FCP1 Phosphorylation by Casein Kinase 2 Enhances Binding to TFIIF and RNA Polymerase II Carboxyl-terminal Domain Phosphatase Activity*

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Dephosphorylation of RNA polymerase II carboxyl-terminal domain (CTD) is required to resume sequential transcription cycles. FCP1 (TFIIF-dependent CTD phosphatase 1) is the only known phosphatase targeting RNAPII CTD. Here we show that in Xenopus laevis cells, xFCP1 is a phosphoprotein. On the basis of biochemical fractionation and drug sensitivity, casein kinase 2 (CK2) is shown to be the major kinase involved in xFCP1 phosphorylation in X. laevis egg extracts. CK2 phosphorylates xFCP1 mainly at a cluster of serines centered on Ser457. CK2-dependent phosphorylation enhances 4-fold the CTD phosphatase activity of FCP1 and its binding to the RAP74 subunit of general transcription factor TFIIF. These findings unravel a new mechanism regulating CTD phosphorylation and hence class II gene transcription.

RNA polymerase II (RNAPII)* is a multiprotein complex in charge of eucaryotic mRNA synthesis (1). Rpb1, the largest RNAPII subunit, is extensively phosphorylatable on its carboxyl-terminal domain (CTD), which consists of up to 52 repeats of the consensus heptapeptide Tyr-Ser-Pro-Thr-Ser-Pro-Ser (2). Two phosphoisoforms of RNAPII coexist in somatic cells in a dynamic equilibrium (3). The hypophosphorylated IIA form assembles into preinitiation complexes, whereas the hyperphosphorylated IIO form elongates mRNA synthesis and recruits mRNA processing factors (4). The conversion from IIA to IIO occurs during initiation and elongation of transcription and involves CTD kinases such as CDK7 and CDK9, respectively, included in the transcription factors TFIH and p-TFEB (5, 6). CTD dephosphorylation is required to recycle RNAPII after a transcriptional round (7).

In contrast to the numerous CTD kinases identified, only one CTD phosphatase has been characterized so far. It has been purified from yeast and mammalian cells (8, 9), and the corresponding cDNA has been cloned by a two-hybrid screen using the RAP74 subunit of the general transcription factor TFIIF as a bait (10). FCP1 (TFIIF-dependent CTD Phosphatase 1) consists of a single 150-kDa polypeptide subunit (11, 12). FCP1 does not display any homology with other known protein phosphatases but possesses an unusual catalytic site present in a subset of phosphotransferases (13, 14). FCP1 is a highly specific phosphatase as the CTD within a functional RNAPII remains its only known protein substrate. FCP1 is recruited to RNAPII through docking sites on the Rpb4 subunit of RNAPII and on the RAP74 subunit of TFIIF (15, 16).

FCP1 activity is regulated at various levels. First, competitive binding of general transcription factors TFIIF and TFIIB to FCP1 carboxyl-terminal domain, respectively, stimulates or inhibits its phosphatase activity (15, 17). Second, free RNAPII IIO molecules are better substrates than molecules engaged in transcription elongation complexes (18, 19). Third, inhibition of FCP1 phosphatase activity by the human immunodeficiency viral protein Tat may contribute to stimulate transcriptional elongation from the human immunodeficiency virus promoter (20, 21).

We had characterized previously (22) the Xenopus laevis FCP1 orthologue, xFCP1. We now report that, in Xenopus somatic cells and eggs, xFCP1 is phosphorylated. The major kinase involved in this process is identified as casein kinase 2 (CK2), a pleiotropic kinase involved in various cellular processes including cell proliferation and gene expression (23). CK2 phosphorylation strongly enhances FCP1 binding to TFIIF and CTD phosphatase activity. These findings unravel a new mechanism potentially regulating CTD dephosphorylation.

EXPERIMENTAL PROCEDURES

Xenopus Cells and Egg Extracts—A6 cells derived from Xenopus kidney were propagated as described previously (22). 24 h after plating, subconfluent cells were lysed in PB buffer (50 mM Tris-HCl, pH 7.9, 10 mM MgCl2, 10 mM KCl, 0.1 mM EDTA, 10% glycerol, 0.5 mM dithiothreitol (DTT)) supplemented with 0.5% Nonidet P-40. The lysate was clarified by centrifugation at 10,000 × g for 10 min. The resulting supernatant was incubated for 30 min at 37 °C in the presence or absence of 2.8 units per μl of calf-intestinal alkaline phosphatase (Amersham Biosciences). The reaction was stopped by addition of the same volume of Laemmli sample buffer. Interphasic egg extracts were prepared according to Murray (24) with modifications described previously (22).

Plasmids—To identify the CK2 phosphorylation sites, the xFCP1 coding sequence was PCR-amplified from pGEX4T2-xFCP1wt (22) and inserted in-frame with an amino-terminal histidine tag in pET19b vector (Novagen). Site-directed mutagenesis was performed using QuikChange protocol (Stratagene). Insertion of the desired point mutations was checked by sequencing. X. laevis RAP74 (xRAP74) was cloned by taking advantage of a pre-existing sequence in GenBank™ data base (accession number Z17426). Total RNAs were extracted from A6 cells using the RNeasy kit (Qiagen). The primers ATG TGC ACA TAT GGC ATC GCT GGG CAC CAG and ATG GAT CCT CAC TCC TTA AGG TAA AAG TGC ATC TTG TC were used to amplify the full-length xRAP74 open reading frame by reverse transcription-PCR (Thermoscript RT; Invitrogen). The 1,593-bp PCR product was sequenced and a BamHI/NdeI restriction fragment was subcloned in pET19b providing

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plasmid pET19-xRAP74. The insert was sequenced and differed slightly from the preexisting one and was therefore submitted to GenBank under accession number AY114105.

Recombinant Proteins—For protein expression, Escherichia coli BL21(DE3) codon + cells (Stratagene) were transformed with the appropriate plasmids, and an exponentially growing culture was induced for 3 h at 37 °C with 0.3 mM isopropyl-β-D-thiogalactopyranoside. xFCP1 fused to GST was purified on glutathione-Sepharose 4B beads (22). For His-tagged proteins production, bacterial cells were harvested, resuspended in 1:20 culture volume of phosphate-buffered saline (PBS), and sonicated. The resulting lysate was supplemented with 1% Triton X-100, 10 mM imidazole, and 500 mM NaCl and clarified by centrifugation at 10,000 × g for 10 min. The supernatant was bound onto Ni²⁺-chelating Sepharose Fast Flow (Amersham Biosciences). Bound fusion proteins were recovered with Elution Buffer (20 mM NaHPO₄, 500 mM NaCl, 500 mM imidazole, pH 7.4) and dialyzed against 50 mM Tris, pH 7.4, 100 mM NaCl, 1 mM MgCl₂, 10% (w/v) glycerol, 0.5 mM DTT. Western Blot and Far Western Blot—Samples in Laemmli buffer were heated for 5 min at 95 °C before loading on SDS-PAGE. Western blots were carried out using as primary antibodies: anti-human FCP1 (kindly provided by Dr. Jack Greenblatt), anti-human RAP74 (kindly provided by Dr. Zachary Burton), and anti-CK2α (Calbiochem). Reactive bands were visualized using horseradish peroxidase-conjugated secondary antibodies (Promega) and chemiluminescence (Fierce). For RAP74 Far Western blots, the nitrocellulose membranes were incubated overnight at 4 °C in Blocking buffer (5% w/v skim milk, 20 mM Tris, pH 7.6, 137 mM NaCl, 0.2% Tween 20, 2 mM DTT, 0.5 mM phenylmethylsulfonyl fluoride) and hybridized for 4 h at room temperature with Blocking buffer supplemented with 1 µg recombinant Xenopus RAP74. After extensive washing, membranes were processed as for Western blots using anti-RAP74 as primary antibody.

Immunoprecipitations—20 µl of protein A-Sepharose beads (Amersham Biosciences) equilibrated in PB buffer were incubated overnight at 4 °C with 20 µl of anti-FCP1 or mock serum. The beads were washed with PB buffer and incubated with 10 µl of crude interphasic Xenopus egg extract previously radiolabeled for 30 min at room temperature in the presence of 5 Ci of [γ-32P]ATP (5000 Ci/mmol). After 30 min of shaking at 4 °C, the beads were washed 3 times with PB buffer and resuspended in Laemmli buffer. Radiolabeled proteins were analyzed by SDS-PAGE followed by autoradiography.

Fast Protein Liquid MonoQ Chromatography—Interphase extracts (0.3 ml) from freshly laid eggs were diluted 10-fold in CB (Chromatography buffer, 20 mM Tris, pH 7.9, 20 mM NaCl, 5 mM MgCl₂, 0.5 mM DTT), filtered through a 0.2-µm Millipore membrane and immediately applied to an HR5/5 MonoQ column (Amersham Biosciences). The column was developed at a flow rate of 1 ml/min, with a 20-ml linear NaCl gradient (20–500 mM) in CB buffer followed by a 10-ml linear NaCl gradient (500–1,000 mM) in CB buffer. 1-ml fractions were collected, supplemented with glycerol (10% final), and stored at −80 °C.

Protein Kinase Assays—Aliquots (5 µl) of column input or fractions from the MonoQ column were added to 10 µl of GP buffer (20 mM sodium pyrophosphate, 5 mM MgCl₂, 1 mM EDTA, 1 mM NaN₃, 10% glycerol, 1 mM DTT, 0.1% bovine serum albumin) supplemented with 0.1 mM ATP, 1 µCi of [γ-32P]ATP (5,000 Ci/mmol), and the appropriate substrate as follows: full-length recombinant xFCP1 (1 µg) or casein (Sigma) (2 µg). When indicated, the reaction contained 20 µM histidine (Sigma) or 100 µg/ml poly-L-lysine (Sigma). The phosphorylation was allowed to proceed for 30 min at 30 °C and was stopped by addition of 15 µl of 2× Laemmli buffer. Samples were analyzed by SDS-PAGE and autoradiography. Quantification of kinase activity was performed using a Fujifilm BAS 3000 Imager. Alternatively, 5 µl of crude interphasic egg extract was incubated for increasing times at 30 °C in the presence of recombinant Xenopus FCP1 (1 µg) and 1 mM ATP in a total volume of 15 µl of GP buffer. Reaction was arrested as above and analyzed by SDS-PAGE followed by anti-FCP1 Western blot. Recombinant FCP1 was phosphorylated by casein kinase 2 as follows: 1 µCi of [γ-32P]ATP was incubated for 30 min at 30 °C in the presence of 0.1 mM ATP, 1 µCi of [γ-32P]ATP (5,000 Ci/mmol), and 20 micromolar of recombinant casein kinase 2 (reconstituted holoenzyme αβ₂, Roche Molecular Biochemicals), in a total volume of 15 µl of GP buffer. For extensive phosphorylation, cold ATP only was used at a 5 mM concentration.

Phosphorylation on Glutathione-Sepharose Beads and GST Pull-down—Glutathione-Sepharose beads coated with GST-xFCP1 fusion proteins were incubated with 7.5 million units per milliliter of purified CK2 (Roche Molecular Biochemicals) in GP buffer in the presence of 5 mM ATP for 40 min at 30 °C under agitation. The beads were next washed three times with PBS supplemented with 1% Triton X-100, and the fusion proteins were eluted with 10 mM glutathione in 50 mM Tris-HCl, pH 8. The GST moiety was removed from the eluted fusion protein by digestion with 10 units of thrombin (Amersham Biosciences) per mg of protein for 30 min at room temperature. Thrombin was next neutralized by addition of 1 mM phenylmethylsulfonyl fluoride (Sigma). Alternatively, for GST pull-down, the beads were incubated for 15 min at 4 °C in 5% bovine serum albumin in PBS. 1 mM of recombinant xRAP74 was then added, and incubation was followed for 1 h. After extensive washing with PBS, beads were resuspended in Laemmli sample buffer, and SDS-PAGE analysis was performed.

RESULTS

Xenopus FCP1 Is a Phosphoprotein—To investigate post-translational modifications, electrophoretic mobilities of endog-
FIG. 3. CK2 copurifies with the FCP1 kinase. 

A, fractions of the MonoQ column were assayed for casein kinase activity in the presence of casein and [γ-32P]ATP. Incorporation of radioactivity in casein was analyzed by SDS-PAGE and autoradiography. Position of caseins is marked by an asterisk. CK2α, the catalytic subunit of CK2, was detected by Western blot. B, FCP1 kinase and casein kinase activities were quantified and plotted against the elution profile of the MonoQ column. The NaCl gradient concentration is visualized as a dotted line. C, FCP1 kinase activity of fraction 19 was assayed and quantified in the presence or absence of the indicated inhibitors. The position of xFCP1 is indicated.

FIG. 4. CK2 phosphorylates xFCP1 at a cluster of serines centered on Ser457. 

A, left panel, recombinant xFCP1 was incubated or not with ATP and purified CK2. Phosphorylation of FCP1 was followed by SDS-PAGE and Western blot. Positions of unphosphorylated (xFCP1) and phosphorylated xFCP1 (xFCP1-P) are indicated. Right panel, recombinant xFCP1 was incubated or not with purified CK2 in the presence of [γ-32P]ATP. Incorporation of 32P in xFCP1 was analyzed by SDS-PAGE and autoradiography. 

B, alignment of FCP1 serines clusters A–C for human (Hs) or Xenopus (Xl) proteins. The serine residues mutated to alanine are underlined. C, xFCP1 (wild type (wt) or mutant (mut)) was phosphorylated by CK2 and analyzed by Western blot. Positions of xFCP1 and phosphorylated xFCP1 (xFCP1-P) are indicated.
Endogenous and recombinant xFCP1 proteins were compared. Endogenous FCP1 present in Xenopus A6 cells was totally extracted in a low salt buffer (22) and migrated as a protein doublet, slower than the recombinant protein, as revealed by Western blot (Fig. 1A, middle and left lanes). To test if such a slower migration was due to phosphorylation, the cell extract

**Fig. 5. Phosphorylation of FCP1 by CK2 increases its specific activity.**

A, recombinant xFCP1 was phosphorylated or not by CK2, purified, and assayed for CTD phosphatase activity by incubation with labeled RNAP IIO in the presence of xRAP74. The time of reaction is indicated (minutes). The positions of Iio and Iia forms are indicated. B, dephosphorylation time courses were obtained for xFCP1 (○) and CK2-phosphorylated xFCP1 (xFCP1-P, □). The amount in the Iia form (% of total RNAP II-associated-radioactivity) was plotted as a function of the reaction time (minutes). C, CTD phosphatase activity was assayed for xFCP1 (○) and xFCP1-P (□) in the presence of increasing amounts of recombinant xRAP74. Activity (AU, arbitrary units) was quantified after autoradiography and plotted against RAP74 amounts. D, p-Nitrophenylphosphatase activity was assayed for different amounts (µg) of recombinant xFCP1 (○) and xFCP1-P (□). The release of p-nitrophenol was estimated by reading the absorbance at 410 nm. E, CTD phosphatase activity was measured for xFCP1 wild-type (wt) and mutant (mut) A phosphorylated (phosph) or not by CK2. The activity reported is expressed as a percentage of wild-type protein activity and represents the mean of four independent experiments.
was treated with alkaline phosphatase before SDS-PAGE. After such a treatment, xFCP1 migrated as fast as the recombinant protein (right lane). Thus, FCP1 is phosphorylated in A6 cells, and phosphorylation is sufficient to account for the difference in electrophoretic properties between cellular and recombinant proteins.

FCP1 phosphorylation was investigated in Xenopus egg extract, a system that supports elaborate cellular functions (24). [γ-32P]ATP was added to an interphasic egg extract, and immunoprecipitation was performed with an anti-FCP1 antibody. Autoradiography revealed a unique 32P-labeled band that comigrated with xFCP1 (Fig. 1B). This band was present in anti-FCP1 but not in mock immunoprecipitates. Therefore, xFCP1 is phosphorylated in Xenopus egg extracts as well as in somatic cells.

A Strong FCP1 Kinase Activity Is Present in Xenopus Egg Extracts—The latter experiment suggested that an FCP1 kinase(s) is(are) present in egg extracts. To assay FCP1 kinase activity, recombinant FCP1 was incubated with egg extracts for increasing times. An ATP-dependent decrease in FCP1 electrophoretic mobility was observed by Western blot (Fig. 2A). After 1 h of incubation, the migration of recombinant FCP1 became identical to that of cellular FCP1. Hence egg extracts contain kinase(s) that may account for FCP1 phosphorylation.

To identify this (these) kinase(s), an egg extract was fractionated on a MonoQ anion exchange column, and fractions were assayed for FCP1 kinase activity. A radioactive band at the expected migration was visualized when fractions 18–20 were incubated with recombinant FCP1 and [γ-32P]ATP (Fig. 2B), but not when the protein substrate was omitted (data not shown). This activity peaked in fraction 19 (corresponding to a 460 mM NaCl elution). Thus, a single FCP1 kinase activity fractionated from egg extracts.

CK2 Is the Major FCP1 Kinase Present in Egg Extracts—Previous experiments from our laboratory had indicated that CK2 eluted from a MonoQ column with NaCl concentrations close to 450 mM (data not shown). We therefore investigated whether FCP1 kinase activity could be attributable to CK2. Aliquots of the MonoQ column were incubated with casein and [γ-32P]ATP. A casein kinase activity also peaked in fraction 19 (Fig. 3A, top). An antibody directed against human CK2α subunit detected a protein with the appropriate size (42 kDa) only in fraction 19 (Fig. 3A, bottom). Furthermore, the elution profiles for both CK2 and FCP1 kinase activities matched perfectly (Fig. 3B). CK2 activity is well established to be strongly inhibited by heparin but enhanced by polyamines (26). Indeed, the FCP1 kinase in fraction 19 was completely inhibited by heparin (Fig. 3C). In contrast, polylysine increased the FCP1 kinase activity (Fig. 3C). Taken together, these experiments suggest that CK2 is the dominant FCP1 kinase in Xenopus egg extracts.

CK2 Phosphorylates xFCP1 in Vitro at a Conserved Serine Cluster—The properties of recombinant xFCP1 phosphorylated by purified CK2 were investigated next. Phosphorylation by CK2 decreased xFCP1 electrophoretic mobility as revealed by Western blot (Fig. 4A, left panel). This change was due to phosphate incorporation as checked by autoradiography (Fig. 4A, right panel). CK2 phosphorylates mainly serine residues in an acidic context, within the consensus motif S(D/E)X(D/E) (27, 28). Several such potential CK2 phosphorylation sites are found in the xFCP1 protein sequence. Three serine clusters were conserved from frogs to humans as follows: three potential serine targets were present in cluster A, centered on Ser457, in the middle of the protein, in cluster B, centered on Ser886, at the carboxyl terminus, but cluster C, centered on Ser963 at the extreme carboxyl terminus, only showed a single serine target (Fig. 4B). It should be noted that phosphorylation of one serine may prime phosphorylation of other neighboring serines by introducing an acidic context (29).

To localize the phosphorylation sites, mutant His-tagged xFCP1 proteins were elaborated; in each mutant the potential CK2 serine targets within one cluster were replaced by alanines (Fig. 4B). Wild-type and mutant xFCP1 proteins were submitted to extensive CK2 phosphorylation followed by Western blot. The phosphorylation-induced shift of cluster B or cluster C mutant proteins was similar to that of the wild-type protein (Fig. 4C). In contrast, mutation of cluster A markedly decreased the shift. A similar decrease was observed when cluster A mutation was combined with mutations in clusters B or C. These results suggest that the major CK2 phosphorylation site in xFCP1 is localized within cluster A, centered on Ser457.

CK2 Phosphorylation Increases the CTD Phosphatase Activity of FCP1—To investigate the effect of CK2 phosphorylation on the CTD phosphatase activity, recombinant GST-xFCP1 was phosphorylated or not by CK2 on glutathione beads and eluted by reduced glutathione. CTD phosphatase activity was detected using 32P-labeled mammalian RNAP IIO and by taking advantage of the faster electrophoretic migration of the dephosphorylated Rpb1 subunit (22). RNAP IIO dephosphorylation time courses were performed in the presence of an excess of recombinant xRAP74 subunit of TFIIH (Fig. 5A). The amount in the dephosphorylated Rpb1 form, Ila, was quantified and plotted against the reaction time, demonstrating that CTD dephosphorylation followed a linear dependence with time (Fig. 5B). Most importantly, the CK2-phosphorylated xFCP1 was 4 times more active than control xFCP1.

RAP74 is known to stimulate FCP1 activity (15). Indeed, addition of xRAP74 markedly increased the CTD phosphatase activity, and a half-activation was observed with 10 ± 2 nM of RAP74. Phosphorylation of FCP1 did not appear to significantly modify the xRAP74 concentration dependence (Fig. 5C). The CTD phosphatase activity of both phosphorylated and...
unphosphorylated xFCP1 reached a plateau when the RAP74 concentration exceeded 20 nM. Thus, CK2 phosphorylation of FCP1 significantly increases the CTD phosphatase catalytic rate. 

*p*-Nitrophenyl phosphate has been used as an artificial substrate for *Saccharomyces cerevisiae or Schizosaccharomyces pombe* FCP1 (14, 30). Indeed, *p*-nitrophenol was generated in proportion to the amounts of FCP1 in the assay (Fig. 5D). However, CK2 phosphorylation of FCP1 did not significantly influence the *p*-nitrophenylphosphatase activity. Hence, the CK2 phosphorylation effect on FCP1 activity is specific for RNA polymerase II used as a substrate.

We next questioned the involvement of cluster A phosphorylation in the increase in CTD phosphatase activity. Wild-type or mutant His-tagged xFCP1 proteins were phosphorylated or not by CK2 and repurified by Ni²⁺ chromatography. The amounts of the four proteins were adjusted to provide a similar signal with in-gel Coomassie Blue staining (data not shown). CTD phosphatase activity was precisely measured for each protein in linear dephosphorylation conditions. Phosphorylation by CK2 strongly activated the wild-type protein but failed to significantly activate the mutant A (Fig. 5E). Hence CK2 phosphorylation of serines in cluster A accounts for the enhancement of FCP1 CTD phosphatase activity.

**CK2 Phosphorylation Enhances the Binding of FCP1 to the RAP74 Subunit of TFIIIF**—Genuine FCP1 is known to bind strongly to the RAP74 subunit of TFIIIF, and this interaction stimulates phosphatase activity (10, 15, 16). To investigate a possible modulation of FCP1 binding to TFIIIF by phosphorylation, a Far Western blot was performed using recombinant xRAP74 as a probe. The anti-RAP74 antibody did not react with xFCP1 phosphorylated or not (data not shown). RAP74 bound stronger on phosphorylated xFCP1 (xfcP1-P) than on control although equal amounts of proteins had been loaded (Fig. 6A).

To investigate RAP74-FCP1 interaction in non-denaturing conditions, GST pull-down experiments were performed. Glutathione-Sepharose beads coated with GST or GST-FCP1 previously phosphorylated or not by CK2 were incubated with recombinant RAP74. The amounts of GST, GST-xFCP1, and GST-xFCP1-P were checked to be identical by Coomassie Blue staining. In these conditions, phosphorylated FCP1 bound to RAP74 with a much stronger efficiency than control FCP1 (Fig. 6B). There was no discrepancy between this result and the kinetic data that were obtained using much higher concentrations of RAP74 not discriminating between FCP1 and FCP1-P anymore (Fig. 5C). Taken together, these data demonstrate that phosphorylation of FCP1 by CK2 strongly enhances its binding to RAP74.

**DISCUSSION**

Here we report that in *X. laevis* cells, the RNAP II CTD phosphatase FCP1 is a phosphoprotein. CK2 appears to be the *in vivo* FCP1 kinase: (i) CK2 cofractionates with the major FCP1 kinase activity eluting from a MonoQ column; (ii) FCP1 kinase activity and CK2 exhibit the same pattern of drug sensitivity; and (iii) purified CK2 is an efficient FCP1 kinase in *vitro*. Phosphorylation of recombinant FCP1 by CK2 enhances CTD phosphatase activity and binding to TFIIIF.

CK2 is a nuclear enzyme conserved in eucaryotes and exhibits pleiotropic functions; among them, it is required for cell cycle progression in yeast (26) and can function as an oncogene in mammals (31).

**FCP1 Phosphorylation, a Target for Regulating CTD Dephosphorylation—RNAP II CTD phosphorylation appears as a highly regulated process essential for gene expression (2, 4, 32, 33). Regulation of the enzymes involved in establishing the CTD phosphorylation profile is therefore critical. Phosphorylation of CTD kinases and/or their associated cyclins regulates global or specific gene transcription. For example, phosphorylation of CDK7 accounts for the inhibition of this TFIIH-associated kinase and thereby transcription during mitosis (34). Furthermore, phosphorylation of cyclin H by CDK8 negatively regulates CDK7 (35). Autophosphorylation of CDK9 might contribute to the positive control of human immunodeficiency virus-long terminal repeat-driven transcription (36).

We now show that phosphorylation of FCP1 by CK2 enhances its CTD phosphatase activity. Substrate recognition by FCP1 is indirect; although it dephosphorylates the CTD of Rbp1, its docking sites are located on distinct polypeptides, the Rbp4 subunit of RNA polymerase II and the RAP74 subunit of TFIIIF (16). As FCP1 phosphorylation does not affect its *p*-nitrophenylphosphatase activity, it might contribute to an adequate positioning of the CTD rather than modifying the catalytic site. *In vivo*, the enhanced binding of phosphorylated FCP1 to RAP74 may increase the influence of phosphorylation on the phosphatase activity.

CK2 activity is poorly regulated *in vivo*, and it has been suggested that phosphorylation of CK2 substrates might be the regulatory step (37). Although FCP1 is itself a phosphatase, it did not exhibit autodephosphorylation in our hands (data not shown). Interestingly, in *S. cerevisiae*, FCP1 has been reported to interact with a PP2C-related phosphatase (open reading frame number YCR079W) in a two-hybrid screen (38). FCP1 dephosphorylation might therefore regulate the CTD dephosphorylation process.

**Involvement of CK2 in Regulation of Gene Expression—**Most relevant to our findings, CK2 seems involved in transcription by the three classes of eucaryotic RNA polymerases. CK2 is clearly required for class III gene expression in yeast as well as in human cells (39, 40). CK2 forms a stable complex with TFIIIB, and phosphorylates its TATA-binding subunit. CK2 phosphorylation might be required for TFIIIB to be recruited on most but not all class III promoters. The CK2 inhibition that follows a genotoxic stress might therefore contribute to shut-off class III gene transcription. CK2 has also been suggested to be involved in class I gene expression as a component of the RNA polymerase I holoenzyme and as a kinase phosphorylating the upstream binding factor (UBF) (41–43). CK2 also targets several proteins in the basal class II transcriptional machinery *in vitro* and *in vivo*, including RNAP II itself (Ref. 44 and data not shown), TATA-binding protein (39, 45), and TFIIIF (46). Moreover, immunofluorescence studies on polytenic chromosomes have indicated that the CK2α catalytic subunit is colocalized with transcribing RNAPII (47). Genome scale analysis reveals that a subset of class II genes relies upon CK2 for their expression (48). Taken together, these data suggest that CK2 may play a role in transcription by phosphorylating specific substrates such as the FCP1 phosphatase.

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