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Phosphorylation by Casein Kinase 2 Facilitates rRNA Gene Transcription by Promoting Dissociation of TIF-IA from Elongating RNA Polymerase I▽

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The protein kinase casein kinase 2 (CK2) phosphorylates different components of the RNA polymerase I (Pol I) transcription machinery and exerts a positive effect on rRNA gene (rDNA) transcription. Here we show that CK2 phosphorylates the transcription initiation factor TIF-IA at serines 170 and 172 (Ser170/172), and this phosphorylation triggers the release of TIF-IA from Pol I after transcription initiation. Inhibition of Ser170/172 phosphorylation or covalent tethering of TIF-IA to the RPA43 subunit of Pol I inhibits rDNA transcription, leading to perturbation of nucleolar structure and cell cycle arrest. Fluorescence recovery after photobleaching and chromatin immunoprecipitation experiments demonstrate that dissociation of TIF-IA from Pol I is a prerequisite for proper transcription elongation. In support of phosphorylation of TIF-IA switching from the initiation into the elongation phase, dephosphorylation of Ser170/172 by FCP1 facilitates the reassociation of TIF-IA with Pol I, allowing a new round of rDNA transcription. The results reveal a mechanism by which the functional interplay between CK2 and FCP1 sustains multiple rounds of Pol I transcription.

Changes in growth and proliferation impinge rapidly and efficiently on transcription of rRNA genes (rDNA) by RNA polymerase I (Pol I). Conditions that harm cellular metabolism, such as nutrient starvation, oxidative stress, inhibition of protein synthesis, or cell confluence, downregulate rDNA transcription, whereas growth factors and agents that stimulate growth and proliferation upregulate Pol I transcription (14, 29, 33, 35, 41). In mammals, Pol I exists in two distinct forms, Pol Iα and Pol Iβ, both of which are catalytically active, but only Pol Iβ can assemble into productive transcription initiation complexes and direct accurate transcription (28). Pol Iβ is associated with the transcription initiation factor TIF-IA, the mammalian homolog of *Saccharomyces cerevisiae* Rrn3, a basal factor that mediates the interaction of Pol I with the TATA binding protein-containing factor TIF-IB/SL1 (6, 37, 42). TIF-IA is phosphorylated at multiple sites, and specific phosphorylation in response to certain metabolic and environmental cues affects the interaction with Pol I and/or TIF-IB/SL1, thereby regulating the assembly of productive transcription initiation complexes (9, 25, 26, 45). In cell-free transcription assays, TIF-IA has been shown to dissociate from Pol I and to lose its ability to associate with Pol I upon transcription (5, 7, 17). Although the molecular mechanism underlying the cyclic association and dissociation of the Pol I/TIF-IA complex has yet to be elucidated, it is very likely regulated by posttranslational modifications that facilitate reinitiation of transcription.

Recent studies have established that casein kinase 2 (CK2) is present at the rDNA promoter and is physically associated with Pol Iβ (23, 31). CK2 targets several components of the Pol I transcription apparatus and therefore regulates transcription at multiple levels. CK2 phosphorylates serine residues within the C-terminal part of the upstream binding factor (UBF) (40). This phosphorylation stabilizes both the binding of UBF to rDNA and the interaction of UBF with TIF-IB/SL1 (23, 31), indicating that stabilization of the preinitiation complex is important for cycling of the transcription machinery. Previous mass spectrometric analysis has revealed that TIF-IA is phosphorylated at two serine residues, Ser170/172, which are contained in a consensus sequence for CK2 (36). In the present study we have examined the functional relevance of Ser170/172 phosphorylation and show that phosphorylation by CK2 is required for TIF-IA activity, nucleolar integrity, and cell cycle progression. Unphosphorylated TIF-IA efficiently interacts with Pol I and is capable of assembling into productive transcription initiation complexes, whereas phosphorylation by CK2 triggers dissociation of TIF-IA from Pol I. After release from elongating Pol I, Ser170/172 is dephosphorylated by FCP1, facilitating reassociation with Pol I and transcription initiation. The results suggest that phosphorylation and dephosphorylation of TIF-IA at Ser170/172 occur during each round of transcription and that reversible phosphorylation is indispensable for pre-rRNA synthesis and multiple rounds of Pol I transcription.

MATERIALS AND METHODS

Chemicals and antibodies. 4-Hydroxytamoxifen (4-OHT) and CK2 inhibitors 4,5,6,7-tetrabromobenzotriazol (TBB) and dimethylamino-TBB (DMAT) were purchased from Calbiochem. Polyclonal antibodies against TIF-IA, RPA116, PAF53, TAF_I95, and Cre recombinase have been described previously (16, 20, 39). Antibodies against FCP1, bromodeoxyuridine, Flag (M2), and anti-Flag M2-agarose were purchased from Sigma or Santa Cruz. Phosphospecific antibodies against TIF-IA phosphorylated at Ser44 or Ser170/172 were raised in

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rabbits by using phosphopeptides DFFNpSPPRKT (amino acids 40 to 49) and VDVpSDpSDDE (amino acids 167 to 175).

Plasmids. The expression vector pcDNA3.1-3xFlag-TIF-IA and the corresponding point mutants contain cDNA encoding human TIF-IA (GenBank accession no. AJ272050) fused to a 5′-terminal triple-Flag sequence. TIF-IA/RPA43 was constructed by fusing TIF-IA to the N terminus of human RPA43 (GenBank accession no. BC130298). pMr600 harbors murine rDNA sequences from −324 to +292, and pHrP$_2$-BH is an artificial ribosomal minigene containing a 5′-terminal human rDNA fragment (from −411 to +375) fused to two rDNA terminator elements. pHr-IRES-Luc-T4 is a similar minigene construct harboring human rDNA sequences from −411 to +314, the firefly luciferase gene with an internal ribosome entry site (IRES) sequence, a polyadenylation signal, and four rDNA terminator elements. Plasmids encoding Flag- or green fluorescent protein (GFP)-tagged FCP1 were a gift of B. Majello, Naples, Italy.

Transfections and retroviral infections. Transfection of HEK293T and NIH 3T3 cells with expression plasmids was performed by the calcium phosphate-DNA coprecipitation method. Transfection of small interfering RNAs (siRNAs) was performed with *Trans*IT TKO reagent (Mirus). Expression of the pHr-IRES-Luc-T4 reporter in HEK293T cells was assayed after cotransfection with TIF-IA expression plasmids and pRL-TK (Promega), using the dual-luciferase reporter assay system (Promega). Cre-mediated depletion of TIF-IA was conducted as described previously (44) or by infection of *TIF-IA$^{fl/fl}$* murine embryonic fibroblasts (MEFs) with retrovirus coding for ER^{T2}-tagged Cre recombinase (creER^{T2}), followed by activation of creER^{T2} with 4-OHT. Ectopic TIF-IA was introduced into *TIF-IA$^{fl/fl}$* MEFs by infection with pBabe-puro retrovirus containing Flag- or GFP-tagged TIF-IA, TIF-IAS170/172A, or TIF-IA/RPA43 cDNAs.

Tryptic phosphopeptide analysis and in vitro kinase phosphatase assays. Tryptic phosphopeptide mapping of metabolically labeled TIF-IA was performed as described previously (25). To phosphorylate TIF-IA in vitro, 200 ng of immunopurified Flag-TIF-IA was incubated with 250 units of CK2 (New England Biolabs) in reaction buffer containing 2.5 μCi [γ-^{32}P]GTP (5,000 Ci/mmol). After sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and blotting, labeled TIF-IA was visualized by autoradiography. Dephosphorylation with immunopurified Flag-FCP1 was performed in buffer containing 50 mM Tris-HCl at pH 8.0, 10 mM potassium acetate, 0.1 mM EDTA, 0.5 mM dithiothreitol, 10 mM $MgCl_2$, and 10% glycerol. The activity of Flag-FCP1 and calf intestinal alkaline phosphatase (CIAP) (Roche) was assayed in the same buffer supplied with 10 mg/ml *p*-nitrophenyl phosphate, and release of *p*-nitrophenyl was measured by spectrophotometry at 405 nm.

In vitro transcription assays. Transcription reaction mixtures contained 50 to 100 μg nuclear extract proteins from confluent FM3A cells, 50 ng of linearized pMr600/EcoRI, and 50 to 100 ng of immunopurified Flag-tagged TIF-IA expressed in HEK293T cells (25).

Immunoprecipitation and ChIP assays. Cells were lysed in IP buffer (20 mM Tris-HCl at pH 7.4, 200 mM NaCl, 1 mM EDTA, 1 mM EGTA, 50 mM NaF, 10 mM β-glycerophosphate, 10 mM KH_2PO_4, 1 mM Na_3VO_4, 1% Triton X-100, protease inhibitors) (Roche). Cleared supernatants were incubated for 4 h at 4°C with soluble or bead-bound antibodies. Beads were washed with IP buffer, and bound material was eluted and analyzed by immunoblotting. Chromatin immunoprecipitation (ChIP) assays were performed as described previously (25).

Immunofluorescence. Cells grown on coverslips were fixed in methanol and permeabilized in acetone at −20°C. After being washed with phosphate-buffered saline and incubated with primary antibodies for 1 h, coverslips were washed and incubated with appropriate secondary antibodies coupled to fluorophores. To monitor Pol I transcription, cells were labeled with 2 mM fluorouridine (FUrd) for 20 to 60 min, fixed, and stained with antibromodeoxyuridine antibody. Immunofluorescence was monitored by microscopy, and FUrd incorporation was quantified using the NIS-Elements BR imaging software (Nikon).

FRAP (fluorescence recovery after photobleaching) analysis. A region (~1 μm) within the nucleolus of cells expressing GFP-tagged proteins was bleached for 0.5 s with the 488-nm laser line at 100% laser power. GFP emission was monitored at 0.5-s intervals using a 505-nm filter. Data from at least 15 cells were quantified as described previously (10).

RESULTS

Phosphorylation of TIF-IA at Ser170/172 is required for efficient Pol I transcription. Previous mass spectrometric analysis has revealed that human TIF-IA is phosphorylated at two serine residues, Ser170 and Ser172 (36), both of which reside in a consensus sequence for CK2. In vitro phosphorylation assays using immunopurified TIF-IA and recombinant CK2 revealed that TIF-IA was efficiently phosphorylated by CK2. Phosphorylation was impaired by specific CK2 inhibitors, such as TBB and DMAT (Fig. 1A). The two CK2 consensus sites are conserved in TIF-IA from higher eukaryotes, suggesting that phosphorylation by CK2 serves an important role in TIF-IA function. To examine the role of CK2-dependent phosphorylation of TIF-IA in Pol I transcription, we replaced Ser170/172 with alanine (TIF-IAS170/172A) or aspartic acid (TIF-IAS170/172D) and compared the tryptic phosphopeptide maps of metabolically labeled wild-type and mutant TIF-IAs. TIF-IA is targeted by various protein kinases that phosphorylate TIF-IA at multiple sites, e.g., at Ser44, Ser199, Thr200, Ser633, and Ser649 (25, 26, 45). Therefore, the pattern and intensity of most tryptic phosphopeptides were similar in wild-type and mutant TIF-IAs, except for three previously uncharacterized phosphopeptides (spots h to j) that were absent in TIF-IAS170/172A (Fig. 1B). The fact that TIF-IAS170/172A lacks three phosphopeptides is due to partial cleavage at two nonoptimal trypsin cleavage sites (Arg145 and Lys163) in the vicinity of Ser170/172. To demonstrate CK2-dependent phosphorylation of TIF-IA in vivo, we generated a phospho-specific antibody against TIF-IA phosphorylated at Ser170/172 (anti-TIF-IApS170/172) and used this antibody to monitor Ser170/172 phosphorylation upon overexpression of wild-type and mutant TIF-IAs in HEK293T cells. As shown in Fig. 1C, TIF-IA but not TIF-IAS170/172A was recognized by this phosphospecific antibody. Moreover, treatment with the CK2 inhibitor DMAT strongly decreased Ser170/172 phosphorylation, supporting the notion that CK2 phosphorylates TIF-IA at Ser170/172.

To assess the functional relevance of CK2-mediated phosphorylation, we assayed Pol I transcription in the absence and presence of DMAT. Consistent with previous studies (23), DMAT treatment caused a dose-dependent decrease of pre-rRNA synthesis (Fig. 1D). To explore whether transcription inhibition is due to inactivation of TIF-IA, we compared the activities of wild-type TIF-IA, TIF-IAS170/172A, and TIF-IAS170/172D in vitro. For this, the activities of wild-type and mutant TIF-IAs immunopurified from HEK293T cells were assayed in a cell-free transcription system containing nuclear extract from confluent cells. TIF-IA is known to be inactive in growth-arrested cells, and therefore, extracts from confluent cells exhibit low Pol I transcriptional activity (Fig. 1E, first lane). Supplementation with exogenous TIF-IA stimulated transcription in a dose-dependent manner whereas mutant TIF-IAS170/172A only marginally activated transcription (Fig. 1E). Surprisingly, the phospho-mimicking mutant TIF-IAS170/172D rather impaired transcription, indicating that a permanent negative charge at positions 170 and 172 inactivates TIF-IA.

The requirement of CK2-dependent phosphorylation of TIF-IA for efficient Pol I transcription was also demonstrated in vivo. Wild-type or mutant TIF-IA was overexpressed in HEK293T cells together with pHrP$_2$-BH, an artificial minigene construct comprising the human rDNA promoter fused to two terminator elements (25), or with pHr-IRES-Luc-T4, a reporter in which transcription of the luciferase gene is under the control of the human rDNA promoter. In agreement with the

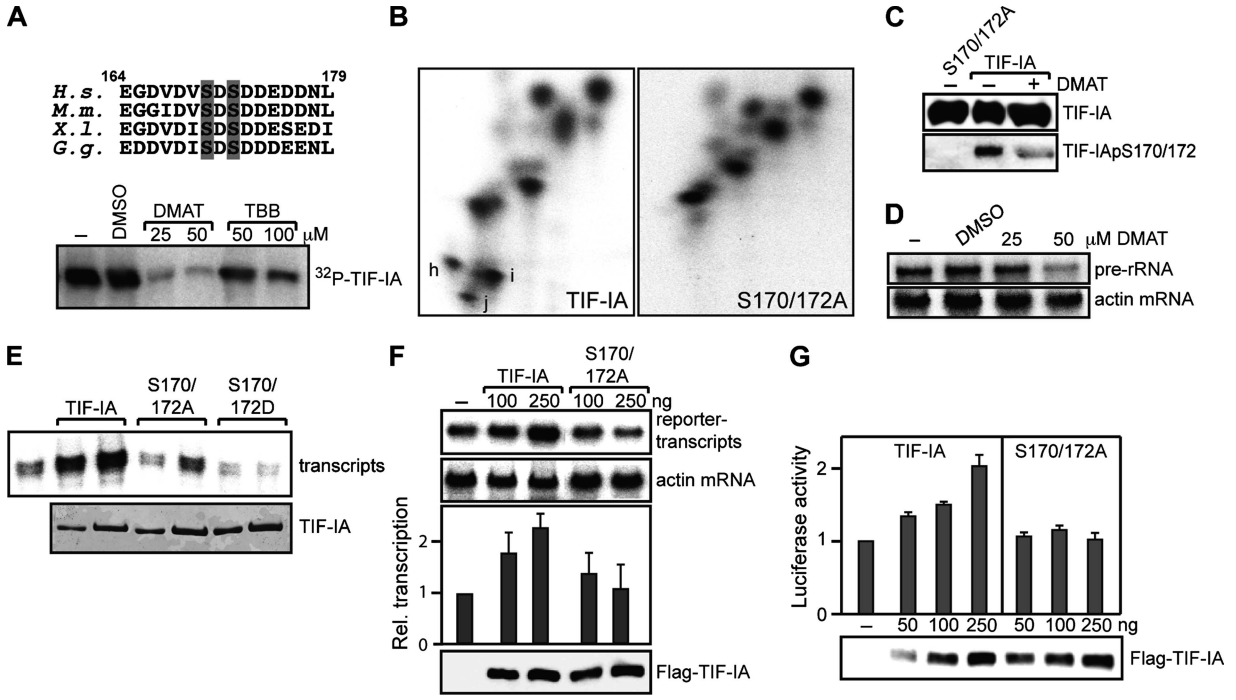

FIG. 1. Phosphorylation of Ser170/172 by CK2 is required for TIF-IA activity. (A) TIF-IA is phosphorylated by CK2 in vitro. Immunopurified Flag-tagged TIF-IA was in vitro phosphorylated by recombinant human CK2 in the absence or presence of the CK2 inhibitor TBB or DMAT as indicated. After SDS-polyacrylamide gel electrophoresis, ^{32}P-labeled TIF-IA was visualized by autoradiography. The amino acid sequences of TIF-IA encompassing putative CK2 consensus sites (S/T-X-X-D/E) from human (*H.s.*), mouse (*M.m.*), *Xenopus laevis* (*X.l.*), and chicken (*G.g.*) are shown above. Phosphoacceptor sites in TIF-IA (serines 170 and 172) are highlighted. DMSO, dimethyl sulfoxide. (B) Serines 170 and 172 are phosphorylated in vivo. HEK293T cells expressing Flag-tagged TIF-IA or TIF-IAS170/172A were metabolically labeled with [^{32}P]orthophosphate for 3 h. TIF-IA was immunoprecipitated and subjected to two-dimensional tryptic phosphopeptide mapping. Phospho-Ser170/172 was contained in three phosphopeptide spots (spots h, i, and j) because of partial cleavage at two adjacent nonoptimal trypsin cleavage sites (Arg145 and Lys163) in the vicinity of Ser170/172. (C) CK2 phosphorylates Ser170/172. Phosphorylation of immunopurified Flag-tagged TIF-IA and TIF-IAS170/172A from mock- or DMAT-treated (50 μM, 2 h) cells was monitored on immunoblots using anti-Flag antibodies or antibodies specific for phospho-Ser170/172 (anti-TIF-IApS170/172). (D) Transcription of rDNA depends on CK2 activity. RNA from untreated or mock- or DMAT-treated (25 and 50 μM, 2 h) HEK293T cells was isolated, and levels of 45S pre-rRNA were monitored on Northern blots. As a loading control, blots were reprobed for β-actin mRNA. (E) Phosphorylation of Ser170/172 is required for TIF-IA activity. Flag-tagged TIF-IA, TIF-IAS170/172A, or TIF-IAS170/172D was immunopurified from HEK293T cells and assayed for transcriptional activity in a TIF-IA-responsive nuclear extract from confluent cells. A silver-stained SDS gel of wild-type and mutant TIF-IAs used in the transcription assays is shown below. (F) Activation of a Pol I minigene depends on TIF-IA phosphorylation at Ser170/172. HEK293T cells were cotransfected with a ribosomal minigene construct (pHrP$_2$-BH) and the indicated amounts of expression vectors encoding Flag-TIF-IA or Flag-TIF-IAS170/172A. Transcript levels were analyzed on Northern blots, normalized to β-actin mRNA, and represented in the bar diagram below. The immunoblot shows the expression levels of Flag-TIF-IA and Flag-TIF-IAS170/172A. (G) Wild-type TIF-IA, but not TIF-IAS170/172A, activates transcription of a Pol I-driven luciferase reporter. HEK293T cells were transfected with pHr-IRES-Luc-T4 and expression vectors encoding Flag-TIF-IA or Flag-TIF-IAS170/172A. The bar diagram shows the level of luciferase activity determined in three independent experiments. The immunoblot shows the expression levels of Flag-TIF-IA and Flag-TIF-IAS170/172A.

in vitro data, TIF-IA enhanced transcription of both Pol I-driven reporter genes in a dose-dependent manner. Overexpression of TIF-IAS170/172A, on the other hand, did not affect transcription (Fig. 1F and G), underscoring the importance of Ser170/172 phosphorylation in transcription activation.

Ablation of Ser170/172 phosphorylation leads to cell cycle arrest and perturbation of nucleolar structure. To analyze the consequence of TIF-IA phosphorylation by CK2 in vivo, we assayed the effect of exogenous wild-type or mutant TIF-IA in MEFs from *TIF-IA*$^{fl/fl}$ embryos where the endogenous *TIF-IA* gene was inactivated by Cre-*loxP*-mediated excision (44). Consistent with previous studies showing that TIF-IA knockout abrogates Pol I transcription and affects nucleolar structure and cell cycle progression (44), infection with a retroviral vector encoding Cre-recombinase (murine stem cell virus [MSCV]-Cre), but not with the empty vector (MSCV), resulted in disintegration of nucleoli (Fig. 2A). In cells infected with MSCV, the nucleoli were clearly visible and TIF-IA colocalized with Pol I ("mock" panel, *TIF-IA*$^{fl/fl}$). After Cre-mediated depletion of TIF-IA, the nucleolar structure was perturbed and Pol I localized in small distinct spots in the nucleoplasm ("mock" panel, *TIF-IA*$^{-/-}$). Significantly, in the presence of ectopic TIF-IA the structure of nucleoli as well as the nucleolar localization of Pol I was preserved in *TIF-IA*$^{-/-}$ MEFs (Flag-TIF-IA panel). Ectopic TIF-IAS170/172A, however, did not rescue nucleolar morphology of TIF-IA-depleted cells, and both TIF-IAS170/172A and Pol I were distributed throughout the nucleoplasm in *TIF-IA*$^{-/-}$ MEFs (Flag-S170/172A panel). GFP-tagged TIF-IAS170/172A properly localized to nucleoli in

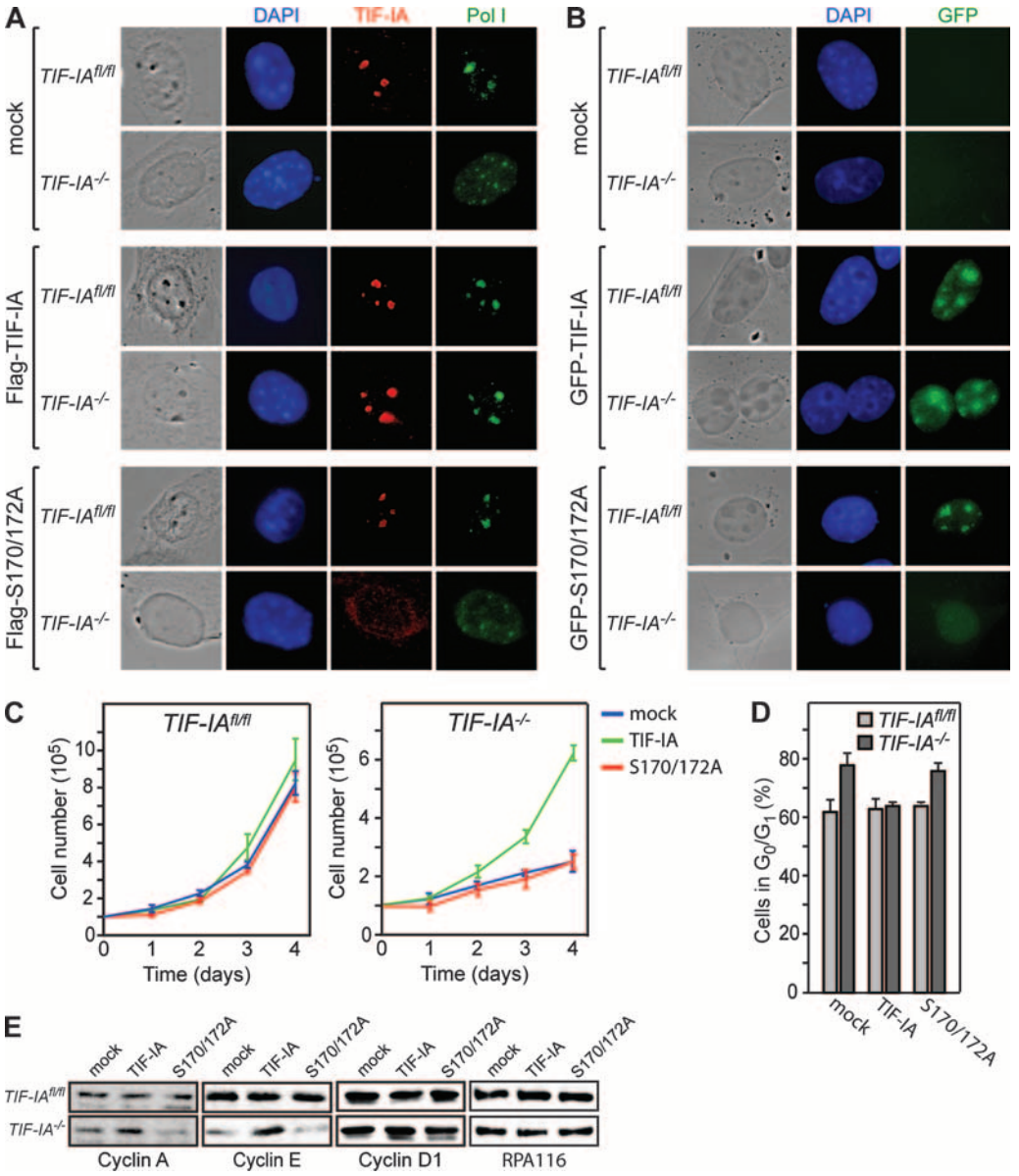

FIG. 2. Phosphorylation of TIF-IA at Ser170/172 is required for nucleolar integrity and cell proliferation. (A) Mutation of TIF-IA at Ser170/172 causes nucleolar disruption. *TIF-IA*$^{fl/fl}$ MEFs expressing no ectopic TIF-IA (top panels), Flag-tagged TIF-IA (middle panels), or TIF-IAS170/172A (bottom panels) were infected with MSCV (*TIF-IA*$^{fl/fl}$) or MSCV-Cre (*TIF-IA*$^{-/-}$). Six days after infection, Pol I and both endogenous and recombinant TIF-IA were visualized by indirect immunofluorescence with antibodies against Pol I (RPA194) and TIF-IA. DAPI, 4′,6′-diamidino-2-phenylindole. (B) Nucleolar localization of TIF-IAS170/172A depends on the presence of endogenous TIF-IA. *TIF-IA*$^{fl/fl}$ MEFs expressing creER^{T2} as well as no ectopic TIF-IA (top panels), GFP-tagged TIF-IA (middle panels), or TIF-IAS170/172A (bottom panels) were either mock treated (*TIF-IA*$^{fl/fl}$) or treated with 4-OHT to induce depletion of endogenous TIF-IA (*TIF-IA*$^{-/-}$). After 4 days, cells were fixed and localization of ectopic TIF-IA was monitored by GFP fluorescence. (C) Ser170/172 phosphorylation is required for cell proliferation. *TIF-IA*$^{fl/fl}$ MEFs expressing creER^{T2} were either mock or 4-OHT treated (*TIF-IA*$^{fl/fl}$ or *TIF-IA*$^{-/-}$), and cell numbers were determined at the indicated time points. Error bars are from counting of three different culture dishes. (D) Abrogation of Ser170/172 phosphorylation arrests cells in the G_0/G_1 phase of the cell cycle. *TIF-IA*$^{fl/fl}$ or *TIF-IA*$^{-/-}$ MEFs were stained with propidium iodide and processed for fluorescence-activated cell sorting analysis. The percentage of cells in the G_0/G_1 phase was quantified with Modfit LT software. Error bars are from two independent experiments. (E) Mutation of TIF-IA at Ser170/172 leads to cell cycle arrest. Lysates from *TIF-IA*$^{fl/fl}$ or *TIF-IA*$^{-/-}$ cells were analyzed on immunoblots with antibodies against the indicated cyclins and Pol I (RPA116).

TIF-IA$^{fl/fl}$ MEFs (Fig. 2B, GFP-S170/172A, *TIF-IA*$^{fl/fl}$), demonstrating that mislocalization of mutant TIF-IA was due to perturbation of nucleolar structure. Again, GFP-tagged wild-type TIF-IA but not GFP-TIF-IAS170/172A rescued nucleolar integrity (middle and bottom panels, *TIF-IA*$^{-/-}$). Consistent with the functional link between CK2-dependent phosphorylation of TIF-IA and nucleolar integrity, disruption of nucleoli and release of Pol I and TIF-IA were also observed after treatment of MEFs with DMAT (data not shown).

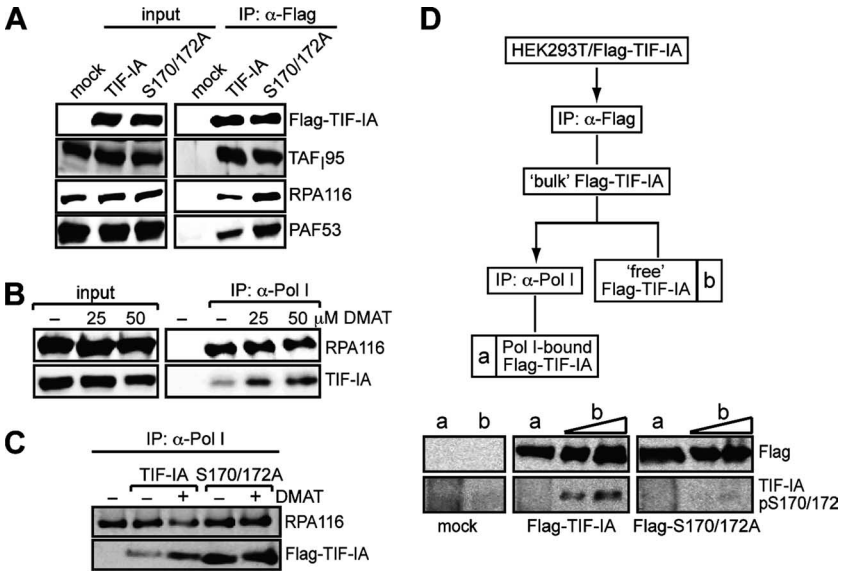

FIG. 3. Ser170/172 phosphorylation facilitates the release of TIF-IA from Pol I. (A) Phosphorylation of TIF-IA by CK2 impairs the interaction between TIF-IA and Pol I. Flag-tagged wild-type or mutant TIF-IA was immunoprecipitated from NIH 3T3 cells, and coprecipitated TIF-IB/SL1 and Pol I were visualized on Western blots using antibodies against TAF$_I$95, RPA116, or PAF53. (B) Inhibition of CK2 increases the association of TIF-IA with Pol I. Pol I was immunoprecipitated with anti-RPA194 antibodies from mock- or DMAT-treated (25 and 50 μM) HEK293T cells, and Pol I and coprecipitated TIF-IA were assayed on immunoblots. (C) Inhibition of CK2 does not enhance the association of TIF-IAS170/172A with Pol I. HEK293T cells overexpressing Flag-tagged wild-type or mutant TIF-IA were either mock treated or treated with DMAT (50 μM, 2 h). Pol I was immunoprecipitated, and the levels of Pol I and coprecipitated Flag-TIF-IA were assayed on immunoblots. (D) Phosphorylation at Ser170/172 releases TIF-IA from Pol I. Flag-tagged TIF-IA and TIF-IAS170/172A were immunoprecipitated with anti-Flag antibodies and eluted with the Flag peptide. Pol I/TIF-IA complexes were precipitated from the eluate with anti-RPA194 antibodies. Ser170/172 phosphorylation of similar amounts of TIF-IA bound to Pol I (a) and "free" TIF-IA in the eluate (b) was visualized on immunoblots using the phospho-specific TIF-IApS170/172 antibody.

Proliferation of *TIF-IA*$^{-/-}$ MEFs harboring exogenous TIF-IA was similar to that of *TIF-IA*$^{fl/fl}$ MEFs, whereas TIF-IAS170/172A did not rescue the growth defect of *TIF-IA*$^{-/-}$ MEFs (Fig. 2C). This demonstrates that wild-type but not mutant TIF-IA is capable of overcoming growth arrest induced by TIF-IA knockout. In addition, flow cytometry (fluorescence-activated cell sorting) analysis showed that upon TIF-IA depletion the fraction of cells in G_0/G_1 phase increased from 64% to 78% in cells harboring TIF-IAS170/172A, whereas expression of ectopic wild-type TIF-IA prevented the enrichment in G_0/G_1 (Fig. 2D). Cell cycle arrest was also demonstrated by decreased levels of cyclins A and E, but not cyclin D1, in *TIF-IA*$^{-/-}$ MEFs (Fig. 2E). Again, ectopic TIF-IA but not TIF-IAS170/172A prevented the decrease of cyclins A and E, in support of the idea of phosphorylation by CK2 being necessary for TIF-IA function and proper cell cycle progression.

Phosphorylation of Ser170/172 impairs the interaction of TIF-IA with Pol I. Next, we examined which step of Pol I transcription is affected by Ser170/172 phosphorylation. TIF-IA mediates the interaction of promoter-bound TIF-IB/SL1 with Pol I and therefore plays an indispensable role in transcription complex formation (42). To decipher the function of Ser170/172 phosphorylation in initiation complex assembly, we immunoprecipitated Flag-tagged wild-type and mutant TIF-IAs and monitored coprecipitated TIF-IB/SL1 (α-TAF$_I$95) and Pol I (α-RPA116 and α-PAF53) on immunoblots (Fig. 3A). Mutation of Ser170/172 did not affect the interaction with TIF-IB/SL1. However, the association of TIF-IAS170/S172A with Pol I was significantly increased, indicating that phosphorylation by CK2 weakens the interaction between TIF-IA and Pol I. In support of this, treatment with DMAT enhanced the association of both endogenous and recombinant TIF-IA with Pol I (Fig. 3B and C). Again, TIF-IAS170/S172A interacted more efficiently with Pol I and this interaction was not affected by DMAT. This suggests that phosphorylation of Ser170/172 does not regulate the assembly of the initiation complex but rather triggers the release of TIF-IA from Pol I after transcription initiation.

If phosphorylation by CK2 weakens the binding of TIF-IA to Pol I, then the bulk of "free" TIF-IA should be phosphorylated at Ser170/172, whereas Pol I-associated TIF-IA should be unphosphorylated. To prove this, we sought to compare the phosphorylation status of Pol I-associated and "free" TIF-IA. We immunoprecipitated Flag-tagged TIF-IA, separated "free" TIF-IA from Pol I-associated TIF-IA by a subsequent immunoprecipitation with anti-Pol I antibodies, and monitored phosphorylation of similar amounts of "free" and Pol I-bound TIF-IA on immunoblots by using the anti-TIF-IApS170/172 antibody. As shown in Fig. 3D, the antibody recognized "free" TIF-IA but not TIF-IA associated with Pol I, supporting the notion that phosphorylation by CK2 promotes dissociation of TIF-IA from Pol I after transcription initiation. Phosphorylation of "free" but not Pol I-associated TIF-IA at Ser170/172 was also observed on endogenous TIF-IA (data not shown), demonstrating that exogenous and endogenous TIF-IAs are similarly regulated.

Constitutive association of TIF-IA with Pol I impairs pre-rRNA synthesis. In contrast to our results demonstrating that CK2-mediated dissociation of TIF-IA from Pol I after initiation is required for rDNA transcription, a recent study has shown that covalent binding of Rrn3, the yeast homolog of TIF-IA, to the RPA43 subunit of Pol I does not impair rDNA transcription (22). This indicates that yeast does not require dissociation of Rrn3 from elongating Pol I, suggesting that yeast and mammals use different mechanisms to facilitate multiple rounds of transcription. To address this issue, we generated a similar TIF-IA/RPA43 protein, fusing RPA43 to the C terminus of TIF-IA, and compared its functional properties with those of TIF-IA and TIF-IAS170/172A in *TIF-IA*$^{-/-}$ MEFs. Although the expression levels of TIF-IA/RPA43 were lower than those of wild-type TIF-IA and the S170/172A mutant, more Pol I coprecipitated with TIF-IA/RPA43 than with TIF-IA or TIF-IAS170/172A, demonstrating that TIF-IA/RPA43 was efficiently incorporated into Pol I and has replaced endogenous RPA43 (Fig. 4A). Significantly, TIF-IA/RPA43—like TIF-IAS170/172A—was not capable of rescuing cell cycle arrest after Cre-mediated knockout of TIF-IA (Fig. 4B and data not shown). Moreover, GFP-tagged TIF-IA/RPA43 was distributed throughout the nucleoplasm in *TIF-IA*$^{-/-}$ MEFs and nucleolar structures disappeared (Fig. 4C). Disruption of nucleoli was further demonstrated by immunostaining of Pol I in *TIF-IA*$^{-/-}$ MEFs harboring Flag-TIF-IA/RPA43 (Fig. 4D).

Given that TIF-IA/RPA43 does not sustain cell proliferation and nucleolar integrity, the fusion protein should be incapable of activating rDNA transcription. To test this, we monitored the synthesis of nucleolar RNA by FUrd labeling and immunostaining with an anti-FUrd antibody. As shown in Fig. 4E, Cre-dependent ablation of TIF-IA in *TIF-IA*$^{fl/fl}$ MEFs abolished Pol I transcription (mock panel). Transcription was retained in the presence of ectopic TIF-IA, whereas the capability of TIF-IAS170/172A and TIF-IA/RPA43 to rescue Pol I transcription was strongly compromised. Quantitation of FUrd incorporation revealed that transcription dropped to 26% in cells expressing TIF-IAS170/172A and to 17% in cells expressing TIF-IA/RPA43 compared to cells expressing ectopic TIF-IA. These observations support the view that TIF-IA has to be released from Pol I after transcription initiation to promote efficient pre-rRNA synthesis.

Phosphorylation by CK2 increases the dynamics of TIF-IA and decreases promoter occupancy of Pol I. Previous FRAP experiments have shown that the Pol I machinery dynamically assembles at the rDNA promoter, individual Pol I subunits, or subcomplexes interacting with rDNA in a stochastic manner (10, 13). If Ser170/172 phosphorylation promotes dissociation of TIF-IA from Pol I, the nucleolar residence time of GFP-tagged TIF-IA should be lower than that of TIF-IAS170/172A and TIF-IA/RPA43. Indeed, the recovery kinetics of both TIF-IAS170/172A and TIF-IA/RPA43 were delayed, the time to reach 50% recovery of the prebleach signal increasing from 46 s for TIF-IA to 78 s and 82 s for TIF-IAS170/172A and TIF-IA/RPA43, respectively (Fig. 5A). A similar increase in nucleolar residence time of TIF-IA was observed if CK2 activity was inhibited by DMAT (Fig. 5B). Notably, GFP-tagged RPA194, the largest subunit of Pol I, was also strongly retained upon DMAT treatment (Fig. 5C), demonstrating that Ser170/172 phosphorylation decreases the residence time of Pol I at transcription foci, most likely by promoting transcription elongation.

If phosphorylation by CK2 triggers the release of TIF-IA from Pol I and facilitates transcription elongation, then inhibition of CK2-dependent phosphorylation should lead to accumulation of Pol I/TIF-IA complexes in the proximity of the rDNA promoter. Indeed, ChIP assays revealed significant differences of Pol I occupancy in mock- and DMAT-treated cells (Fig. 5D). Consistent with phosphorylation by CK2 being required for dissociation of TIF-IA from Pol I, the level of TIF-IA at the promoter was significantly increased upon DMAT treatment. Increased association of TIF-IA was accompanied by elevated promoter occupancy of Pol I in DMAT-treated cells. Regarding Pol I binding to rDNA, Pol I levels increased within the pre-rRNA coding region in mock-treated cells, whereas no increase of Pol I levels was observed after DMAT treatment. This finding supports the notion that CK2-dependent release of TIF-IA is required for efficient transcription elongation.

Transcription elongation through rRNA genes has been shown to be associated with increased trimethylation of histone H3 at lysine 9 (H3K9me3) within the pre-rRNA coding region (43). To assess whether decreased Pol I occupancy upon DMAT treatment is due to impaired transcription elongation, we monitored H3K9me3 levels in the pre-rRNA coding region in mock- and DMAT-treated cells. As shown in Fig. 5E, the H3K9me3 level was significantly reduced upon DMAT treatment, being fivefold lower than that in mock-treated cells, whereas H3K4me3, a histone modification that marks active rDNA repeats, was hardly affected. Thus, CK2-dependent phosphorylation of TIF-IA is required for the establishment of a chromatin structure that is characteristic for transcription elongation.

FCP1 counteracts CK2-mediated phosphorylation of TIF-IA. Having established that phosphorylation by CK2 triggers the release of TIF-IA from Pol I, reassociation with Pol I and a new round of transcription should require dephosphorylation of Ser170/172. Previous studies of yeast have shown that the phosphatase FCP1 plays a role in rDNA transcription (11). We therefore reasoned that FCP1 might be the enzyme that counteracts CK-dependent phosphorylation of TIF-IA. In accord with this prediction, FCP1 was found to bind to the rDNA promoter, whereas no association with the pre-rRNA coding region was observed (Fig. 6A). Moreover, FCP1 coimmunoprecipitates with both Pol I and TIF-IA (Fig. 6B and C), indicating that FCP1 is associated with the initiation-competent subpopulation of Pol I. To examine whether FCP1 dephosphorylates Ser170/172, TIF-IA was prephosphorylated with CK2 in vitro and used as a substrate in dephosphorylation assays. Clearly, FCP1 efficiently removed the phosphate groups from Ser170/172 in vitro, whereas the same activity of CIAP had no effect (Fig. 6D). Overexpression of FCP1 in HEK293T cells caused dephosphorylation of Ser170/172, while phosphorylation of Ser44, a residue that is targeted by Cdk2/cyclin E (23), was not affected (Fig. 6E). If CK2-dependent phosphorylation impairs the association of TIF-IA with Pol I, and FCP1 antagonizes Ser170/172 phosphorylation, then overexpression of FCP1 should augment the interaction of TIF-IA with Pol I. To test this, we analyzed the association

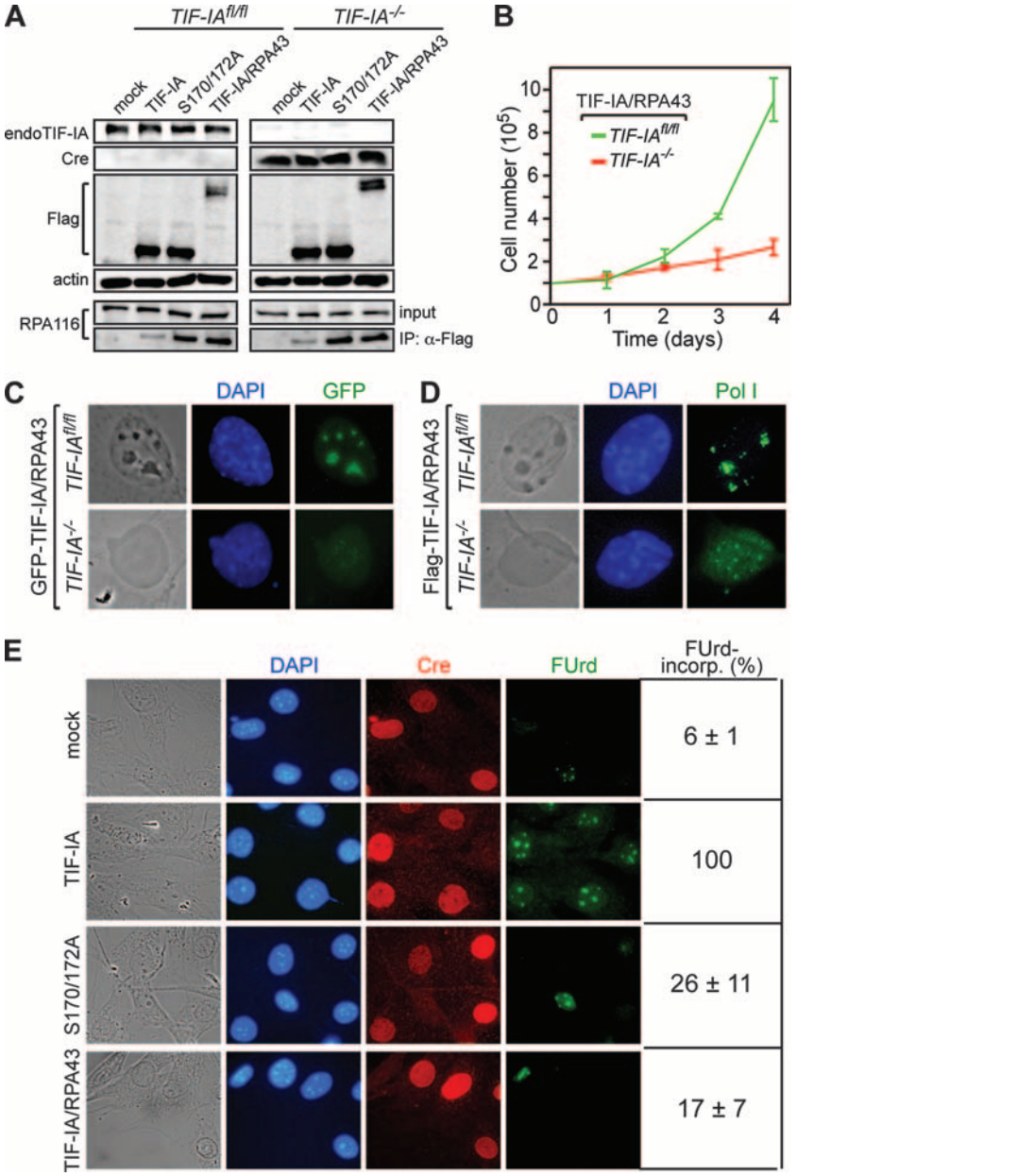

FIG. 4. Covalent association of TIF-IA with Pol I impairs rDNA transcription. (A) TIF-IA/RPA43 is incorporated into Pol I. Western blots of *TIF-IA*$^{fl/fl}$ MEFs infected with MSCV (*TIF-IA*$^{fl/fl}$) or MSCV-Cre (*TIF-IA*$^{-/-}$) showing Cre-mediated depletion of endogenous TIF-IA and expression of exogenous TIF-IA. Blots were probed with antibodies specific for TIF-IA, Cre recombinase, the Flag epitope, or β-actin. In the bottom panel, Flag-tagged TIF-IA, TIF-IAS170/172A, and TIF-IA/RPA43 were immunoprecipitated, and coprecipitated Pol I (RPA116) was visualized on immunoblots. (B) Fusion of TIF-IA with Pol I inhibits cell proliferation. *TIF-IA*$^{fl/fl}$ MEFs expressing TIF-IA/RPA43 and creER^{T2} were either mock or 4-OHT treated (*TIF-IA*$^{fl/fl}$ or *TIF-IA*$^{-/-}$), and cell numbers were determined at the indicated time points. Error bars represent numbers from three different experiments. (C) Tethering of TIF-IA to RPA43 causes nucleolar disruption. *TIF-IA*$^{fl/fl}$ MEFs coexpressing creER^{T2} and GFP-TIF-IA/RPA43 were mock or 4-OHT treated (*TIF-IA*$^{fl/fl}$ or *TIF-IA*$^{-/-}$), and localization of TIF-IA/RPA43 was monitored by GFP fluorescence. DAPI, 4′,6′-diamidino-2-phenylindole. (D) Overexpression of TIF-IA/RPA43 leads to mislocalization of Pol I. MEFs expressing creER^{T2} as well as Flag-TIF-IA/RPA43 were either mock treated (*TIF-IA*$^{fl/fl}$) or treated with 4-OHT (*TIF-IA*$^{-/-}$), and distribution of Pol I (RPA194) was visualized by indirect immunostaining. (E) TIF-IAS170/172A and TIF-IA/RPA43 do not rescue Pol I transcription in TIF-IA-deficient cells. *TIF-IA*$^{fl/fl}$ MEFs expressing ectopic TIF-IA, TIF-IAS170/172A, or TIF-IA/RPA43 were depleted from endogenous TIF-IA by MSCV-Cre infection and pulse-labeled with FUrd, and FUrd incorporation into nascent RNA was monitored by indirect immunofluorescence. Cells were costained with anti-Cre antibodies. FUrd staining of at least 200 Cre-positive cells was quantified using NIS-Elements BR imaging software (Nikon); the value for *TIF-IA*$^{-/-}$ MEFs expressing wild-type TIF-IA was set to 100%.

of TIF-IA with Pol I in the absence and presence of exogenous FCP1. Indeed, overexpression of FCP1 enhanced the interaction of TIF-IA with Pol I, the levels being similar to that of TIF-IAS170/172A (Fig. 6F). This result is consistent with FCP1 facilitating the reassembly of Pol I/TIF-IA complexes by removing the phosphate groups from Ser170/172. In accord with this, knockdown of FCP1 by siRNA impaired the association of TIF-IA with Pol I (Fig. 6G), leading to a

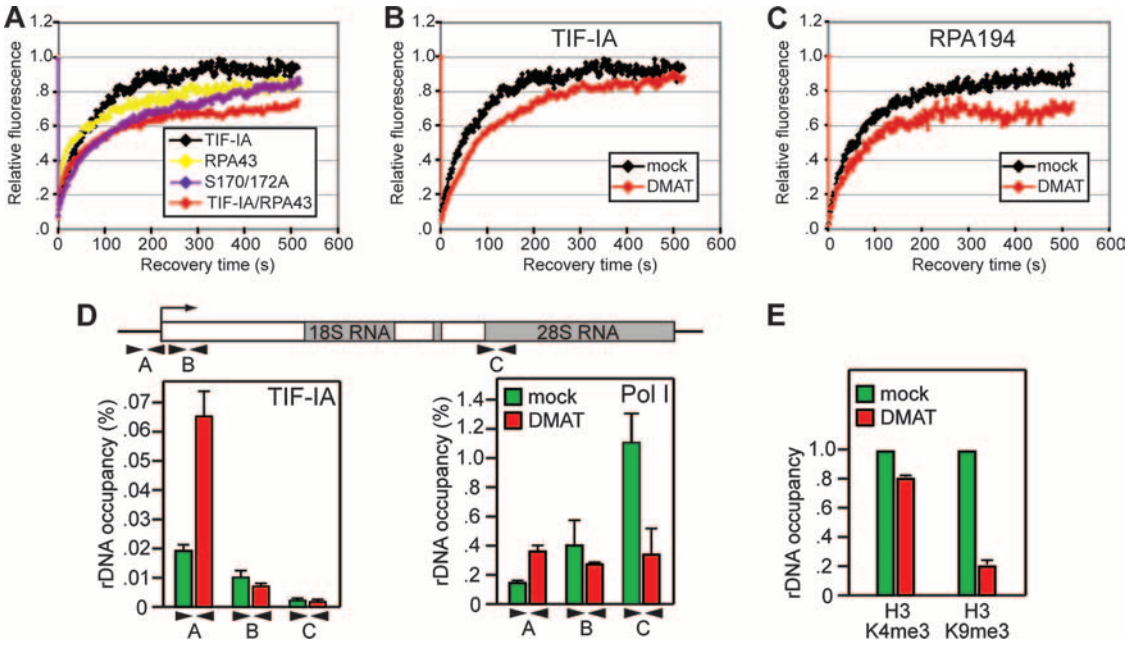

FIG. 5. Dissociation of TIF-IA from elongating Pol I is required for efficient rDNA transcription. (A) Enhanced association with Pol I decreases the mobility of TIF-IA. Graphs of FRAP analyses showing the recovery kinetics of GFP-tagged TIF-IA, TIF-IAS170/172A, TIF-IA/RPA43, or RPA43 in HeLa cells. In each case values from at least 15 cells were averaged. (B) Inhibition of CK2 decreases the mobility of TIF-IA. FRAP recovery kinetics of GFP-TIF-IA in HeLa cells that were either mock treated (black curve) or treated with 50 μM DMAT for 2 h (red curve). (C) Inhibition of S170/172 phosphorylation reduces the dynamics of Pol I. FRAP recovery kinetics of GFP-RPA194 was monitored in mock- or DMAT-treated HeLa cells. (D) Ablation of Ser170/172 phosphorylation leads to enrichment of Pol I at the rDNA promoter. Cross-linked chromatin from mock- or DMAT-treated NIH 3T3 cells was immunoprecipitated with antibodies against TIF-IA or Pol I, and precipitated DNA was assayed by quantitative PCR, amplifying the rDNA promoter (−160/−1, primer pair A) or the coding region (+451/+670 and +8124/+8268, primer pairs B and C, respectively). The bar diagram shows the amounts of precipitated rDNA in mock-treated (green bars) and DMAT-treated (red bars) cells normalized to input rDNA. Mean values from three independent experiments are shown. (E) The association of H3K9me3, but not H3K4me3, with the pre-rRNA coding region is altered upon inhibition of CK2. Data from ChIP experiments showing rDNA occupancy of H3K4me3 and H3K9me3 in mock-treated (green bars) and DMAT-treated (red bars) cells. Immunoprecipitated DNA was assayed by quantitative PCR, amplifying the coding region (positions +8124 to +8268). The levels of methylated H3K4 and K9 were normalized to that of histone H3, and values for mock-treated cells were set as 1. Error bars represent standard deviations of three independent experiments.

strong inhibition of Pol I transcription (Fig. 6H). Finally, moderate overexpression of FCP1 activated Pol I transcription, whereas high levels of FCP1 decreased pre-rRNA synthesis, supporting the view that a fine-tuned balance between phosphorylation and dephosphorylation of Ser170/172 regulates TIF-IA activity.

DISCUSSION

TIF-IA, a key player in Pol I transcription, is targeted by a variety of signaling pathways that regulate TIF-IA activity in response to external signals. The present study demonstrates that CK2 phosphorylates TIF-IA at Ser170 and Ser172, and reversible phosphorylation of these serine residues is required for efficient Pol I transcription. CK2 is known to be present in the nucleolus (2, 32) and copurifies with Pol I (15, 34). Previous studies have demonstrated that CK2 is associated with the initiation-competent Pol Iβ complex, suggesting that CK2 promotes early steps in Pol I transcription (31). Pol Iβ-associated CK2 has been shown to phosphorylate UBF, topoisomerase IIα, and the TAF_I110 subunit of the promoter selectivity factor TIF-IB/SL1 (23, 31, 40). Moreover, transcription reactions with immobilized rDNA templates indicated that phosphorylation by CK2 stabilizes TIF-IB/SL1-dependent binding of UBF at the rDNA promoter, the first step in preinitiation complex formation, thereby promoting multiple rounds of transcription initiation (23, 31). In support of the idea of CK2 regulating Pol I transcription at multiple levels, our study demonstrates that phosphorylation by CK2 is required for TIF-IA function and Pol I transcription. We have used the specific CK2 inhibitor DMAT as well as mutational analysis to show that CK2-dependent phosphorylation of TIF-IA at Ser170/172 weakens the association of TIF-IA with Pol I, leading to dissociation of TIF-IA from the elongation complex. Previous studies have shown that TIF-IA is released from Pol I after transcription initiation and is capable of reassembling with preinitiation complexes lacking TIF-IA (38). Other studies have suggested that TIF-IA is inactivated or consumed during transcription, losing the ability to associate with Pol I and facilitate a new round of transcription (7, 17). Our finding that reversible phosphorylation regulates the formation of the Pol I/TIF-IA complex may reconcile these apparently contradictory results. We show that phosphorylation by CK2 does not affect the recruitment of Pol I/TIF-IA complexes to promoter-bound TIF-IB/SL1, i.e., the formation transcription initiation complexes, but triggers the dissociation of TIF-IA from Pol I after transcription initiation. Dephosphorylation by FCP1, on the other hand, promotes interaction with Pol I, thereby facil-

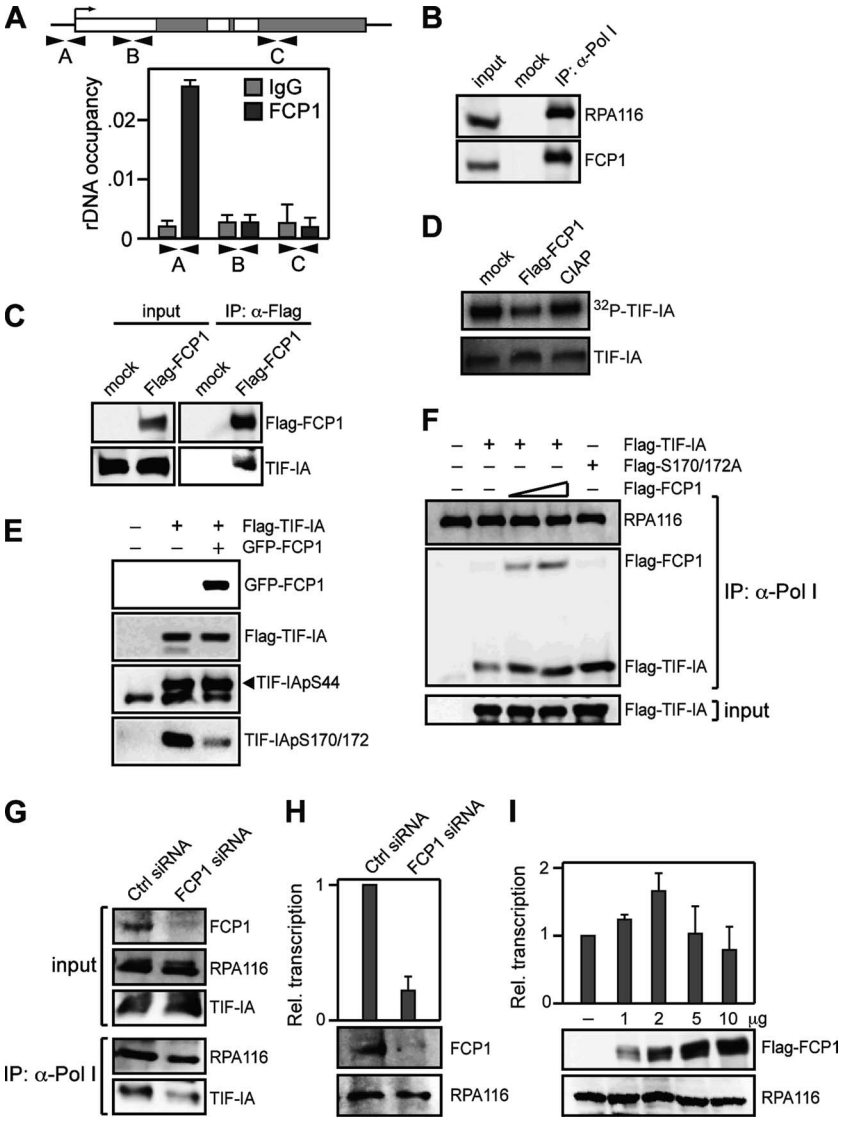

FIG. 6. FCP1 stimulates rDNA transcription by counteracting CK2-mediated phosphorylation of TIF-IA. (A) FCP1 associates with the rDNA promoter. Cross-linked chromatin from HEK293T cells was immunoprecipitated with antibodies against FCP1 (dark bars) or control immunoglobulin Gs (light bars), and precipitated DNA was assayed by quantitative PCR with primers amplifying the rDNA promoter (primer pair A) or the coding region (primer pairs B and C). Amounts of precipitated DNA normalized to input rDNA are shown. Mean values are from three independent experiments. (B) FCP1 interacts with Pol I. Pol I was immunoprecipitated from HEK293T cells, and coprecipitated FCP1 was monitored on Western blots with anti-FCP1 antibodies. (C) FCP1 interacts with TIF-IA. Lysates from HEK293T cells overexpressing Flag-FCP1 were incubated with anti-Flag antibodies, and coprecipitation of TIF-IA with Flag-FCP1 was analyzed on Western blots. (D) FCP1 dephosphorylates Ser170/172 of TIF-IA in vitro. Flag-tagged FCP1 was immunopurified from HEK293T cells, and phosphatase activity was assayed using *p*-nitrophenyl phosphate as a substrate. To monitor dephosphorylation of Ser170/172, TIF-IA was ^{32}P labeled with CK2 and incubated with Flag-FCP1 or the same activity of CIAP. Phospho-TIF-IA was visualized by autoradiography (upper panel), and total TIF-IA was visualized by Ponceau staining (lower panel). (E) FCP1 dephosphorylates Ser170/172 in vivo. The expression of GFP-FCP1 and Flag-TIF-IA in HEK293T cells was analyzed on Western blots (top two panels). After immunoprecipitation with anti-Flag antibodies, phosphorylation of TIF-IA was monitored with antibodies that specifically recognize phospho-Ser170/172 (pS170/172) and phospho-Ser44 (pS44). The arrowhead marks the specific band recognized by the anti-phospho-Ser44 antibody. (F) Overexpression of FCP1 increases the formation of Pol I/TIF-IA complexes. Pol I was precipitated from HEK293T cells expressing Flag-tagged TIF-IA or TIF-IAS170/172A in the absence or presence of Flag-FCP1, and coprecipitated Flag-TIF-IA and Flag-FCP1 were visualized on Western blots. (G) Knockdown of FCP1 disrupts Pol I/TIF-IA complexes. U2OS cells were transfected with control duplex siRNA directed against GFP (Ctrl siRNA) or with FCP1-specific siRNA. Cell were lysed, and Pol I was immunoprecipitated with anti-RPA194 antibodies. Amounts of coprecipitated TIF-IA as well as levels of FCP1, RPA116, and TIF-IA in the inputs were monitored on Western blots. (H) FCP1 is required for Pol I transcription. Cells were transfected with control or FCP1-specific siRNAs. The knockdown of FCP1 was monitored on immunoblots. The synthesis of pre-rRNA was measured by quantitative reverse transcription-PCR and normalized to glyceraldehyde-3-phosphate dehydrogenase. Mean values are from two independent experiments. (I) Modulation of FCP1 levels affects Pol I transcription. HEK293T cells were transfected with the indicated amounts of expression vectors encoding Flag-FCP1, and pre-rRNA was measured by quantitative reverse transcription-PCR. Mean values are from three independent experiments. The Western blots below show the expression levels of Flag-FCP1 and Pol I (RPA116).

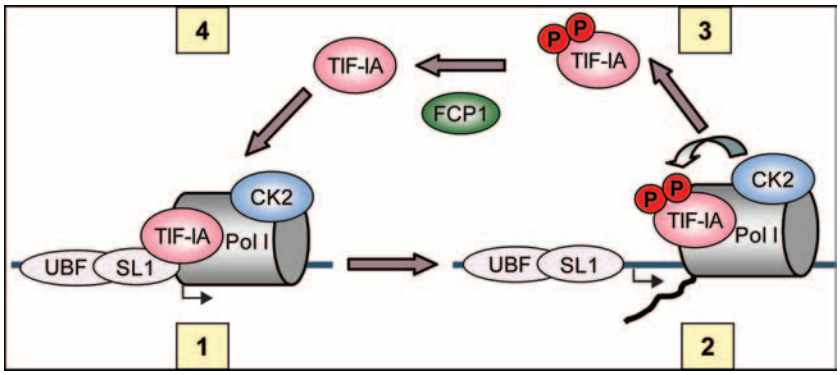

FIG. 7. Model depicting the role of reversible phosphorylation of TIF-IA in the Pol I transcription cycle. UBF and TIF-IB/SL1 bound to the rDNA promoter recruit Pol Iβ, the initiation-competent subpopulation of Pol I containing CK2, to rDNA (step 1). After transcription initiation and promoter escape, CK2 phosphorylates Pol I-associated TIF-IA at Ser170/172 (step 2). This phosphorylation weakens the association of TIF-IA, leading to dissociation of the Pol I/TIF-IA complex (step 3). Then, Ser170/172 is dephosphorylated by FCP1, which enables TIF-IA to assemble into a new initiation complex and to reinitiate transcription (step 4).

itating the reassembly of productive transcription initiation complexes. The impact of CK2-dependent dissociation of Pol I/TIF-IA complexes may have been overlooked in a previous study focusing on the role of CK2 in the interaction between UBF and TIF-IB/SL1 (23).

We propose a model in which Ser170/172 phosphorylation determines whether Pol I is associated with TIF-IA, i.e., is capable of initiating transcription, or whether Pol I is capable of proceeding into transcription elongation (Fig. 7). The following lines of experimental evidence support this model. First, CK2 is present at the rDNA promoter (23, 31), and our ChIP data reveal that inhibition of CK2 leads to an increase of Pol I in the 5′-terminal region of rDNA. Second, studies with yeast and mammals have shown that TIF-IA/Rrn3 dissociates from Pol I as a consequence of transcription (3, 17, 27). Third, our study shows that phosphorylation of TIF-IA at Ser170/172 is not involved in the assembly of transcription initiation complexes but is required for dissociation from elongating Pol I. Together, these results reveal that CK2 is the key factor that regulates the dissociation and reassembly of initiation complexes after each round of transcription.

The situation is different in yeast, where phosphorylation of Pol I, rather than Rrn3, regulates the interaction of Rrn3 with Pol I (12), suggesting that yeast and mammals use different mechanisms to release TIF-IA/Rrn3 from elongating Pol I. In support of this, the CK2 target sites are not conserved between TIF-IA and Rrn3, and transcription-coupled dissociation of the Pol I/Rrn3 complex does not require reversible phosphorylation of Rrn3. Moreover, an Rrn3-deficient yeast strain expressing a nondissociable Pol I/Rrn3 complex was viable and had the same growth characteristics as did wild-type cells, indicating that dissociation of Rrn3 from elongating Pol I is not required for efficient transcription and reinitiation (22). In contrast, our similar experimental approach in mammalian cells revealed that covalent tethering of TIF-IA to Pol I impaired TIF-IA function and Pol I transcription. This indicates that the cyclic formation and disruption of the Pol I-TIF-IA/Rrn3 complex during each round of transcription are mandatory in mammals but not in yeast. Interestingly, recent FRAP data from mammalian cells have shown that the dynamics of Pol I assembly determines the transcriptional output, increased transcription initiation and pre-rRNA synthesis being accompanied with prolonged retention of Pol I components at the promoter. Once Pol I is properly assembled, the rate of entry into processive elongation is elevated (13). Our FRAP data are consistent with and extend these results, demonstrating that dynamic assembly and disruption of initiation complexes are required to sustain high levels of Pol I transcription. Given that CK2 targets several components of the Pol I machinery, it remains to be investigated how phosphorylation by CK2 coordinates the interplay of the individual proteins that are required for assembly and disassembly of the initiation complex.

CK2 also activates Pol III transcription (18, 19), suggesting that CK2-dependent phosphorylation may provide a mechanism to coregulate the transcriptional output of Pol I and Pol III. With regard to Pol II, reversible phosphorylation of its C-terminal domain (CTD) is known to play an essential role in the transcription process. After transcription initiation, the CTD is phosphorylated and hyperphosphorylation is required for efficient transcription elongation. The major protein phosphatase known to dephosphorylate the CTD is FCP1 (8, 24). FCP1 is associated with the RAP74 subunit of TFIIF, and FCP1-dependent dephosphorylation of the CTD is required for recycling of Pol II and reinitiation of transcription. Notably, CK2 also phosphorylates FCP1, and phosphorylation enhances the CTD-phosphatase activity of FCP1 (1, 30). Thus, reversible phosphorylation of components of the transcription apparatus by CK2/FCP1 is an essential and conserved mechanism promoting early stages of transcription and recycling of nuclear RNA polymerases. In support of this, RPA49 (also known as PAF53), a Pol I subunit that controls binding and release of TIF-IA/Rrn3 (4), shows sequence homology to RAP74 (21). This suggests a common mechanism of FCP1 recruitment to Pol I and Pol II promoters, i.e., by interaction with RPA49/PAF53 and with RAP74, respectively. Phosphorylation by CK2 is known to increase the association of FCP1 with RAP74 (1, 30) and therefore may also augment binding of FCP1 to RPA49/PAF53, which then would trigger dephosphorylation of Ser170/172. Thus, CK2 appears to serve a dual function in Pol I transcription, promoting elongation by phosphorylation-de-

pendent release of TIF-IA and facilitating a new round of transcription initiation by phosphorylation of FCP1.

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