombinant GSTβ and GST were immobilized separately onto a glutathione-agarose beads and adjusted to a final concentration of 1 mg/ml of wet resin. The resin was pretreated with buffer A (40 mM HEPES, pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM PMSF, 0.1% NP-40, and 1 mg/ml BSA). Wet resin (50 μl) containing the immobilized proteins were incubated in buffer A with 1 μg of each recombinant factor. After 30 min at 4°C, the resin was washed five times with buffer A without BSA. Beads were then collected by centrifugation and eluted with 25 μl of 25 mM glutathione. The eluates were mixed with Laemmli SDS loading buffer, separated in a 10% SDS–PAGE gel, and analyzed by Western blot with affinity purified antibodies against each factor and RNAPII.

Phosphorylation of TFIIA, TFIIE, TFIIF, and RNAPII With Non-Radioactive ATP

TFIIA, TFIIE, TFIIF, and purified RNAPII were phosphorylated by mixing 10 pmol of each factor with 1 pmol of CK2 reconstituted holoenzyme containing either the catalytically active or the inactive CK2α subunit. The reactions were performed for 30 min in the same buffer (containing 1 mM of unlabeled ATP) used to phosphorylate the factors with radioactive ATP. After, the samples were incubated with antibodies against CK2α bound to Protein A-agarose (Sigma) to remove the enzyme. The samples were dialyzed 2 h against buffer B (20 mM Tris pH 7.8, 10% glycerol, 10 mM β-mercaptoethanol, 0.5 mM

EDTA, 0.1 mM PMSF, and 100 mM KCl) and stored at −50°C until use.

Gel Retardation Assays

Gel retardation assays were performed in a 20 μl reaction volume using: 0.1–1 ng of a 3′-end-labeled DNA fragment containing the Ad-MLP TATA motif (approximately 5,000 cpm) extending from −40 to +20. Reactions mixtures contained 20 mM HEPES pH 7.8, 4 mM $MgCl_2$, 4 mM ammonium sulfate, 8% (v/v) glycerol, 2% (w/v) polyethylene glycol 8000, 60 mM KCl, 5 mM β-mercaptoethanol, 0.2 mM EDTA, and 100 ng of poly(dG-dC)-poly (dG-dC). Reactions were incubated for 30 min at 30°C and analyzed in 5% polyacrylamide gel containing 0.5× TBE buffer (45 mM Trisborate pH 8.0, 1 mM EDTA). The same buffers were used to run the gels. Complexes were visualized by autoradiography and fold activation values were measured with a phosphoimager.

In Vitro Transcription Reactions

The reactions were performed in 40 μl containing 500 ng of the Ad-MLP promoter directing transcription of a G-less cassette as a template (pML(C2AT)) [Sawadogo and Roeder, 1985]. Reactions mixtures contained 20 mM HEPES pH 7.8, 8 mM $MgCl_2$, 60 mM KCl, 10 mM ammonium sulfate, 10% (v/v) glycerol, 5 mM β-mercaptoethanol, 10% (w/v) polyethylene glycol 8000, RNAase T1 (20 U), 0.6 mM ATP, GTP, CTP, and 0.15 μM [α-^{32}P]UTP (NEN, 800 Ci/mmol). The GTFs and RNAPII were added as indicated in each figure. After 45 min incubation at 30°C, reactions were stopped by adding 40 μl of stop mix (10 mM EDTA, 100 mM sodium acetate pH 5.5, 0.2% SDS, and 1 mg/ml yeast tRNA). Samples were extracted with 80 μl of phenol–chloroform and the aqueous phase was removed and precipitated with 3 volumes of ethanol. The precipitates were dried in a Speed Vac and resuspended in 20 μl of 98% formamide. The products of the transcription were separated in a 5% polyacrilamide gel containing 7 M urea and 0.5× TBE. Gels were dried and analyzed by autoradiography.

RESULTS

CK2 Phosphorylates TFIIA, TFIIE, TFIIF, and RNAPII

Purified human recombinant factors (Fig. 1A) were incubated with recombinant *Xenopus* CK2 and [γ-^{32}P] ATP under optimal conditions for phosphorylation. In Figure 1B, it is observed that the largest subunits of TFIIA (IIAαβ, lane 1), TFIIE (p56, lane 4), and TFIIF (Rap74, lane 5) are strongly phosphorylated by CK2 holoen-

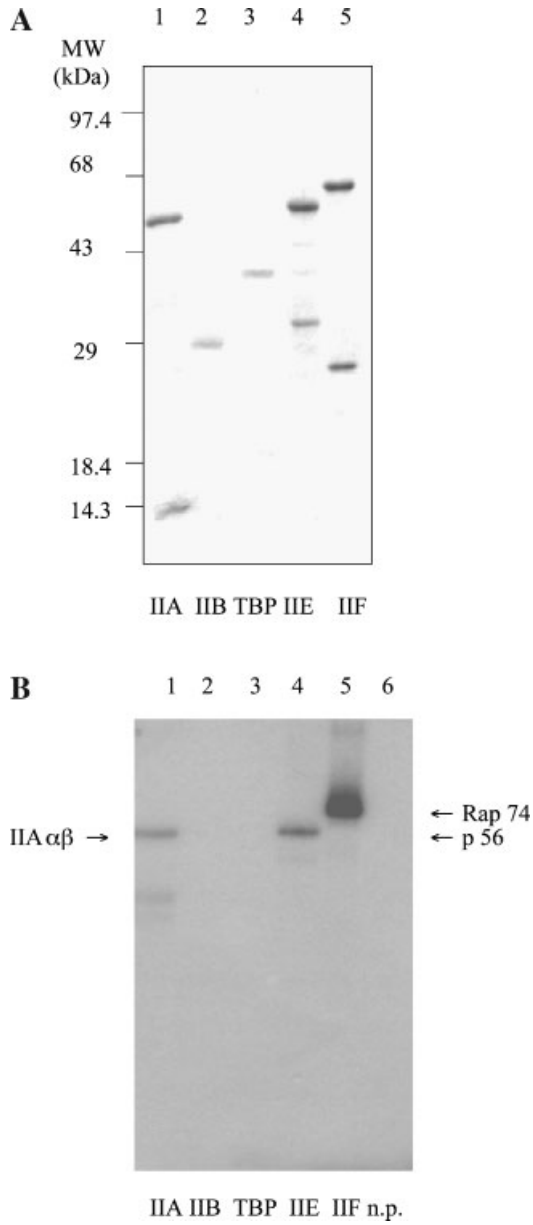


Fig. 1. Phosphorylation of purified human recombinants GTFs by CK2 holoenzyme. **A**: Recombinants factors were purified as described in Materials and Methods and 5 μg were analyzed in a 10% SDS–PAGE gel followed by Coomassie blue staining. **B**: Four pmol of each purified recombinants GTFs were incubated with 1 pmol of CK2 reconstituted holoenzyme and [γ-^{32}P]ATP. The products of the reaction were separated in a 10% SDS–PAGE gel and analyzed by autoradiography. **Lane 1**: TFIIA. **Lane 2**: TFIIB. **Lane 3**: TBP. **Lane 4**: TFIIE. **Lane 5**: TFIIF. **Lane 6**: no protein.

zyme. However, when the catalytically inactive CK2α is used no detectable phosphorylation is observed (not shown). Although, CK2α by itself is able to phosphorylate TFIIA, TFIIE, and TFIIF, CK2β subunit is able to stimulate by at least three fold the phosphorylation (data not shown). The smallest subunits of TFIIA, TFIIE, and TFIIF are not phosphorylated by CK2 holoenzyme, neither GTFs TFIIB (lane 2) and TBP (lane 3) are not phosphorylated by CK2 holoenzyme. Likewise, TFIIB and TBP were not phosphorylated when incorporated as part of the promoter complex (data not shown). RNAPII was phosphorylated in the subunits of 214,000 (IIA) and 20,500 daltons as also described by Dahmus (not shown). Phosphorylation by the CK2 holoenzyme of human histones H2A, H2B, H3, and H4, either free or as present in oligonucleosomes, was also studied but we did not observed phosphorylation under any of the conditions used (data not shown).

Protein–Protein Interactions of CK2β With GTFs and RNAPII

The GST pulldown assay was used to study protein–protein interactions between CK2β with the GTFs and RNAPII. Each transcription factor was incubated with GST–CK2β bound to glutathione-agarose and interaction tested using Western blot analysis. As a negative control we used GST bound to glutathione-agarose. As seen in Figure 2, CK2β can interact directly with CK2α (panel 1, lane 3), TFIIA (panel 2, lane 3), RNAPII (panel 3, lane 3), TFIIF (panel 4, lane 3), and TBP (panel 7, lane 3). However, TFIIE (panel 5, lane 3) and TFIIB (panel 6, lane 3) do not interact with CK2β. The factors are not able to interact with the negative control GST. Also, CK2α cannot interact with TBP and TFIIB (data not shown).

Phosphorylation of TFIIA, TFIIF, and RNAPII by CK2 Increases the Formation of Complexes on the TATA box of Ad-MLP

GTFs TFIIA, TFIIF, and RNAPII were phosphorylated by CK2 holoenzyme in the presence of unlabeled ATP. Control experiments were performed using the catalytically inactive CK2αA^{156}. Complex formation was measured by gel retardation assays using as probe the Ad-MLP promoter. As seen in Figure 3A, increasing amounts of phosphorylated TFIIA (lanes 1–3) increases the TBP/IIA complex on the Ad-MLP promoter which is at least 14 fold with the highest amount of TFIIA used. An increment in the complex using TFIIA treated with the catalytically inactive CK2α is also seen but it is less, reaching a maximum of eightfold with the highest amount of TFIIA used (lanes 4–6). Figure 3B shows the IIA/TBP/IIB/IIF–RNAPII complex formation using phosphorylated TFIIF (lanes 3–5). This increase is at least twofold with the highest amount of phosphorylated TFIIF used in the assay. A greater increase is seen if the comparison is made to that seen with 5 ng nonphosphorylated TFIIF (lane 2). TFIIF treated with the catalytically inactive CK2α under similar conditions does not cause an increase in complex formation (lanes 6–8). Lanes 1 and 2 represent the complexes IIA/TBP/IIB and IIA/TBP/IIB/IIF–RNAPII, respectively. Figure 3C shows the results of complex formation using phosphorylated RNAPII. Here, the phosphorylation of RNAPII by CK2 holoenzyme (lanes 3–5) augments the formation of the complex IIA/TBP/IIB/IIF–RNAPII by at least sixfold compared with RNAPII phosphorylated with CK2 holoenzyme containing the catalytically inactive CK2α (lanes 6–8). Lanes 1 and 2 represent the complexes IIA/TBP and IIA/TBP/IIB, respectively.

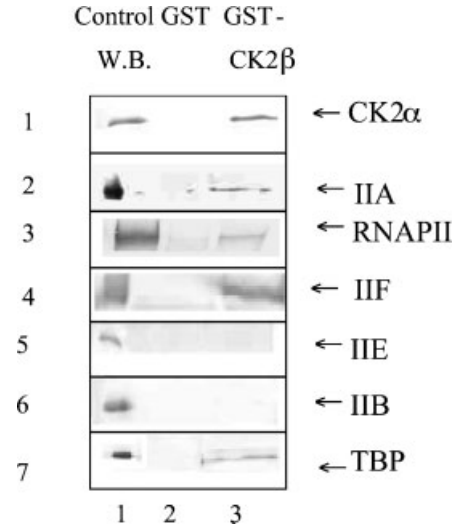
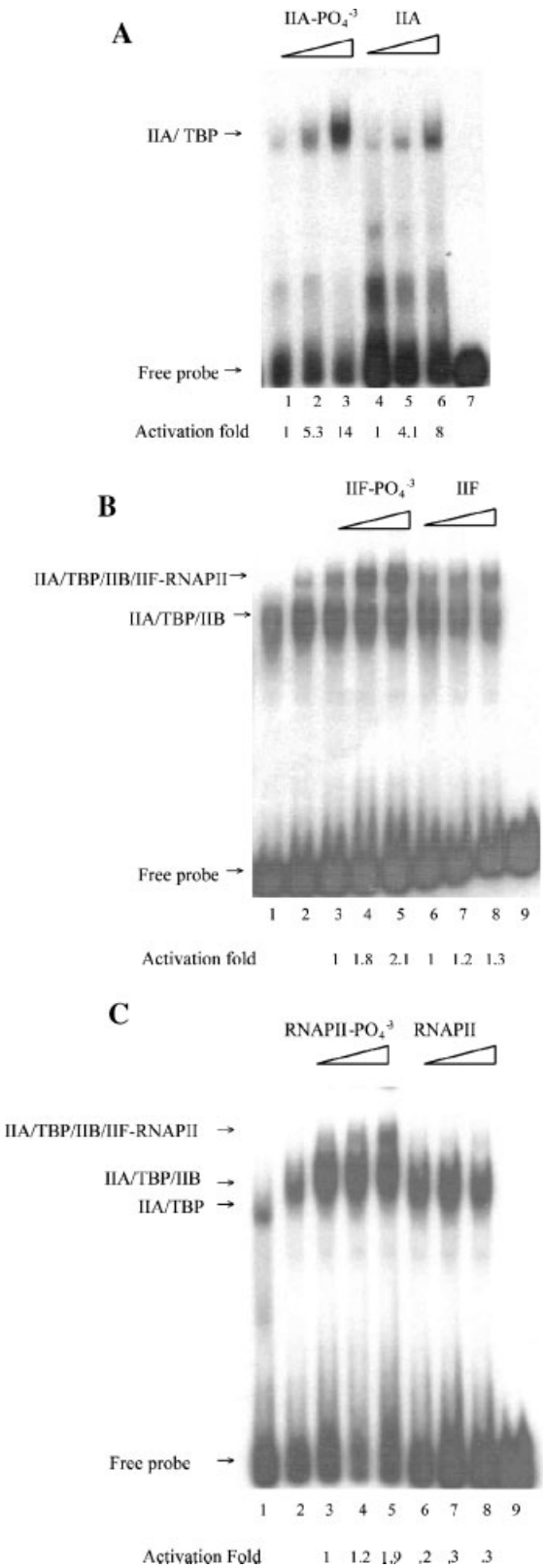


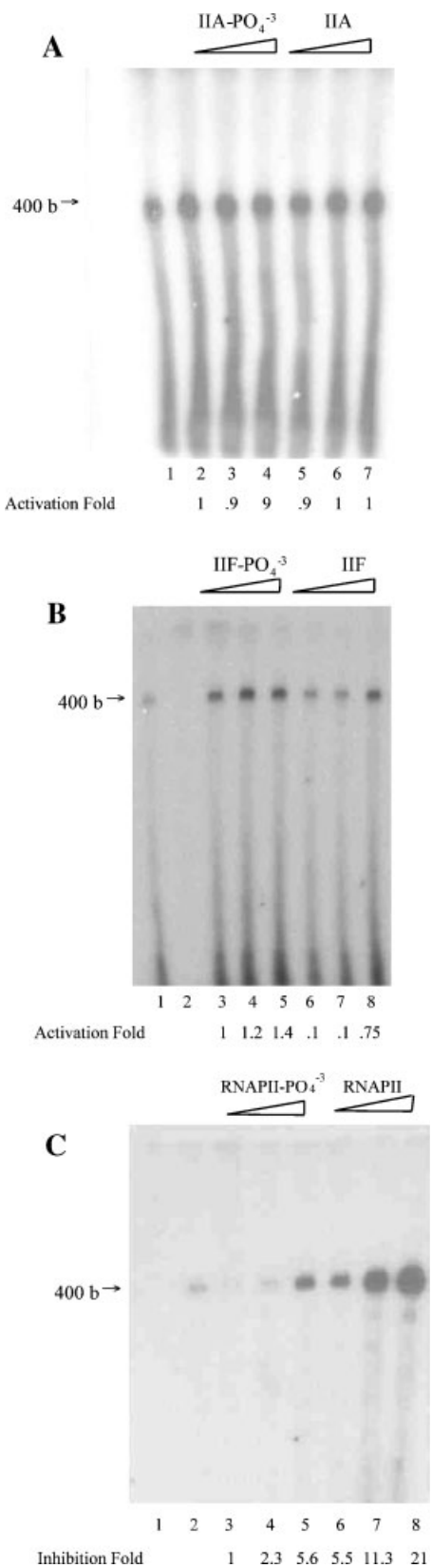
Fig. 2. Protein–protein interactions of CK2β with GTFs and RNAPII. GST-CK2β and GST were purified as described in Materials and Methods and immobilized on glutathione-agarose. Fifty microlitres of wet resin was incubated with 1 μg of each recombinant factor and 2 μg of RNAPII as described in Materials and Methods. Bound proteins were eluted with glutathione and analyzed by Western blot with affinity purified antibodies against each factor which are indicated in the figure. Lane 1 of each panel represents 1/10 of the input. Interactions with each recombinant factor and RNAPII are indicated in each panel.



Transcription Is Modified by Phosphorylation of TFIIF and RNAPII

Transcription assays to measure the effect of phosphorylation of GTFs and RNAPII were performed using the Ad-MLP promoter fused to a G-less cassette. We assayed separately each phosphorylated factor and RNAPII. As seen in Figure 4A, CK2 phosphorylation of TFIIA does not affect transcription (lanes 2–4). Also, TFIIA treated with catalytically inactive CK2α as holoenzyme does not cause any effect on transcription (lanes 5–7). The transcription assay is not dependent on TFIIA (lane 1). Phosphorylation of TFIIF by CK2 holoenzyme (Fig. 4B) causes an increase in the amount of transcripts formed (lanes 3–5). This increase is 10 fold compared with the transcription obtained with TFIIF treated with the inactive CK2α (compare lanes 3 and 4 with lanes 6 and 7). The assay is completely dependent on TFIIF (lane 2). We also analyzed the effect of phosphorylation by CK2 on the transcription activity of TFIIE, however, we did not detect any effect (data not shown). Next, we analyzed the effect of the phosphorylation of RNAPII. Phosphorylation of RNAPII by CK2 holoenzyme causes a dramatic inhibition on the formation of transcripts (Fig. 4C, lanes 3–5) compare with RNAPII phosphorylated with the catalytically inactive CK2α (Fig. 4C, lanes 6–8). The inhibition is at least fivefold as

Fig. 3. Effects of phosphorylation by CK2 of the GTFs and RNAPII on the formation of complexes on the TATA box of the Ad-MLP promoter. Gel retardations assays were performed with a labeled probe which was incubated with the different factors and RNAPII which had been previously phosphorylated with CK2 holoenzyme or holoenzyme formed using catalytically inactive CK2αA^{156}. **A**: **Lanes 1–3** contain 5 ng TBP, and 12, 25, and 50 ng, respectively, of phosphorylated TFIIA. **Lanes 4–6** contain the same amount of TBP as in lanes 1–3 and 12, 25, and 50 ng, respectively, of TFIIA phosphorylated with the catalytically inactive CK2αA^{156}. **Lane 7** contains the free probe. **B**: All the lanes contain 5 ng TBP, 25 ng TFIIA, 20 ng TFIIB and 500 ng RNAPII. **Lane 1** contains TBP, TFIIA, and TFIIB. **Lane 2** contains TBP, TFIIA, TFIIB and 5 ng of recombinant nonphosphorylated TFIIF. **Lanes 3–5** contain 10, 60, and 120 ng of phosphorylated TFIIF. **Lanes 6–8** contain TFIIF phosphorylated with the catalytically inactive CK2αA^{156}. **Lane 9** contains the free probe. **C**: **Lane 1** contains 5ng TBP, 25 ng TFIIA. **Lane 2** contains 5 ng TBP, 25 ng TFIIA and 20 ng TFIIB. **Lanes 3–5** contain 5 ng TBP, 25 ng TFIIA, 20 ng TFIIB, 30 ng TFIIF and 100, 250, and 500 ng of phosphorylated RNAPII. **Lanes 6–8** contain the same amount of TBP, TFIIA, TFIIB, and TFIIF as in lanes 3–5 and 100, 250, and 500 ng of phosphorylated RNAPII with the catalytically inactive CK2αA^{156}. **Lane 9** contains the free probe. Fold activation was calculated giving the unit fold to the specific retardation complex formed in the lowermost condition for each phosphorylated factor.



measured by phosphoimager analysis (compare lanes 4 and 7). The assay is dependent on RNAPII, since omission of the enzyme does not produce transcripts. Lane 2 represents a control with crude nuclear extract.

DISCUSSION

Several of the GTFs, RNAPII, and histones have multiple putatives phosphorylation sites for CK2. In this report, we have demonstrated that the human GTFs TFIIA, TFIIE, TFIIF, and RNAPII are phosphorylated by vertebrate CK2 holoenzyme. Also, TFIIA, TFIIF, TBP, and RNAPII can interact directly with CK2β. Phosphorylation of TFIIA, TFIIF, and RNAPII augments the formation of a complex as measured by gel retardation assays. Furthermore, we demonstrate that phosphorylation of TFIIF causes an increase in transcription, however, phosphorylation of RNAPII causes a decrease on transcription. Phosphorylation of TFIIA and TFIIE does not have an effect on transcription.

It has been shown that phosphorylated TFIIF purified from HeLa cells is more competent in the formation of a preinitiation complex as compared to recombinant TFIIF or when TFIIF has been treated with alkaline phosphatase [Kitajima et al., 1994]. Also transcription levels are higher when TFIIF is phosphorylated. This is possible due to a reduced interaction with RNAPII. Our results are in complete agreement

Fig. 4. Transcriptional effects of phosphorylation by CK2 on the factors TFIIA, TFIIF, and RNAPII. Reconstituted transcription assays were performed as described in Materials and Methods using the Ad-MLP promoter which directs the transcription of a G-less cassette. **A**: Effect on transcription of the phosphorylation of TFIIA. **Lane 1** contains 5 ng TBP, 20 ng TFIIB, 200 ng TFIIF, 100 ng TFIIE, 300 ng TFIIH, and 500 ng RNAPII. **Lanes 2–7** contain the same amount of GTFs and RNAPII as lane 1. Lanes 2–4 contain 12, 25, and 50 ng of phosphorylated TFIIA. Lanes 5–7 contain 12, 25, 50 ng of TFIIA phosphorylated with the catalytically inactive CK2αA^{156}. **B**: Effect on transcription of the phosphorylation of TFIIF. **Lane 1** contain nuclear extract. **Lane 2** contain 5 ng TBP, 20 ng TFIIB, 100 ng TFIIE, 300 ng TFIIH, and 500 ng of RNAPII. **Lanes 3–5** contain 10, 60, 120 ng of phosphorylated TFIIF. **Lanes 6–8** contain 10, 60, 120 ng of TFIIF phosphorylated with the catalytically inactive subunit of CK2αA^{156}. **C**: Effect on transcription of the phosphorylation of RNAPII. **Lane 1**: minus RNAPII; **lane 2** contains nuclear extract. **Lanes 3–5** contain 100, 200, and 500 ng of phosphorylated RNAPII. **Lanes 6–8** contain 100, 200, and 500 ng of RNAPII phosphorylated with the catalytically inactive CK2αA^{156}. Fold activation or inhibition was calculated giving the unit fold to the specific retardation complex formed in the lowermost condition for each phosphorylated factor.

with these studies. It has also been reported that the largest subunit of TFIIF possesses a serine and threonine protein kinase activity which autophosphorylates a serine in position 385 and threonine in position 389 [Rossignol et al., 1999]. It is indicated in this report that TFIIF undergoes autophosphorylation and that autophosphorylated TFIIF is more competent in transcription elongation. These authors demonstrated that TFIIF is phosphorylated by exogenous CK2 in positions 207–230, 271–283, and 335–344. In contrast with these previous findings, no autophosphorylation of TFIIF was seen with our recombinant factor when tested in conditions of phosphorylation and in the presence of kinase-inactive CK2α. The present work suggests that the reported autophosphorylation [Rossignol et al., 1999] may have occurred because of the binding of endogenous CK2 or CK2-like activity bound to the TFIIF in the HeLa cell system. It has also been reported that TFIIF and CK2α colocalize with transcribing RNAPII in chromosomes of *Chironomus* salivary glands [Egyházi et al., 1999]. This association is sensitive to DRB, an inhibitor of CK2 phosphorylation. However, the functional consequence of TFIIF phosphorylation by CK2 has not been defined. We addressed this question using human recombinant TFIIF phosphorylated by recombinant CK2 and demonstrated that this modification has positive effects both on transcription complex formation and transcription. These results suggest that CK2 plays a positive role on transcription initiation by RNAPII possible due to the stabilization of the transcription complex.

The largest subunit of TFIIA in yeast (also designated Toa1) is phosphorylated at serine residues 220, 225, and 232 [Solow et al., 1999]. Alanine substitution of these three residues completely eliminated phosphorylation in vivo of Toa1, however the yeast are viable. Phosphorylated TFIIA is at least 30-fold more efficient in the formation of a TBP/IIA/DNA complex. Recombinant yeast TFIIA can be stimulated to form the complex formation by previous phosphorylation with CK2. Substitution of serines residues 220, 225, and 232 for alanine can be synthetically lethal when combined with an alanine substitution at tryptophan 285 which disrupts the TFIIA–TBP interface. These results suggest that phosphorylation of TFIIA may be important in transcription. Our results using human TFIIA are in agreement with this observations, since phosphorylation of the largest subunit of human TFIIA by CK2 can stimulate the formation of a IIA/TBP/DNA complex, however it has no effect on transcription possibly due to the fact that TFIIA is not required in transcription assays reconstituted with TBP. However, we do not rule out the possibility that phosphorylation by CK2 of human TFIIA in vivo has functional effects on transcription.

Although TFIIE is phosphorylated in vitro by CK2, this phosphorylation has no effect on the transcription activity. This result suggest that in vitro phosphorylation of TFIIE by CK2 is not required for transcription. Again, it is possible that in vivo phosphorylation by CK2 plays a positive role on transcription by RNAPII.

A number of protein kinases can phosphorylate the CTD of RNAPII [Dahmus, 1996; Bregman et al., 2000]. The CTD has the sequence consensus YSPTSPS and is essential, since its deletion in yeast causes cell death. RNAPII is phosphorylated by CK2 in the subunits of 214,000 and 20,500 daltons [Dahmus, 1981]. One site of phosphorylation in the largest subunit is in a 10 residue motif present in the CTD [Fong et al., 2003]. This motif is phosphorylated in vitro by CK2 and it is essential for transcription, splicing and poly(A) site cleavage. However, mutation in the serine residues phosphorylated by CK2 has no effect on mRNA processing. However, such mutations were not tested in transcription. Indeed, stronger transcription and processing are observed when the serine residues phosphorylatable by CK2 are mutated, a finding which may indicate that phosphorylation by CK2 exerts a negative effect on transcription and mRNA processing. It is most probable that the largest subunit of RNAPII can also be phosphorylated by CK2 elsewhere in the polypeptide, since it contains several CK2 consensus sequences. We assayed the effect of phosphorylation by CK2 on RNAPII and we found that phosphorylated RNAPII is more competent in the formation of transcription complexes on the Ad-MLP promoter, however, this phosphorylation dramatically inhibits transcription. This result strongly suggests that phosphorylation prior to initiation of transcription inhibits the elongation properties of RNAPII, since the formation of complexes is augmented when RNAPII is phosphorylated by CK2. The increased initiation complex formation introduced in RNAPII

by CK2 phosphorylation may reflect the presence of a required check-point before elongation. Alternatively, in vivo this inhibitory effect can be counterattacked by phosphorylation of RNAPII by other kinases that phosphorylate the CTD. Our in vitro results could thus explain in vivo observations related to silencing chromatin and the repression of heat shock promoters [Rougvie and Lis, 1988; Sekinger and Gross, 2001]. It is possible that in these situations the transcription complex is inactive due to the phosphorylation of RNAPII by CK2 and the transition from an inactive complex to an active one could be trigger by the phosphorylation of RNAPII by other kinases.

Also, ours results demonstrate that CK2β can interact with TFIIA, TBP, TFIIF, and RNAPII, suggesting that CK2 may be targeted to the promoters by protein–protein interaction and may form part of the PIC in vivo. In conclusion, ours results strongly suggest that CK2 regulates the process of transcription by RNAPII and it can be a key regulatory event in the expression of protein coding genes.

ACKNOWLEDGMENTS

We are grateful to Dr. Jorge E. Allende for his critical review of the manuscript and to Dr. Danny Reinberg who provided highly purified human factor IIH. We acknowledge Evelyn Tamayo for her excellent technical assistance.

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