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Casein Kinase 2 Associates with Initiation-Competent RNA Polymerase I and Has Multiple Roles in Ribosomal DNA Transcription

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Mammalian RNA polymerase I (Pol I) complexes contain a number of associated factors, some with undefined regulatory roles in transcription. We demonstrate that casein kinase 2 (CK2) in human cells is associated specifically only with the initiation-competent Pol Iβ isoform and not with Pol Iα. Chromatin immunoprecipitation analysis places CK2 at the ribosomal DNA (rDNA) promoter in vivo. Pol Iβ-associated CK2 can phosphorylate topoisomerase IIα in Pol Iβ, activator upstream binding factor (UBF), and selectivity factor 1 (SL1) subunit TAF_I110. A potent and selective CK2 inhibitor, 3,8-dibromo-7-hydroxy-4-methylchromen-2-one, limits in vitro transcription to a single round, suggesting a role for CK2 in reinitiation. Phosphorylation of UBF by CK2 increases SL1-dependent stabilization of UBF at the rDNA promoter, providing a molecular mechanism for the stimulatory effect of CK2 on UBF activation of transcription. These positive effects of CK2 in Pol I transcription contrast to that wrought by CK2 phosphorylation of TAF_I110, which prevents SL1 binding to rDNA, thereby abrogating the ability of SL1 to nucleate preinitiation complex (PIC) formation. Thus, CK2 has the potential to regulate Pol I transcription at multiple levels, in PIC formation, activation, and reinitiation of transcription.

The major structural and catalytic components of the protein synthesis machinery, the 18S, 5.8S, and 28S ribosomal RNAs, are transcribed by RNA polymerase I (Pol I). Two forms of the multisubunit Pol I enzyme complex, both >1 MDa, can be extracted from human cell nuclei: Pol Iα, comprising the bulk of Pol I, which can direct random RNA synthesis; and Pol Iβ, accounting for the remaining 10%, which directs ribosomal DNA (rDNA) promoter-driven specific transcription (34). Our mass spectrometry analysis revealed the presence of a number of associated factors, distinct from the core subunits, specific to either Pol Iα or Pol Iβ (34). These associated factors are likely to integrate the fundamental rRNA synthesis function of the enzyme at the rDNA chromatin with other cellular processes. One such associated factor is the Pol Iβ-specific hRRN3 (mouse equivalent, TIF-IA) (34), which forms the crucial link between this initiation-competent Pol I complex and essential transcription factor selectivity factor 1 (SL1), a complex of TATA-binding protein (TBP) and three or more Pol I-specific TBP-associated factor (TAF_I) proteins (8, 9, 54). SL1 directs Pol I to the rDNA core promoter, and together these complexes are necessary and sufficient for promoter-specific Pol I transcription in a reconstituted transcription assay (basal transcription) (14). SL1 also stabilizes binding at the rDNA promoter of the Pol I transcription activator upstream binding factor (UBF) (14). SL1 and UBF interact cooperatively to support efficient initiation of transcription by Pol I (4, 24, 27).

This study focuses on another Pol I-associated factor, serine-threonine kinase casein kinase 2 (CK2) (also known as CKII and formerly known as nuclear kinase II). CK2 is present in the nucleolus, the site of ribosome biogenesis (15, 42), and copurifies with mammalian Pol I (3, 12, 45). Pol I transcription is tightly controlled, responding to the protein synthesis requirements of the cell, upregulated by the effectors of growth factor and nutrient-responsive signaling pathways, subject to cell cycle control in mammalian systems, and responsive to cellular stress-activated signaling pathways (17, 30, 35, 46). An increased level and activity of CK2 also correlate with cell growth and proliferation (2, 29, 32, 43). CK2 copurifies with Pol I complexes from broccoli (47), frogs (1), and rats, where it was proposed to phosphorylate the largest subunit of Pol I (18). Despite the intriguing association of CK2 with Pol I, a role(s) for this polymerase-associated CK2 in Pol I transcription remained to be resolved. Besides this association of CK2 with Pol I, recombinant CK2 in vitro can phosphorylate the carboxy-terminal domain of UBF (36, 53), which contributes to its activation function (24), and mutation of CK2-phosphorylated serine residues in this domain impairs the ability of UBF to activate transcription (52). The mechanism by which CK2 phosphorylation stimulates UBF to activate transcription was unknown.

Here we present evidence that the kinase activity that copurifies with Pol I from human cells is CK2 and, intriguingly, that CK2 is specifically associated with the initiation-competent Pol Iβ complex and is located at the rDNA gene in vivo, primarily at the promoter. Our data suggest that CK2 is required for efficient reinitiation of transcription by Pol I. Furthermore, Pol Iβ-associated CK2 phosphorylation can enhance the stability

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of UBF in the preinitiation complex (PIC), thereby increasing the activation potential of UBF and upregulating transcription. However, CK2 can also decrease the ability of SL1 to bind the rDNA promoter, thereby downregulating PIC formation and transcription. We therefore propose that CK2 functions to regulate Pol I transcription at multiple levels.

MATERIALS AND METHODS

RNA polymerase I, SL1, UBF, topoisomerase IIα (TopoIIα), and CK2. Human RNA Pol Iα and -β and SL1 were purified from HeLa cell nuclear extracts as described previously (14, 34). Recombinant human UBF (UBF1) was purified from Sf9 insect cells infected with recombinant baculoviruses (14). Human topoisomerase IIα and recombinant human CK2 holoenzyme were from TopoGEN and New England Biolabs, respectively.

Kinase assays, phosphorylations, and inhibitors. Kinase assay reaction mixtures contained 5 to 7 μM ATP and 2 μCi of [γ-^{33}P]ATP (3,000 Ci/mmol) in TM10i/0.05 buffer (50 mM Tris-HCl pH 7.9, 12.5 mM $MgCl_2$, 1 mM EDTA, 10% [vol/vol] glycerol, 0.05 M KCl, 0.015% [vol/vol] NP-40, 1 mM dithiothreitol, 1 mM metabisulfate, 10 μg/ml bovine serum albumin) and either human Pol Iβ or recombinant human CK2 holoenzyme. Reactions were incubated at 30°C for 15 to 30 min and stopped by addition of LDS protein sample buffer (Invitrogen). After incubation at 70°C for 10 min, samples were resolved on 4 to 12% gradient bis-Tris polyacrylamide gels (Invitrogen), and phosphorylated proteins were detected by autoradiography or phosphorimage analysis (FujiFilm phosphorimager FLA5100).

CK2-specific inhibitors 4,5,6,7-tetrabromo-2-azabenzimidazole (TBB) (Calbiochem) and the potent and highly selective CK2 inhibitor 3,8-dibromo-7-hydroxy-4-methylchromen-2-one (DBC), a kind gift from L. Pinna (31, 38), were dissolved in dimethyl sulfoxide (DMSO) and used at concentrations of 10 to 100 μM.

CK2 phosphorylation of recombinant UBF. Recombinant Flag-UBF was purified from baculovirus-infected insect cells following the same purification procedure as for untagged UBF, as described previously (14). A 1.6-μg amount of this purified Flag-UBF was incubated for 25 min at 30°C with or without 500 U of recombinant human CK2 holoenzyme (New England Biolabs) and 0.5 mM ATP in a 50-μl TM10/0.05 buffer. A 100-μl volume of anti-Flag antibody Sepharose beads (Sigma), equilibrated in TM10/0.05 buffer, was added to the phosphorylation reactions, and incubation was continued for 1 h at 4°C with continuous mixing. Beads were washed four times with 200 μl of TM10/1.0 buffer to remove the CK2. UBF or CK2-phosphorylated UBF (CK2-P-UBF) was eluted with Flag-peptide (Sigma) according to the manufacturer's instructions in 50-μl total volumes. The eluates were further purified (and desalted) on a G50 spin column (Amersham Biosciences) equilibrated in TM10/0.1 buffer according to the manufacturer's instructions. Protein concentration was determined by the Bradford assay (Bio-Rad).

Antibodies for immunoblotting and immunoprecipitation. hRRN3-specific rabbit polyclonal antibodies, raised against His-hRRN3 (purified from recombinant baculovirus-infected insect cells through the His tag), were affinity purified on an *N*-hydroxysuccinimide-activated HiTrap HP column coupled to purified His-hRRN3 (Amersham Biosciences). For immunoprecipitations, affinity-purified RRN3-specific antibodies or control immunoglobulin G (IgG) (Sigma) was bound to protein A or G paramagnetic beads (Dynal). After three washes in TM10/0.05 buffer (as TM10i buffer, except no bovine serum albumin), Pol Iβ was added and the beads were incubated for 2 h with rotation at 4°C. The beads were then washed extensively in TM10i/0.05 and TM10/0.1. Immunocomplexes were eluted with LDS sample loading buffer and analyzed by immunoblotting. Antibodies used for immunoblotting and/or (chromatin) immunoprecipitation were specific for hRRN3 (rabbit polyclonal antibody against His-hRRN3), hRRN3 (sheep polyclonal against hRRN3 peptides) (34), CK2α subunit (rabbit polyclonal), CK2β subunit (mouse monoclonal; Calbiochem), TopoIIα (rabbit polyclonal; Biotrend), TAF_Is (rabbit polyclonals [9, 54]); UBF (rabbit polyclonal); A190 (largest Pol I subunit) and AC19 (shared Pol I and III subunit) (34); and Pol I second-largest subunit A135/A127 and Pol I-associated factor of 53 kDa (PAF53) (Santa Cruz Biotechnologies). Secondary antibodies were conjugated to horseradish peroxidase (Jackson ImmunoResearch Laboratories), and detection of immunocomplexes was by chemiluminescence (Amersham Biosciences).

In vitro transcription. In vitro Pol I transcription assays with human rDNA promoter fragments (Fr4, −193 to +239), immobilized Fr4 (IT-rDNA) (39), and nonspecific transcription assays with sheared calf thymus DNA (ctDNA) (which yields transcripts of ~500 nucleotides long and therefore measures transcript elongation in vitro) were performed as described previously (34, 39). Specific transcription was analyzed by S1 nuclease protection (34, 39).

Nucleolar chromatin immunoprecipitation. Nucleoli were prepared from formaldehyde-treated HEK293 cells (at ~75% confluence for 10 min with 1% fresh formaldehyde, after which the reaction was stopped with 0.2 M glycine for 5 min) as described previously (37), with the following modifications. Nucleoli were released by sonication in a Bioruptor sonication bath (Diagenode) using three runs of 5 min (30-s pulses, 30-s intervals) in ice water. Nucleoli were pelleted by centrifugation (microcentrofuge at 15,000 × *g* for 1 min), and the sonication procedure was repeated. After the nucleolar structure was disrupted (as determined under the microscope), high-molecular-weight DNA was then sonicated for nine runs of 5 min (30-s pulse and 30-s intervals). This procedure resulted in the majority of fragments in the 250- to 300-bp size range. The resulting sheared nucleolar chromatin was analyzed in nucleolar chromatin immunoprecipitation (ChIP) assays as described previously (37). The following antibodies were used: antibodies specific for CK2α (a generous gift from Nouria Hernandez); the second-largest subunit of Pol I, RPA135 (also known as A127) (Santa Cruz Biotechnology); anti-TAF_I63 and anti-TAF_I110; and sheep or rabbit IgG (both from Sigma) as controls. Protein A or Protein G paramagnetic beads (Dynal) were used for immunoprecipitation reactions. Eluted immunoprecipitated material was deproteinated and concentrated as described previously (37), and the resulting DNA was used in quantitative real-time PCR.

Immunoprecipitated materials were analyzed by quantitative PCR with primers specific for the promoter regions P1 (42787 to 42993) and P2 (42837 to 42993), the transcribed regions Tr1 (4501 to 4700; 18S) and Tr2 (12100 to 12304; 28S), and the intergenic spacer (35822 to 36031) (numerations according to the complete human rDNA repeat sequence, GenBank accession number U13369). Each PCR of 20 μl contained 10 μl of the SYBR GREEN PCR Master Mix (Applied Biosystems), 10 pmol of each primer, and 1 μl of immunoprecipitated chromatin. No DNA and a titration of input chromatin (50, 5, 0.5, or 0.05 ng) were included for each primer set. Reactions were performed and monitored in the Applied Biosystems 7300/7500 real-time PCR system. The 7000 System SDS software was used for data analysis.

RESULTS

Pol I-associated kinase activity is present in highly purified initiation-competent Pol Iβ. We first sought to identify the kinase activity associated with human Pol I complexes. Kinase activity was detected in fractions containing initiation-competent Pol Iβ, which supports rDNA promoter-specific transcription (Fig. 1A, fractions 21 to 25). Since the kinase activity profile was identical to that obtained when Pol Iβ fractions were assayed using exogenously added substrate (UBF; data not shown), the autophosphorylation data reflect the distribution of the kinase activity (and not only that of the substrate) over the Pol Iβ fractions. Interestingly, in Pol Iβ a single predominant protein of ~180 kDa was phosphorylated by the Pol Iβ-associated kinase (Fig. 1B, lane 2). By contrast, no phosphorylated proteins were detected in Pol Iα-containing fractions in autophosphorylation reactions (Fig. 1B, lane 1).

CK2 is a component of human Pol Iβ complexes. The human Pol Iβ-associated kinase is able to utilize GTP and ATP as phosphate donors (data not shown), which is characteristic of CK2 (44). The "classic" inhibitors of CK2, heparin and a CK2 substrate peptide, down-regulated the kinase activity of Pol Iβ (Fig. 1C), further suggesting that the Pol Iβ-associated kinase was likely to be CK2. Importantly, a novel potent and selective CK2 inhibitor, DBC (31, 38), and the highly selective inhibitor TBB (31, 48) both inhibited the kinase activity in Pol Iβ in a dose-dependent manner (Fig. 1C). The presence of CK2 in human Pol I complexes is consistent with evidence to suggest that the Pol I-associated kinase from rats, *Xenopus*, and broccoli is CK2 (1, 18, 47). CK2 is a tetrameric complex comprised of two highly related catalytic subunits, α and/or α′, tightly associated with a dimer of regulatory subunits, β (or β′) (29).

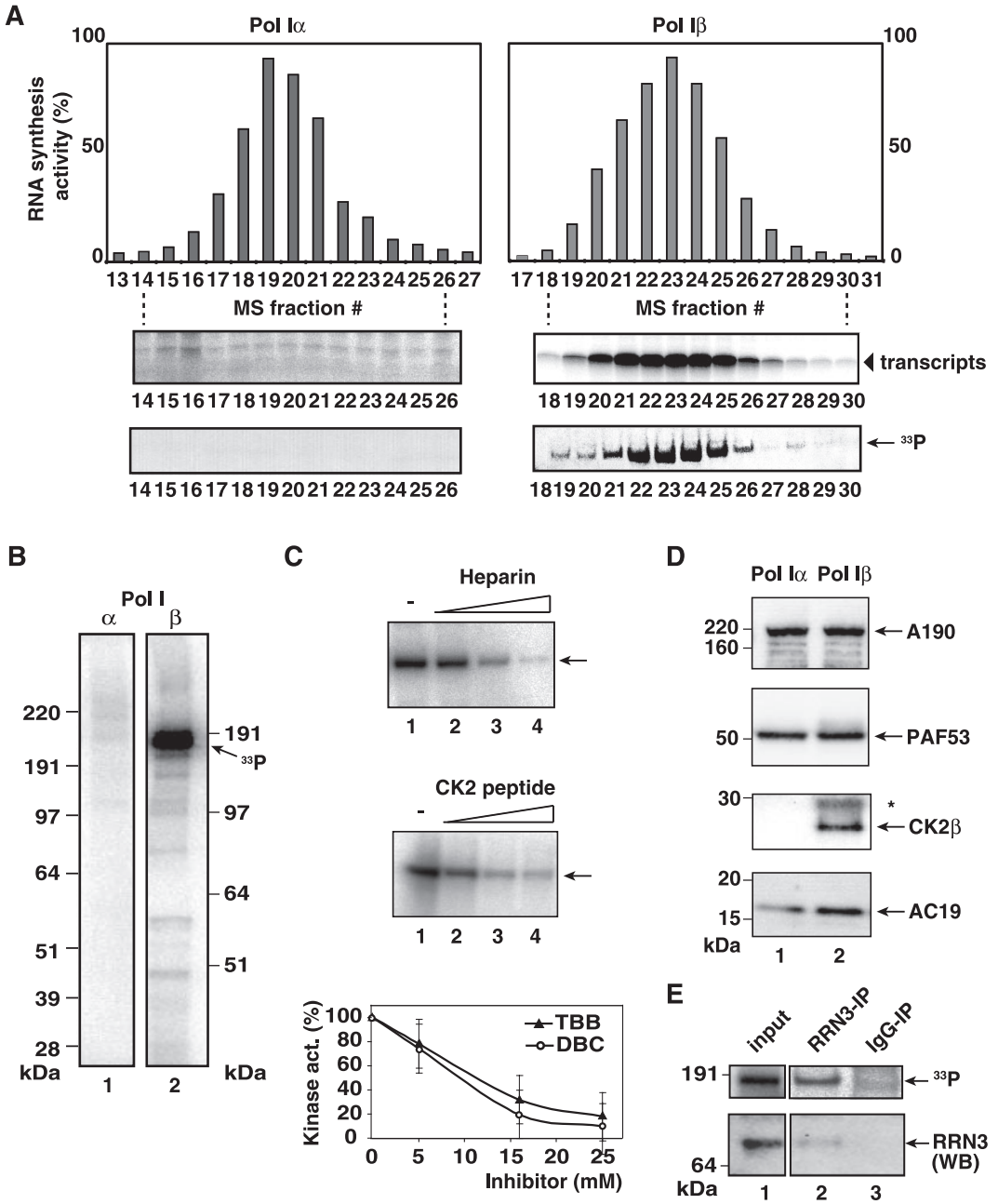

FIG. 1. CK2 is specifically associated with initiation-competent Pol Iβ. A. Kinase activity copurifies with Pol Iβ. Fractions from the Mono S (MS) column for Pol Iα and for Pol Iβ (a final step in the purification of Pol I complexes from HeLa nuclei [34]) were assayed in a nonspecific transcription assay and the activities expressed as a percentage of maximal transcription activity for each form of Pol I. The same fractions were assayed in a reconstituted rDNA promoter-specific transcription assay with SL1 (arrowhead; transcripts). The fractions were assayed for kinase activity in autophosphorylation reactions (arrow; ^{33}P). B. The Pol Iβ-associated kinase phosphorylates a single predominant protein. The Mono S fractions containing the peak transcription activities of Pol Iα and β were pooled and analyzed in autophosphorylation reactions. Phosphorylated polypeptides were visualized by phosphorimaging. C. The Pol Iβ-associated kinase is CK2. CK2 inhibitors heparin (0, 1, 5, or 10 μg; lanes 1 to 4), CK2 peptide RRREEETEEE (0, 1, 5, or 10 ng; lanes 1 to 4), DBC (0, 5, 10, 15, 20, and 25 μM), and TBB (0, 5, 10, 15, 20, and 25 μM) were analyzed for their effect on Pol Iβ-associated kinase activity in autophosphorylation reactions. For DBC and TBB, the data were quantitated and expressed as percentages of the phosphorylation activity detected in the absence of the inhibitors (set at 100%). The bars represent the standard error. D. CK2 subunit β is detectable in Pol Iβ. Pol Iα (lane 1) and Pol Iβ (lane 2) immunoblots were probed with antibodies specific to Pol I subunit A190, PAF53, or AC19 or to CK2 subunit β. Nonspecific band marked by asterisk. E. Kinase activity coimmunoprecipitates with RRN3 in Pol Iβ. Pol Iβ (3 μl) was immunoprecipitated with affinity-purified rabbit polyclonal RRN3-specific antibodies. RRN3-immunoprecipitated Pol Iβ (RRN3-IP; lane 2), a control IgG immunoprecipitation (IgG-IP; lane 3), or 2 μl of Pol Iβ (Input; lane 1) was assayed in an autophosphorylation assay (^{33}P) and with Western blotting (WB). Phosphorylated protein was visualized as in panel A.

As with the catalytic subunit CK2α, the regulatory subunit CK2β was detected specifically in Pol Iβ and not in Pol Iα (Fig. 1D). CK2 subunits were not detectable on silver-stained protein gels that showed several Pol Iβ subunits, suggesting that CK2 is present in substoichiometric amounts in Pol Iβ (data not shown). This was also suggested by a comparison of kinase activities of Pol Iβ-associated CK2 and of recombinant CK2 of known specific activity, which suggested that active CK2 was present in only 10 to 20% of the Pol Iβ complexes (data not shown). The kinase activity and substrate coprecipitated with the RRN3 component of Pol Iβ (Fig. 1E), consistent with the possibility that both are components of Pol Iβ. Taken together, the data suggest that CK2 activity is the kinase activity specifically associated with initiation-competent Pol Iβ in human cells.

CK2 is at the rDNA promoter and throughout the rDNA in cells. To test whether Pol Iβ-associated CK2 is at the rDNA in vivo, we performed chromatin immunoprecipitations using antibodies specific for CK2α. CK2α was present at the rDNA promoter and to some extent throughout the rDNA, as determined by quantitative real-time PCR (Fig. 2A). In comparison, the TAF_I110 subunit of SL1 was located exclusively in the promoter region of the rRNA genes (Fig. 2B), and the second-largest subunit of Pol I (A135/A127) was distributed throughout the rDNA (Fig. 2C). CK2 is therefore suitably poised at the rDNA to regulate Pol I transcription in vivo.

Topoisomerase IIα is a substrate for Pol Iβ-associated CK2 in Pol I. Pol Iβ-associated CK2 phosphorylated a single predominant protein of ~180 kDa in human Pol Iβ (Fig. 1B, lane 2). The substrates for CK2 in the rat Pol I complex were proposed to include the largest (A194) (18) and perhaps the second-largest subunit (~120 kDa) of Pol I (45). Intriguingly, when we analyzed phosphorylation of proteins in human Pol Iα by added recombinant CK2 and compared this to phosphorylation of proteins of the Pol Iβ complex by the Pol Iβ-associated CK2, the substrate for CK2 was present only in Pol Iβ (Fig. 3A, compare lanes 4 and 5 or lanes 7 and 9), even though both complexes contain the human equivalent (A190) of rat Pol I A194 (Fig. 3A, lanes 1 and 2) and A127 subunits (A127/A135). Moreover, the ~180-kDa substrate for CK2 in Pol Iβ did not comigrate with the human A190 protein (Fig. 3A, compare lanes 1 and 4). Our data suggest a substrate for CK2 in human Pol Iβ other than the largest or second-largest subunits of Pol I (A190 or A135/A127, respectively).

Mass spectrometry analysis revealed the presence of topoisomerase IIα specifically in the Pol Iβ complex (K. Panov, J. Andersen, M. Mann, and J. Zomerdijk, unpublished results). The ~180-kDa protein phosphorylated by Pol Iβ-associated CK2 comigrated with CK2-phosphorylated recombinant TopoIIα (Fig. 3A, lanes 4 and 6 and lanes 7 and 8). The association of TopoIIα with Pol Iβ specifically, and not Pol Iα, was confirmed by immunoblotting (Fig. 3B). Thus, the target of Pol I-associated CK2 in Pol Iβ is likely to be TopoIIα. Addition of recombinant CK2 to Pol Iβ enhanced the phosphorylation of TopoIIα but did not significantly enhance phosphorylation of other proteins (Fig. 3C, lane 5).

The CK2 protein was not detected in Pol Iα immunoblots (Fig. 1D). Nonetheless, it was feasible that Pol Iα possessed CK2 kinase activity that was undetectable, since, as demonstrated, Pol Iα lacks TopoIIα or indeed any other substrate for CK2 (Fig. 3A, lane 9, and 2B, lane 2). However, when the CK2-substrate TopoIIα was incubated with Pol Iα under kinase assay conditions, no phosphorylation of TopoIIα was observed (Fig. 3C, lane 3). Therefore, the data demonstrate that Pol Iα contains neither a substrate for CK2 nor CK2 kinase activity and that Pol Iβ-associated CK2 can phosphorylate TopoIIα in this complex.

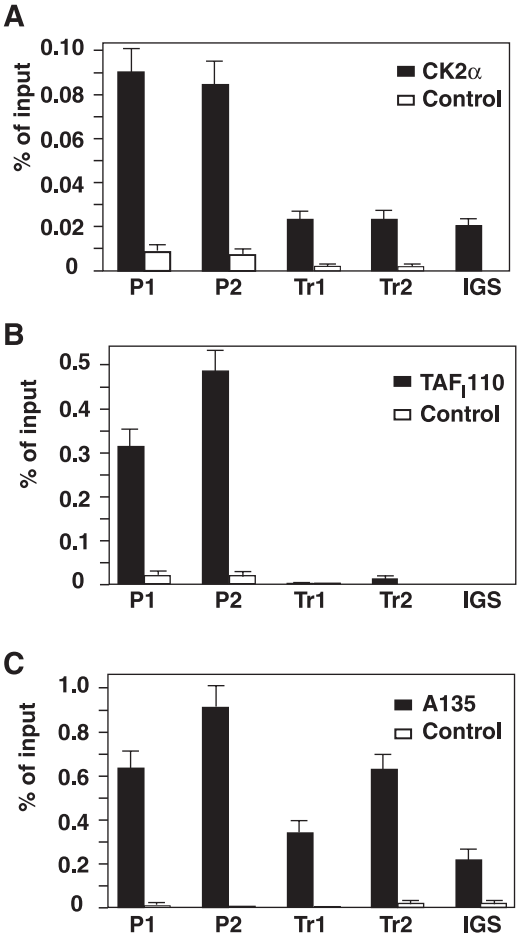

FIG. 2. ChIP analysis indicates that CK2α is present at the rDNA promoter and to some extent throughout the rDNA repeat in cells. Chromatin immunoprecipitation (from HEK293 cells) with antibodies specific to CK2α (A), specific to TAF_I110 of SL1 (B), or specific to the A135 subunit of Pol I (C) or the corresponding sheep or rabbit IgG controls, followed by quantitative real-time PCR with primers specific for the promoter region (P1 and P2), transcribed regions Tr1 (18S gene), or Tr2 (28S gene) and the intergenic spacer (IGS). The data, expressed as percentages of input chromatin, are from two independent experiments.

Inhibition of CK2 activity limits multiround transcription by Pol I. The association of CK2 with Pol Iβ and with rDNA in cells implicates this kinase in Pol I transcription regulation. Indeed, inhibition of CK2 in human cells (with TBB) affects Pol I transcription (data not shown), but we could not be certain that this was a direct effect. To dissect the mechanism(s) by which CK2 might regulate Pol I transcription, we therefore assessed the effect of CK2 inhibition in reconstituted transcription reactions. A widely used competitive inhibitor of CK2, the phospho-acceptor peptide (RRREEETEEE), inhib-

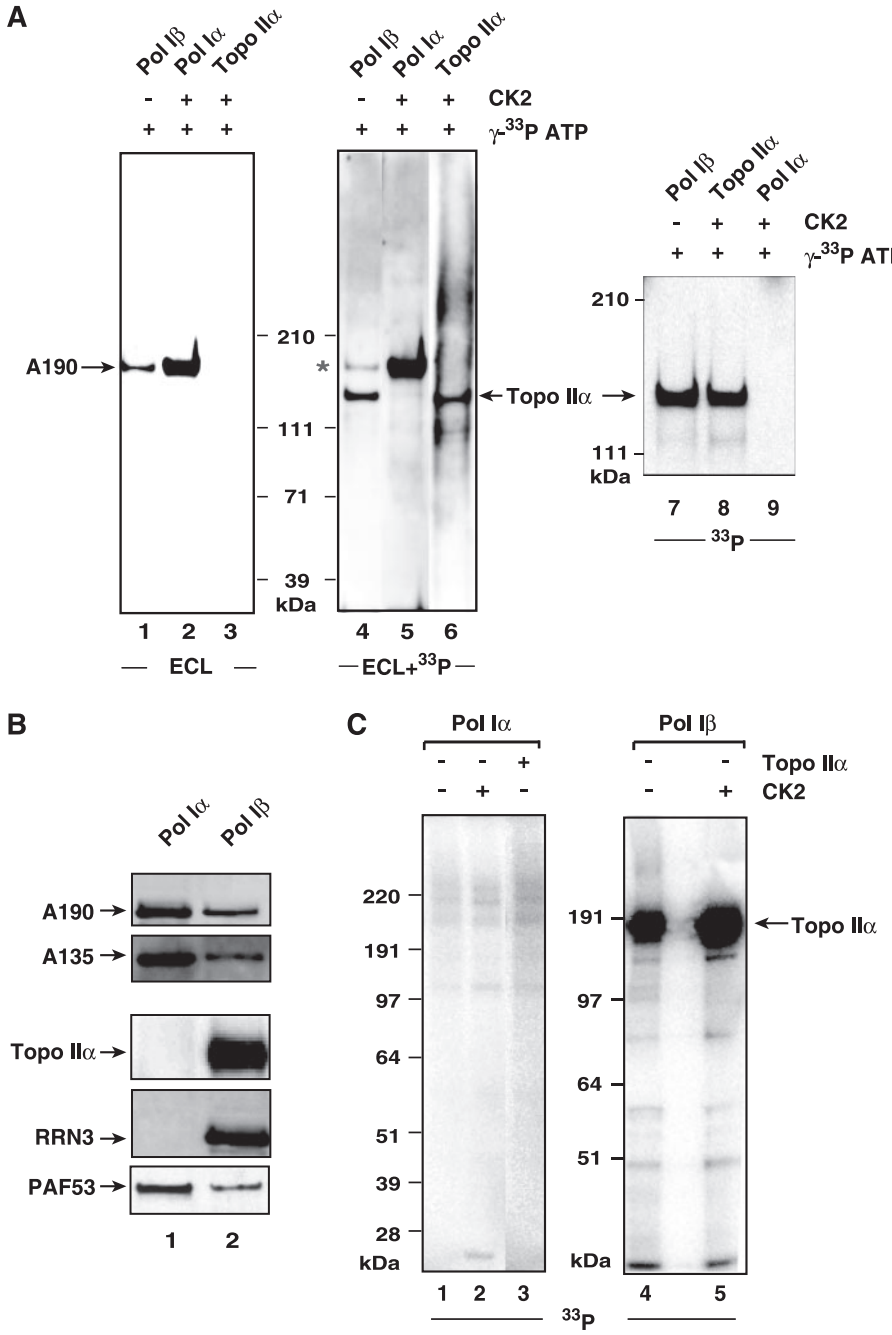

FIG. 3. TopoIIα, rather than the largest Pol I subunit, A190, is the substrate for Pol Iβ-associated CK2 in Pol Iβ. A. Pol Iβ (lanes 1, 4, and 7), Pol Iα (lanes 2, 5, and 9), and TopoIIα (lanes 3, 6, and 8) were incubated in the presence of [γ-^{33}P]ATP for 15 min at 30°C. Pol Iα and TopoIIα reactions were supplemented with 25 U of recombinant CK2. Proteins were separated by Tris-acetate sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Invitrogen) and immunoblotted using A190-specific antibodies. After immunodetection (ECL panel), phosphorylated proteins were detected by autoradiography under conditions where a residual ECL signal from immunodetection of A190 (asterisk) was detectable (ECL+^{33}P panel) or was undetectable (^{33}P panel). B. TopoIIα is present in Pol Iβ and not in Pol Iα. Pol Iα (lane 1) and Pol Iβ (lane 2) complexes were immunoblotted with antibodies specific for the largest (A190), the second-largest (A135), or the PAF53 core Pol I subunit or with TopoIIα- or RRN3-specific antibody. C. Pol Iβ-associated kinase phosphorylates the same substrate as exogenous CK2 in Pol Iβ, whereas Pol Iα contains neither CK2 enzyme activity nor a substrate for CK2. Pol Iα was incubated, in the absence (lanes 1 and 3) or presence (lane 2) of CK2 and in the absence (lanes 1 and 2) or presence of TopoIIα (lane 3) with [γ-^{33}P]ATP for 30 min at 30°C. Pol Iβ was incubated, in the absence (lane 4) or presence (lane 5) of CK2 with [γ-^{33}P]ATP for 30 min at 30°C. De novo phosphorylated proteins were visualized by phosphorimaging.

ited promoter-specific Pol I transcription (Fig. 4A), which correlated with inhibition of the kinase activity in CK2 (see Fig. 1C), but this block in transcription was independent of any effect on CK2 activity, since the peptide also repressed non-specific Pol I transcription by Pol Iα (Fig. 4B), which did not contain CK2 (Fig. 1 and 3). This peptide was therefore unsuitable for studies of the role of CK2 in Pol I transcription. By contrast, the selective CK2 inhibitor DBC (Fig. 1C) did not

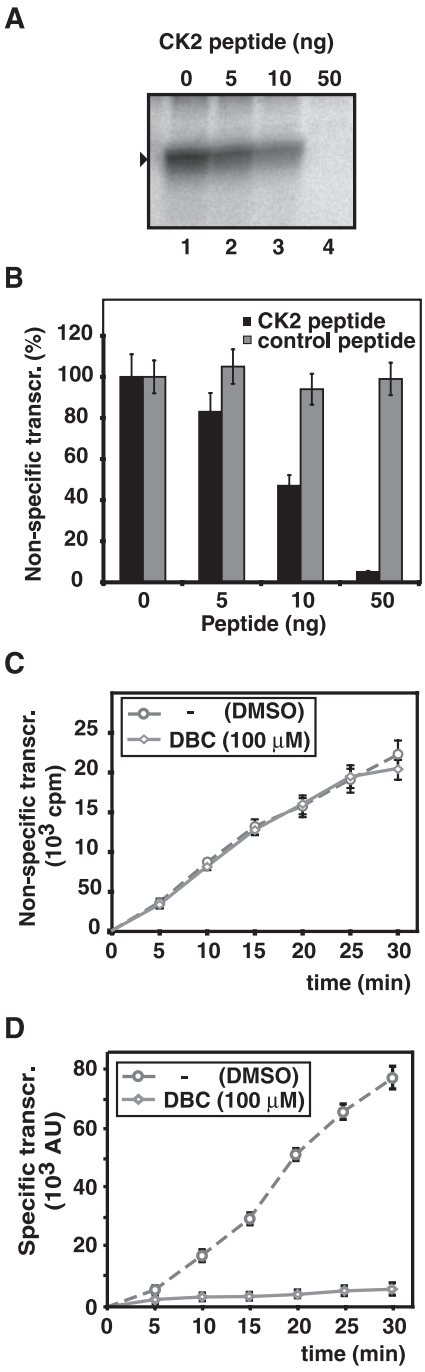

FIG. 4. CK2-specific inhibitor DBC inhibits multiple rounds of specific Pol I transcription but has no effect on elongation following random initiation events. A. CK2 phospho-acceptor peptide inhibits specific Pol I transcription. A 2.5-μl sample of highly purified Pol Iβ, in a 10-μl reaction mixture, was incubated with 0, 5, 10, or 50 ng of CK2 phospho-acceptor peptide (RRREEETEEE; New England Biolabs) for 15 min on ice. Two hundred nanograms of template DNA (prHu3) and 1 μl of highly purified SL1 were added to each reaction mixture. Transcription was initiated with the addition of NTPs. Transcript synthesis was analyzed in an S1 nuclease protection assay. The autoradiograph shows the transcript levels. B. CK2 phospho-acceptor peptide inhibits nonspecific transcription by Pol I, independent of its effect on CK2. A 2.5-μl sample of highly purified Pol Iα (which does not contain CK2) in a 10-μl reaction mixture was incubated with 0, 5, 10, or 50 ng of CK2 phospho-acceptor peptide (black) or control peptide (gray) for 15 min on ice. Nonspecific transcription was initiated by the addition of a transcription mixture containing [α-^{32}P]CTP, NTPs, and calf thymus DNA. Radioactivity incorporated in the acid-insoluble fraction was Cerenkov counted and expressed as a percentage of that without peptide, which was set at 100%. Experimental errors are indicated. C. DBC has no effect on nonspecific Pol I transcription in nuclear extract. HeLa nuclear extract was incubated with DMSO alone or 100 μM DBC (in DMSO) for 15 min at room temperature. Nonspecific transcription reactions were initiated and analyzed over time as in panel A, and synthesis was expressed in cpm and plotted against time (for two independent experiments). D. DBC inhibits multiple rounds of specific Pol I transcription. HeLa nuclear extract was incubated with immobilized rDNA promoter template (Fr4) (39) for 15 min on ice. The templates were washed in TM10/0.05, and then 0 or 100 μM DBC was added to the preformed PICs on these promoter templates. Incubation was continued for another 15 min at room temperature. Transcription was initiated with NTPs, and at each time point, transcription was quantitated by phosphorimaging, expressed in arbitrary units (AU) and plotted against time (for two independent experiments).

interfere with the RNA synthesis activity of Pol Iα, which lacks CK2 (data not shown), and also did not inhibit randomly initiated Pol I transcription in HeLa cell nuclear extract (Fig. 4C). This suggests that CK2 activity is not required for transcript elongation by Pol I in vitro.

Next, we assessed the effect of CK2 inhibition in specific transcription with PICs preassembled from nuclear extracts on an immobilized rDNA promoter fragment. In this system in the absence of CK2 inhibitor, transcript synthesis continued linearly for 30 min (Fig. 4D), consistent with multiple rounds of transcription following reinitiation events (39). Crucially, DBC (and TBB; data not shown) severely inhibited this promoter-directed specific Pol I transcription (Fig. 4D). The time course experiment indicated that transcription in the presence of the CK2 inhibitor occurred primarily in the first few minutes (Fig. 4D). This is reminiscent of that observed for a single round of transcription (39).

Pol Iβ-associated CK2 phosphorylates UBF and SL1 subunit TAF$_I$110. UBF is a substrate for recombinant CK2 in vitro (36, 52, 53), but a role for the Pol Iβ-associated CK2 described here in targeting UBF had not been explored. Neither had a link between Pol Iβ-associated CK2 and SL1 been established. Phosphorylation of recombinant UBF (Fig. 5A, lane 4) and of the TAF$_I$110 subunit of immunopurified SL1 (Fig. 5B, lane 3) was detected following incubation with Pol Iβ. The presence of the rDNA promoter-containing fragment did not significantly influence the level of phosphorylation of UBF and TAF$_I$110 (Fig. 5A, lane 5, and B, lane 4, respectively), though the level of Pol Iβ-associated CK2 phosphorylation of TopoIIα was enhanced (Fig. 5A, lane 3). Phosphorylation of each of these proteins of the Pol I transcription machinery was inhibited by the CK2 inhibitor DBC (Fig. 1C; also data not shown). Therefore, Pol Iβ-associated CK2 can target UBF and TAF$_I$110, in addition to TopoIIα, for phosphorylation.

Inhibition of Pol Iβ-associated CK2 activity decreases UBF-dependent activation but does not affect basal transcription. The potential effects of CK2 phosphorylation of UBF and SL1 by the Pol Iβ-associated CK2 were assessed using the CK2-specific inhibitor DBC in a reconstituted transcription system, with highly purified UBF, SL1, and Pol Iβ, which does not

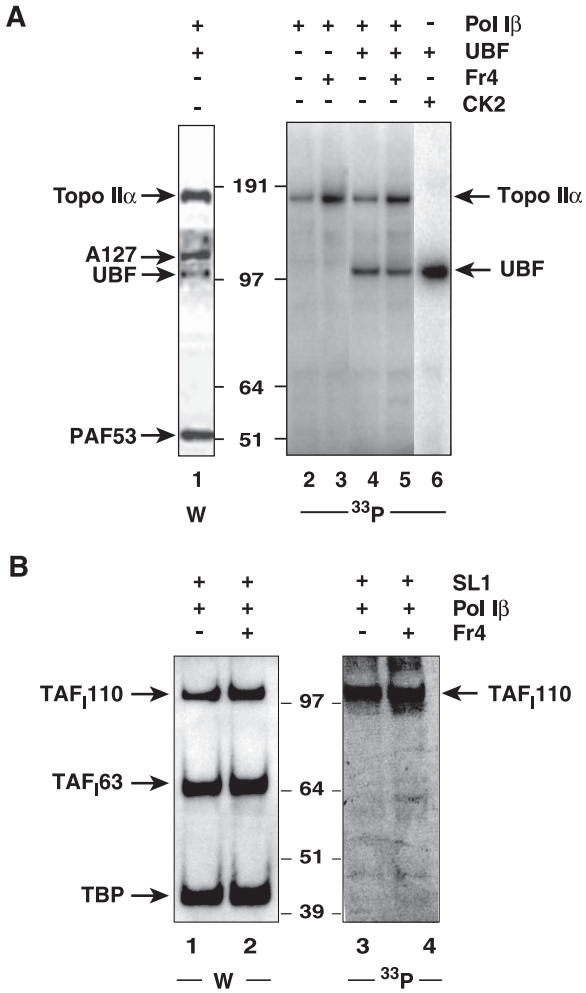

FIG. 5. Pol Iβ-associated kinase phosphorylates UBF and SL1 subunit TAF_I110. A. Pol Iβ-associated kinase phosphorylates UBF. Pol Iβ (lanes 1 to 5) or recombinant CK2 (lane 6) was incubated with (lanes 1, 4, 5, and 6) or without (lanes 2 and 3) recombinant UBF for 15 min on ice. rDNA promoter-containing fragment (Fr4) was also present in the reactions of lanes 3 and 5. Incubation was then continued with [γ-^{33}P]ATP for 30 min at 30°C. Proteins were immunoblotted and probed with antibodies specific for TopoIIα, UBF, or Pol I subunit A127 or PAF53 (lane 1, W), and in parallel de novo phosphorylated proteins were detected by autoradiography (lanes 2 to 6; ^{33}P). B. Pol Iβ-associated kinase phosphorylates TAF_I110 in SL1. Pol Iβ was incubated with TBP-antibody (monoclonal 3G3, a kind gift from L. Tora) immunoaffinity-purified SL1 in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of rDNA promoter template for 15 min on ice. Incubation was then continued with [γ-^{33}P]ATP for 30 min at 30°C. Proteins were immunoblotted and probed with antibodies specific for TAF_I110, TAF_I63, or TBP (lanes 1 and 2; W), and de novo phosphorylated proteins were detected by autoradiography (lanes 3 and 4; ^{33}P).

support efficient reinitiation (39a). Promoter-directed basal transcription was not affected by 50 or 100 μM DBC (Fig. 6A), suggesting that CK2 activity in Pol Iβ is not essential for transcription. By contrast, UBF activation of transcription was reduced but not entirely blocked by DBC (Fig. 6B), suggesting that phosphorylation of UBF by Pol Iβ-associated CK2 augments activation of transcription (UBF activation was 3.7-fold in the absence and 1.8-fold in the presence of DBC). Indeed, prior phosphorylation of recombinant purified UBF (from baculovirus-infected insect cells) by recombinant CK2, which did not alter the mobility of UBF appreciably (Fig. 6C), increased (two- to threefold) the ability of UBF to activate Pol I transcription (Fig. 6D, lanes 5 and 6, compared to lanes 2 and 3).

While CK2 phosphorylation of UBF had been shown to affect its activation function, DNA binding by UBF appeared unaffected (36, 52), and the molecular mechanism underpinning the activation remained unknown. SL1 stabilizes UBF at the rDNA promoter (14), and therefore we asked whether CK2 phosphorylation of UBF would influence the rate of dissociation of UBF from SL1-rDNA promoter complexes. In the absence of SL1, UBF dissociated from the immobilized rDNA promoter fragment rapidly when challenged with ctDNA (Fig. 6E, lanes 8 to 13), and this appeared to be independent of its CK2 phosphorylation state (Fig. 6E, lanes 1 to 6). SL1 significantly reduced the dissociation rate of UBF (Fig. 6E, lanes 21 to 26), and crucially, CK2 phosphorylation of UBF further reduced this dissociation rate in the context of promoter-bound SL1 (Fig. 6E, lanes 14 to 19).

Collectively, our data argue that in addition to the stimulation of reinitiation of Pol I transcription by CK2, the positive effects of Pol Iβ-associated CK2 activity during reconstituted transcription with purified factors are manifested through targeting of UBF in the PIC, which results in a more stable interaction of UBF with promoter-bound SL1.

CK2 phosphorylation of SL1 can inhibit specific Pol I transcription by preventing SL1 binding at the promoter. Preincubation of SL1 with Pol Iβ and ATP before promoter template was added did not result in detectable effects on SL1 activity in reconstituted transcription (data not shown), suggesting that the extent of phosphorylation by Pol Iβ-associated CK2 of SL1 was too low to yield detectable alterations in SL1 activity. The addition of recombinant CK2 to a transcription reaction with purified Pol Iβ, SL1, and rDNA promoter template inhibited promoter-specific transcription in a dose-dependent manner (Fig. 7A). This was not due to inhibition of transcript elongation, however, because CK2 had no effect on nonspecific (random) RNA synthesis by Pol Iβ (Fig. 7B). CK2 inhibited specific Pol I transcription during preinitiation complex formation (Fig. 7A). Since human SL1 has been shown to instigate PIC formation (14), consistent with the ability of TIF-IB (mouse SL1) and rat SL1 to bind their cognate rDNA promoters independently of UBF (49, 51), we asked whether the promoter DNA binding and transcription activity of SL1, shown here to be a potential substrate of CK2 (see Fig. 5B), was affected by recombinant CK2 activity (Fig. 7C). CK2 negatively affected SL1-Pol I-directed rDNA promoter-specific transcription (Fig. 7D, lane 4, compared to lane 1), and this was because CK2 decreased the ability of SL1 to bind the rDNA (Fig. 7E, lane 4, compared to lane 1). The CK2 inhibitor DBC reversed these effects of CK2 on SL1 (Fig. 7E, lane 3, and D, lane 3). There was no significant effect of CK2 activity on Pol I transcription when added after SL1 was bound to the promoter (Fig. 7D, lane 7, compared to lane 1), and further, once bound to the rDNA, SL1 was not dissociated by CK2 (Fig. 7E, lane 8, compared to lane 5). The data from Fig. 6A also suggest that CK2 has no effect on basal transcription once SL1 is bound to the rDNA promoter (note that CK2 phosphorylation of SL1 cannot occur until addition of nucleoside triphosphates [NTPs]). Taken together, the data suggest that

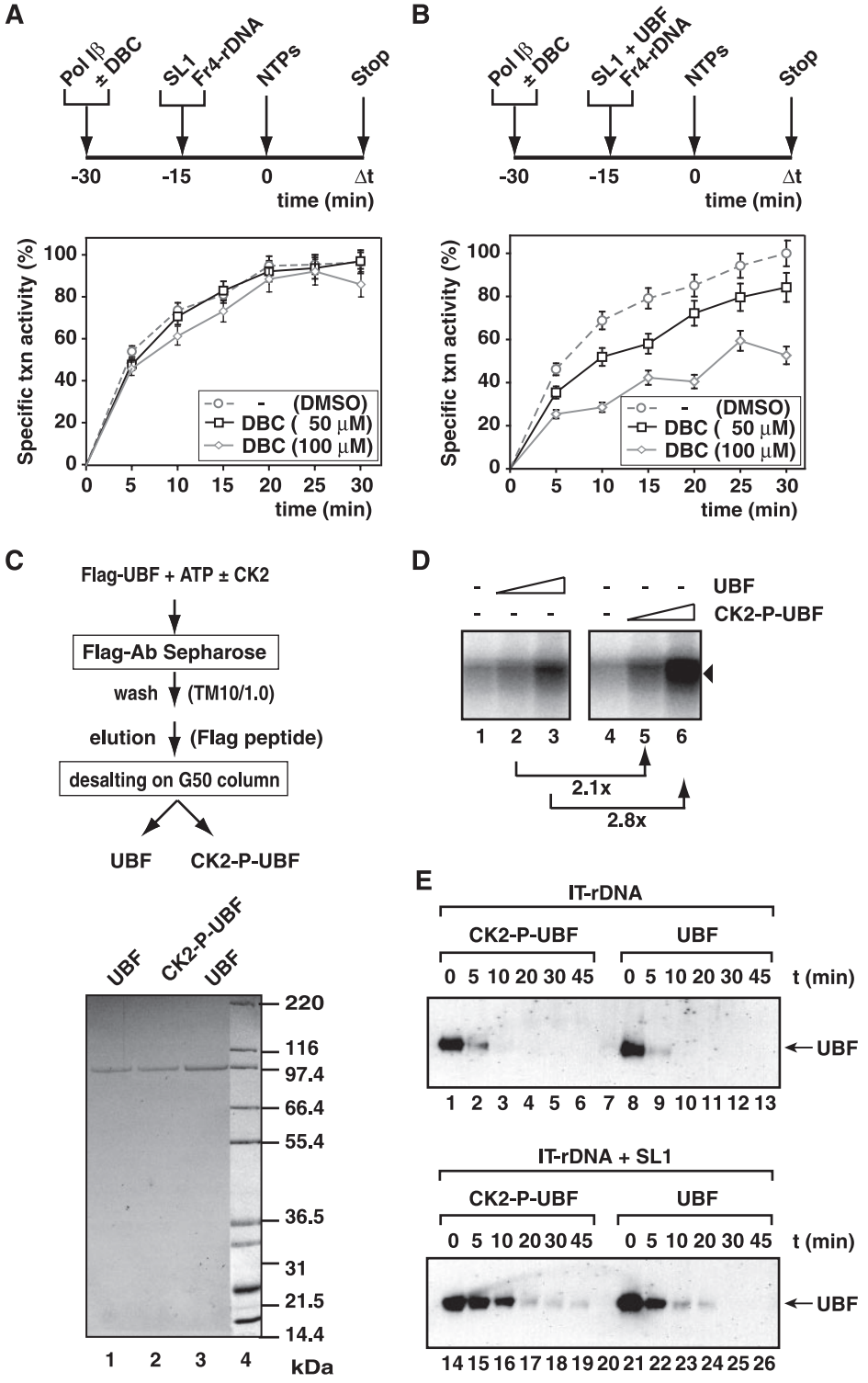

FIG. 6. CK2 phosphorylation activates UBF, increasing UBF-dependent activated but not basal transcription by Pol I, and stabilizes UBF at the rDNA promoter in an SL1-dependent manner. A. CK2 inhibitor DBC does not affect SL1- and Pol I-dependent basal transcription. Pol Iβ was preincubated in the absence of DBC (DMSO) or in the presence of 50 μM or 100 μM of DBC for 15 min at room temperature as outlined. rDNA promoter template (Fr4) and SL1 were added to each reaction, and incubation was continued for another 15 min on ice. Specific transcription (specific txn) was initiated at 30°C upon addition of NTPs, and samples were taken at the time points indicated. At each time point, transcription was quantitated by phosphorimaging and expressed as a percentage of the highest level of transcription, which was set at 100%. B. CK2 inhibitor DBC affects UBF-dependent activation of Pol I transcription. As in panel A, except that recombinant UBF was added to determine the effect of DBC on UBF-dependent activation of Pol I transcription (at the same time as SL1). C. Schematic representation of the purification of CK2-phosphorylated Flag-tagged UBF (CK2-P-UBF; see Materials and Methods). A 1.5-μl sample of either (Flag-)UBF (lane 1) or CK2-P-(Flag-)UBF (lane 2) and 2.5 μl (400 ng) of highly purified Flag-tagged UBF, which was the input for the phosphorylation reaction (lane 3), were

TAF_I110 can be targeted for phosphorylation by CK2 and this can inhibit the ability of SL1 to bind DNA and thereby to support Pol I transcription.

DISCUSSION

CK2 has been implicated in increased mammalian cell growth and proliferation (29), and Pol I transcription must be upregulated in order to achieve such an increase. Loss of CK2 activity in yeast mutant strains correlates with reduced rRNA synthesis by Pol I (16). Our identification of the human Pol I-associated kinase as CK2 agrees with results of similar studies on the Pol I complexes of broccoli, *Xenopus*, and rat (1, 18, 47), supporting the concept of a direct and conserved role for CK2 in Pol I transcription, although mechanisms have remained elusive.

Intriguingly, while the human Pol I isoforms share many of the core subunits, CK2 is specifically associated with the initiation-competent isoform Pol Iβ and not with the highly abundant Pol Iα. Since CK2 is substoichiometric in Pol Iβ, it is possible that regulation by CK2 of Pol I transcription might involve the differential association of CK2 with initiation-competent Pol Iβ rather than the kinase activity of CK2 itself. Furthermore, we demonstrate for the first time that CK2 is present at the rDNA in proliferating human cells and thus has the potential to regulate rDNA transcription in vivo. One target of the Pol Iβ-associated CK2 is UBF, consistent with previous reports suggesting that recombinant CK2 can target UBF (36, 53). Additionally, two novel targets have been identified in the human Pol I transcription machinery: TAF_I110 in SL1 and TopoIIα in the Pol Iβ complex. Phosphorylation of the largest subunit of Pol I, as previously suggested for rat Pol I (18), was not observed for human Pol I. At present it is not known whether or not the rat Pol I preparation contained TopoIIα, which in protein gels does not resolve readily from the largest subunit of Pol I. Intriguingly, TopoIIα, like CK2, is specifically associated with human Pol Iβ. Importantly, we provide evidence for novel molecular mechanisms in the potential stimulatory and inhibitory effects of CK2 on Pol I transcription: we demonstrate that (i) CK2 can positively affect Pol I transcription through its abilities to stabilize and activate UBF in the PIC and to influence reinitiation of transcription and (ii) can negatively affect transcription through its ability to inhibit SL1 binding, and hence PIC formation, at the rDNA promoter.

A potential regulatory role for CK2 has been demonstrated recently in two in vitro transcription systems for Pol II and Pol III (22, 28). Our reconstituted Pol I transcription assays also reveal a potential modulatory role for CK2 in transcription. Our findings that a novel potent and selective CK2 inhibitor, DBC (31, 38), reduces both single-round UBF-activated transcription and multiround Pol I transcription and that CK2 is specifically associated with initiation-competent Pol Iβ could signify positive roles for CK2 in initiation and reinitiation of Pol I transcription. Previous studies had shown a CK2/kinase NII-induced increase in the activity of rat Pol I at the level of elongation in vitro (12). Though CK2 does not regulate elongation of transcription in the in vitro system used here, the in vivo association of CK2 not only with the rDNA promoter but also with other rDNA sequences associated with Pol I leaves open the possibility that elongation in vivo can be modulated by CK2. Preliminary data suggest that pharmacological inhibition of CK2 in cells affects Pol I transcription (data not shown), but it is unclear what the mechanism is and whether the effect is direct.

To explore mechanisms by which CK2 could function in Pol I transcription, we first identified the targets of CK2 in the Pol I transcription machinery. Our results strongly suggest that Pol Iβ-associated CK2 can phosphorylate TopoIIα in the human Pol Iβ complex and not the largest subunit of human Pol I and also that TopoIIα and CK2 are associated with Pol Iβ and not Pol Iα. TopoIIα, a homodimeric enzyme that catalyzes the cleavage and religation of double-stranded DNA, is a known substrate of CK2 in vitro and in vivo. CK2 interacts directly with TopoIIα and had been shown to stimulate topoisomerase activity (7, 10, 11, 40). Topoisomerase activity is required for rDNA transcription elongation in yeast (5, 50). Our studies show that inhibition of CK2 has no detectable effect on elongation in vitro, suggesting that CK2 phosphorylation of TopoIIα is not required for elongation in this chromatin-free system. However, this does not exclude a role for Topo IIα in the relief of transcription-induced torsional stress in promoter-dependent transcription. Pol Iβ-associated TopoIIα is active in decatenation of the interlocked circular DNA molecules of kinetoplast DNA, though its presence in Pol Iβ remains enigmatic, since we have not seen a requirement for TopoII activity in the current transcription system (K. Panov, T. Panova, and J. Zomerdijk, unpublished data).

Recombinant CK2-mediated phosphorylation of UBF in

resolved on a 4 to 20% bis-Tris Novex gel (Invitrogen). The gel was stained with Sypro-Ruby (Invitrogen). Lane 5 contained the "Mark 12" protein ladder (Invitrogen). D. CK2 phosphorylation of UBF increases UBF activity. Pol Iβ and SL1 were incubated with rDNA promoter template (Fr4) in the absence of UBF (basal transcription; lanes 1 and 4) or in the presence of (Flag-tagged) UBF (5 and 20 ng; lanes 2 and 3) or CK2-phosphorylated (Flag-tagged) UBF (5 and 20 ng; lanes 5 and 6) (see panel C). Incubation was for 15 min on ice, and transcription was initiated upon addition of NTPs. Transcript synthesis after 30 min was quantitated by phosphorimaging from two independent experiments (in duplicate). *n*-fold stimulation is indicated (2.1 ± 0.3 and 2.8 ± 0.4). E. CK2 phosphorylation of UBF reduces the rate of dissociation of UBF from an SL1-rDNA promoter fragment but not from the promoter fragment alone. Recombinant UBF (300 ng) was incubated with 100 U of recombinant CK2 and 0.5 mM ATP in the absence of DBC (in DMSO) or in the presence of 100 μM of DBC for 20 min at room temperature. One hundred fifty nanograms of CK2-phosphorylated UBF (CK2-P-UBF; lanes 1 to 6 and 14 to 19) or nonphosphorylated UBF (UBF; lanes 8 to 13 and 21 to 26) was incubated for 20 min at 0°C with 70 μl of IT-rDNA or IT-rDNA to which SL1 had been prebound for 20 min at 0°C (IT-rDNA + SL1; excess SL1 removed by TM10/0.05 wash). Templates were subsequently washed with TM10/0.05, and sheared ctDNA was added (at time zero; final concentration, 0.5 mg/ml). Equal aliquots were removed at 0, 5, 10, 20, 30, and 45 min, and the recovered templates were washed with TM10/0.05 to remove factors no longer associated with the IT-rDNA. Template-associated UBF was analyzed by immunoblotting following elution with 5 M urea. Lanes 7 and 20 represent controls in which 21 ng of CK2-P-UBF was incubated with 10 μl of M280 "empty" beads, subsequently washed in TM10/0.05. The immunoblots are representative of two independent experiments.

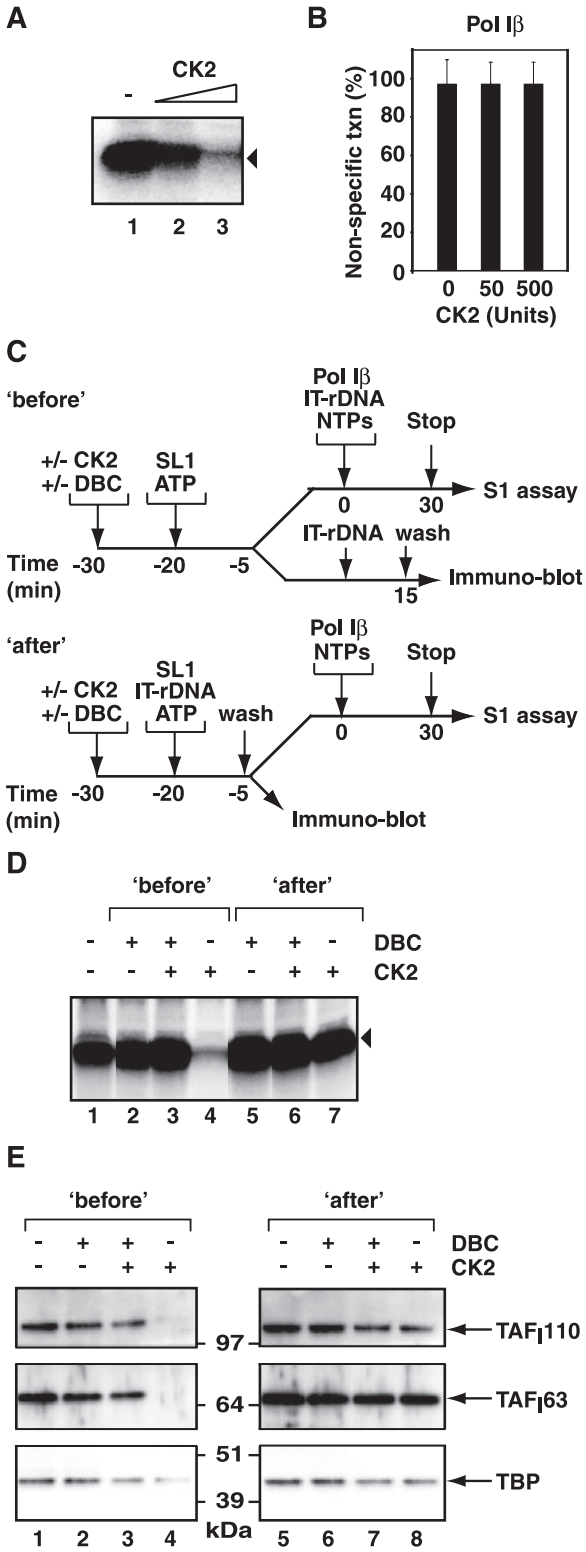

FIG. 7. CK2 phosphorylation of SL1 can inhibit specific transcription by preventing SL1 binding at the promoter. A. CK2 can inhibit specific Pol I transcription during formation of the SL1 and Pol I-containing preinitiation complex. Pol Iβ, SL1, and rDNA promoter template (Fr4) were incubated with CK2 (0, 100, or 500 U; lanes 1, 2, and 3, respectively) in the presence of ATP for 15 min at room temperature, and then transcription was initiated by addition of NTPs. The reactions were incubated for 30 min at 30°C and transcripts analyzed by S1 nuclease protection assay and autoradiography (arrowhead). B. CK2 has no detectable effect on nonspecific RNA synthesis. Pol Iβ was preincubated with 0, 50, or 500 U of CK2 and ATP for 15 min at room temperature and then added to a nonspecific transcription assay. Nonspecific transcription (txn) detected from CK2-treated Pol Iβ is expressed as a percentage of transcription detected in the absence of CK2. C. Schematic representation of experiments to determine the effect of phosphorylation of SL1 by CK2 on Pol I transcription (D) and on rDNA-promoter binding (E). The experiments were repeated twice (in duplicate), and a representative is shown. To test the effect of CK2 added "before" SL1 binding to the rDNA promoter, CK2 (100 U) was incubated with or without DBC (50 μM) for 10 min at room temperature. SL1 was added, and incubation continued for 15 min at room temperature in the presence of ATP. After incubation the reactions were divided in two. For transcription analysis, IT-rDNA and Pol Iβ were added and transcription was initiated upon addition of NTPs. The transcription reactions were incubated for 30 min at 30°C and specific transcripts detected by S1 nuclease protection (see panel D). For analysis of rDNA promoter binding by SL1, IT-rDNA was mixed into the reactions, left on ice for 15 min, and then washed with TM10/0.05. IT-rDNA-bound proteins were eluted in SDS-sample buffer and immunoblotted (see panel E). To test the effect of CK2 added "after" SL1 binding to the rDNA promoter, CK2 (100 U) was incubated with or without DBC (50 μM) for 10 min at room temperature, SL1 prebound (for 15 min on ice) to IT-rDNA was added, and incubation was continued for another 15 min at room temperature in the presence of ATP. The beads were washed in TM10/0.05 buffer and divided in two, for transcription and immunoblot analysis, as described above. D. CK2 enzymatic activity inhibits Pol I transcription when added before, but not after, SL1 binding to DNA. Using the procedures outlined for panel C, the effects of CK2 on promoter-specific Pol I transcription, when added before (lane 4) or after (lane 7) SL1 was bound to IT-rDNA, were analyzed. Control reactions contained no CK2 (lane 1), CK2 preincubated with CK2 inhibitor DBC (lanes 3 and 6), or DBC alone (lanes 2 and 5). E. CK2 enzymatic activity decreases the ability of SL1 to bind DNA but does not cause SL1 to dissociate from DNA. Using the procedures outlined for panel C, the effects of CK2 on SL1 binding to IT-rDNA, when added before (lane 4) or after (lane 8) SL1 was bound to the IT-rDNA, were analyzed and compared to SL1 binding without CK2 (lanes 1 and 5), with CK2 preincubated with CK2 inhibitor DBC (lanes 3 and 7), or with DBC alone (lanes 2 and 6). Antibodies specific for TAF_I110, TAF_I63, or TBP were used in immunodetection.

vitro and its positive role in the regulation of UBF activity have been reported previously (36, 52, 53), although no mechanism had been known for this activation. We propose, based on our rDNA dissociation data, that CK2 phosphorylation of UBF stimulates the ability of UBF to activate Pol I transcription through enhanced stabilization by SL1 of CK2-phosphorylated UBF at the rDNA promoter, providing a molecular mechanism for the stimulatory effect of CK2 on UBF activation of transcription. Furthermore, we show that CK2, recombinant and Pol Iβ associated, can phosphorylate UBF and activate UBF in solution and in the context of the PIC and that phosphorylation of UBF by CK2 upregulates the activity of UBF in Pol I transcription. Phosphorylation by CK2 is insufficient for UBF activity, since dephosphorylated UBF is unable to stimulate transcription and phosphorylation by recombinant CK2 of dephosphorylated UBF or recombinant UBF purified from *Escherichia coli* is not sufficient to activate UBF (52). We deduce that our baculovirus-expressed UBF boasts phosphorylated residues crucial for UBF activity in addition to residues at which CK2 phosphorylation can activate UBF.

We found that inhibition of CK2 activity dramatically re-

duced RNA synthesis by Pol I in transcription reactions with preassembled PICs from nuclear extracts, which normally support multiple rounds of transcription (Fig. 4D). Since CK2 inhibition affected neither elongation of transcription (Fig. 4C) nor basal transcription (Fig. 6A) and only decreased UBF-activated single-round transcription in a highly purified system (Fig. 6B) about twofold, the dramatic effect of CK2 inhibition on multiround transcription is likely due to inhibition of reinitiation. Therefore, we conclude that CK2 activity is required not only for efficient UBF-activated transcription but also to sustain multiple rounds of transcription via a positive effect of CK2 on reinitiation of Pol I transcription. At present we can only speculate about the mechanism by which CK2 functions in reinitiation. Pol Iβ-associated RRN3, for example, is inactivated and dephosphorylated shortly after transcription initiation and dissociates from Pol I (6, 20, 33, 41); its subsequent association with other Pol I complex components in the reassembly of initiation-competent Pol Iβ could conceivably be regulated by CK2.

The activity of SL1 (TIF-IB in mouse) can be regulated through phosphorylation during the cell cycle (19, 26). There is also evidence that promoter occupancy by SL1 is dictated by the availability of nutrients and growth stimulatory factors (23); yet although some of the SL1 subunits are phosphoproteins, as far as we know, there are no reports of SL1 phosphorylation in response to such factors. Our data imply a negative role for CK2 in specific Pol I transcription via phosphorylation of the SL1 subunit TAF_I110, which would be exerted prior to PIC formation at the level of SL1 binding to the rDNA, eliminating the potential of SL1 to nucleate PICs. SL1 bound to the rDNA promoter is not influenced negatively by CK2 phosphorylation, and the CK2 associated with SL1-recruited Pol Iβ complexes can stimulate transcription activation and reinitiation.

The positive influences of CK2 on UBF activation and reinitiation of transcription might prevail in rapidly growing cells, where Pol I transcription is upregulated to fulfill the demand for ribosome biogenesis during cell growth and proliferation. Adverse circumstances, for example, cellular stress, might tip the balance in favor of the negative effects of CK2 on SL1 DNA-binding and, consequently, down-regulation of Pol I transcription. Certainly, yeast Pol I transcription can be down-regulated in response to cellular stress (16). Interestingly, in yeast Pol III transcription, TBP-associated CK2 transduces DNA damage signals to the Pol III transcriptional machinery (16). It is striking that CK2 also displays opposing roles in mammalian Pol III transcription, upregulating Pol III transcription by facilitating recruitment of Brf1-TFIIIB by TFIIIC2 (25) and by stimulating the Pol III enzyme complex through an as yet unknown target (22) and downregulating transcription at mitosis by inactivating TFIIIB (13, 21). One theme that emerges is that CK2 can phosphorylate and modulate proteins of TBP-TAF complexes involved in targeting the RNA polymerases specifically to the promoter. In Pol I transcription, CK2 can affect core promoter binding of SL1, as shown here. In Pol II transcription, CK2 can regulate core promoter selectivity of TFIID by phosphorylation of TAF1 (28). In Pol III transcription, CK2 targets and differentially modulates the activity of TFIIIB complexes (13, 21, 22, 25). It will be interesting to unravel the specific circumstances under which CK2 modulates Pol I transcription in cells and to determine the dependency on the physiological status of the cell and its environment.

We propose that CK2, implicated in mammalian cell growth and proliferation, has the potential to regulate rRNA synthesis by Pol I in the nucleolus at multiple levels, in transcription preinitiation complex formation directed by SL1, in the activation of transcription mediated by UBF, and in the reinitiation of transcription by Pol I.

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