Interaction of Fcp1 Phosphatase with Elongating RNA Polymerase II Holoenzyme, Enzymatic Mechanism of Action, and Genetic Interaction with Elongator*

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Fcp1 de-phosphorylates the RNA polymerase II (RNAPII) C-terminal domain (CTD) in vitro, and mutation of the yeast FCP1 gene results in global transcription defects and increased CTD phosphorylation levels in vivo. Here we show that the Fcp1 protein associates with elongating RNAPII holoenzyme in vitro. Our data suggest that the association of Fcp1 with elongating polymerase results in CTD de-phosphorylation when the native ternary RNAPII0-DNA-RNA complex is disrupted. Surprisingly, highly purified yeast Fcp1 de-phosphorylates serine 5 but not serine 2 of the RNAPII CTD repeat. Only free RNAPII0(Ser-5) and not RNA-PPI0-DNA-RNA ternary complexes act as a good substrate in the Fcp1 CTD de-phosphorylation reaction. In contrast, TFIIH CTD kinase has a pronounced preference for RNAPII incorporated into a ternary complex. Interestingly, the Fcp1 reaction mechanism appears to entail phosphoryl transfer from RNAPII0 directly to Fcp1. Elongator fails to affect the phosphatase activity of Fcp1 in vitro, but genetic evidence points to a functional overlap between Elongator and Fcp1 in vivo. Genetic interactions between Elongator and a number of other transcription factors are also reported. Together, these results shed new light on mechanisms that drive the transcription cycle and point to a role for Fcp1 in the recycling of RNAPII after dissociation from active genes.

During the RNA polymerase II (RNAPII)1 transcription cycle, both the phosphorylation state of the polymerase and the proteins that RNAPII interacts with change dramatically. Throughout promoter recruitment and initiation where RNA-PPI exists in a hypo-phosphorylated state, it interacts with general transcription factors, such as TFIIID, TFIIIB, TFIIF, TFIIE, TFIIH, and Mediator (1). In the process of promoter clearance and early transcript elongation where RNAPII becomes hyper-phosphorylated, interactions with initiation-specific factors are severed, and interactions with other proteins are established. Factors that have been shown to preferentially associate with hyper-phosphorylated elongating RNAPII include RNA processing factors (2) and factors thought to play a role in transcript elongation, such as Elongator, Set1, and Set2 (3–8).

The C-terminal repeat domain (CTD) of RNAPII is the specific target for kinases that phosphorylate the polymerase at the transition from initiation to elongation. The CTD consists of the conserved heptapeptide, Tyr-Ser-Pro-Thr-Ser-Pro-Ser, repeated 26 times in yeast and up to 52 times in metazoans (9). After transcript initiation, the CTD is heavily phosphorylated serine 2 and serine 5 by different protein kinases (pTEFb (yeast Bur1 or Cdk1)) and TFIIH, respectively (10, 11). Later, during transcript elongation and at transcriptional termination, CTD phosphatases remove the phosphate groups, enabling recycling of RNAPII for a new round of transcription (11, 12). A CTD phosphatase was first identified biochemically through its ability to specifically dephosphorylate the RNAPII CTD in vitro (13). The gene encoding a TFIIF-interacting protein was then identified and shown to encode this phosphatase (14). Indeed, the protein, now called Fcp1, directly de-phosphorylates RNAPII in vitro (13–17) in a reaction that is stimulated by TFIIF and inhibited by TFIIB (18). Fcp1 is an unusual magnesium-dependent phosphatase (16) that is not inhibited by most phosphatase inhibitors (13). Cells lacking Fcp1 function cease transcription of the majority of genes and accumulate hyper-phosphorylated RNAPII0 globally (16) and specifically in the coding region of genes (17). Fcp1 also has the ability to stimulate RNAPII transcript elongation in vitro independent of its phosphatase activity (19), suggesting that it associates with and modulates elongating RNAPII. In agreement with this, chromatin immunoprecipitation experiments have shown that Fcp1 associates with the promoter and coding region of active genes in vivo (17).

We have proposed previously that CTD phosphorylation during promoter clearance might displace Mediator and concomitantly allow the association of elongation-specific proteins, such as the Elongator complex (5, 20). Elongator was originally isolated as a component of the chromatin-associated, hyper-phosphorylated form of RNAPII in yeast (5). The proteins of the Elongator complex are highly conserved from yeast to man, and the human complex associates with early elongation complexes in HeLa nuclear extracts (21) and plays an acetyl-CoA-dependent role in transcription reactions reconstituted on chromatin...
templates in vitro (22). Yeast strains lacking the genes encoding Elongator are temperature-sensitive and slow at adapting to new growth conditions. These phenotypes can be explained by slow activation of genes required for growth under the new conditions (5). Most surprisingly, the majority of yeast Elongator seems to be cytoplasmic, and the factor fails to co-immunoprecipitate DNA (chromatin immunoprecipitation) from active yeast genes (23). However, recent results have shown that Elongator associates with the nascent mRNA of several yeast genes in vivo (24), and chromatin immunoprecipitation experiments in human cells showed that Elongator arrives at the S2 gene after assembly of the preinitiation complex and phosphorylation of the RNAPII CTD (25). Together, these results support the idea that Elongator is loaded onto the transcribing polymerase during promoter clearance and then travels with it through the coding region of the gene.

The present studies were inspired by our difficulties in extracting hyperphosphorylated, elongating RNAPII from chromatin without losing the majority of CTD phosphorylation. Because this de-phosphorylation could not be inhibited by the addition of various phosphatase inhibitors, we investigated the possibility that Fcp1 was responsible. Here we provide evidence that Fcp1 associates with the elongating RNAPII holoenzyme. Genetic experiments indicate that another holoenzyme component, Elongator, and Fcp1 functionally interact in vivo. We also show that whereas TFIIH kinase prefers RNAPII in a ternary complex as substrate, Fcp1 prefers free serine 5-phosphorylated RNAPII and that Fcp1-mediated CTD de-phosphorylation appears to proceed through a phosphoryl transfer step from the CTD directly to Fcp1. These results shed new light on the mechanisms that drive the eukaryotic transcription cycle.

**EXPERIMENTAL PROCEDURES**

**Materials**—Chemicals were from Sigma, unless otherwise specified. Radioactive label was from Amersham Biosciences or ICN. Streptavidin beads were from Dynal. The antibodies to detect phosphorylated and nonphosphorylated RNAPII in crude chromatin were 8G3 (Pharminigen) and 8G161 (Babco), respectively. The anti-Elp3, anti-Fcp1, and anti-Rpb3 antibodies have been described elsewhere (16, 26, 27).

**Protein Purification**—Extraction of elongating RNAPII from chromatin, gel filtration of crude sheared chromatin, and the chromatographic steps used in purification of elongating RNAPII holoenzyme have been described (5). Purification of TFIIH (28), Fcp1 (16), TFIF (29), and Elongator (30) was done as described. Cdk1 was purified to virtual homogeneity using the TAP tag purification procedure according to Ref. 31.

**TFIIH Kinase Assay**—Histidine-tagged holohTFIIH was purified as described previously (28). TFIIH kinase assays were done with 100–200 ng of RNAPII in 40 mM Hepes, pH 7.5, 10 mM MgCl2, 5 mM 2-mercaptoethanol, 5% (v/v) glycerol, 10 μCi of [γ-32P]ATP (3000 Ci/mmol), 1 μM RNase inhibitor (5–3′), 5% (v/v) glycerol, 1 pmol of template oligonucleotide DNA for 30 min at room temperature in a 25-μl reaction volume. When the transcription product (ternary complex) was to be used as substrate for Fcp1 phosphatase or TFIIH kinase, no radioactively labeled GTP was used, and biotin on the transcription template here allowed the ternary complexes to be separated from free polymerase by the use of streptavidin beads. This step also ensured removal of TFIIH as well as free label from the reactions using labeled RNAPII as the polymerase source, whether TFIIH-mediated phosphorylation was done before or after transcription.

**Fcp1 Phosphatase Activity Assay**—Fcp1 phosphatase activity, as well as the phosphatase activity of crude chromatin fractions, was assayed with 100–200 ng of RNAPII0 (free or as ternary complex) as a substrate in 20 mM Tris acetate, pH 7.8, 10 mM MgCl2, 5 mM dithiothreitol, 29% glycerol (v/v), 0.1 mM EDTA, 0.025% Tween 20, 10 mM potassium acetate, 10 mM 2-glycerophosphate, 10 mM okadaic acid for 30 min at 30 °C in a total volume of 20–50 μl. Reactions were stopped by the addition of SDS loading buffer, and samples were incubated at 95 °C for 3 min before loading onto a 7.5% SDS-polyacrylamide gel. The gel was dried and exposed to either a PhosphorImager screen or BioMax (Eastman Kodak Co.) film with an intensifying screen.

**Yeast Genetics**—All Saccharomyces cerevisiae strains used for genetic analysis were congenic with strain W303 (ade2-1 can1-100 his3-11,15 leu2-2,212 trp1-1 ura3-52) (33) and were grown and manipulated essentially as described previously (16). Plasmid shuffle of fcp1 alleles carried on TRP1-marked plasmids in fcp1A-elplA cells carrying wild type FCP1 on a URA3-marked plasmid was done using standard techniques (24). To detect phosphorylated in vivo, autoradiograms of cells that lost the URA3 plasmid were selected on 5-fluoroorotic acid-containing media.

**Synthetic Genetic Array Analysis**—Synthetic genetic array analysis was carried out as described previously (35). Automated analysis of the results was carried out by procedures that will be described elsewhere.

**RESULTS**

**Evidence That Fcp1 Is Responsible for De-phosphorylation of RNAPII Following Its Release from DNA**—During attempts to develop procedures by which to purify larger amounts of native, hyper-phosphorylated RNAPII from yeast chromatin, we repeatedly observed significant de-phosphorylation of RNAPII during the first stages of a variety of purification procedures. This observation inspired us to investigate further the biochemical characteristics of RNAPII de-phosphorylation (Fig. 1A). The phosphorylation level of RNAPII in crude, sheared yeast chromatin remained fairly stable after incubation at 30 °C for 30 min. In contrast, conditions that would be expected to disrupt ternary DNA-RNA-RNAPII complexes, such as DNase treatment and to an even larger extent DNase treatment plus addition of nucleotides (which would permit transcriptional run-off), led to significant CTD de-phosphorylation (Fig. 2A, compare lanes 1 and 4). These experiments support the idea that the RNAPII CTD becomes susceptible to de-phosphorylation upon disruption of the ternary complex and provides a possible explanation for our difficulties in isolating hyper-phosphorylated RNAPII after chromatin extraction.

In the course of investigating RNAPII de-phosphorylation in crude chromatin, we also noticed that protein phosphatase inhibitors such as sodium fluoride and okadaic acid failed to inhibit the reaction (data not shown). One of the hallmarks of Fcp1 is being unresponsive to such inhibitors (13), so in order to investigate the contribution of this protein to the observed CTD de-phosphorylation, chromatin was prepared from an fcp1 temperature-sensitive strain (fcp1-4) and its wild type counterpart. The mutated fcp1-4 allele results in a protein that has little, if any, CTD phosphatase activity at elevated temperature in vitro (16). CTD de-phosphorylation was then compared to 25, 30, and 37 °C (Fig. 1B). In chromatin from the wild type strain,
significant CTD de-phosphorylation was observed at both 30 and 37 °C, but in chromatin from fcp1-4, little if any de-phosphorylation took place at any of the temperatures tested. This lack of de-phosphorylation was not due to the chromatin from this strain generally lacking biological activity, as run-off transcription activity in vitro was similar in fcp1-4 and wild type chromatin under the assay conditions used in Fig. 1B (data not shown). These results suggest that Fcp1 is at least partly responsible for the observed de-phosphorylation of RNAPII in vitro.

**Association of Fcp1 in a Large Protein Complex**—When sheared yeast chromatin is subjected to gel filtration in high salt conditions, hyper-phosphorylated RNAPII co-fractionates with DNA, RNA, and Elongator (5) (Fig. 2). When the same fractions were blotted with antibodies specific for Fcp1 phosphatase, this protein was found to be broadly distributed, col-

![Image](https://www.jbc.org/content/430/12/4301/F1.large.jpg)

**FIG. 1.** Phosphatase activity in crude yeast chromatin extracts. A, the level of CTD phosphorylation after incubation of 2 μl of crude yeast chromatin (20–30 mg/ml protein) for 30 min at 30 °C was monitored by Western analysis using antibodies against phosphorylated CTD (8G3), hypo-phosphorylated CTD (8WG16), and Rpb3. Lane 1 represents a mock-treated control; lane 2, 10 mM MgCl2 and 10 mM MnCl2 added; lane 3, 10 mM MgCl2, 10 mM MnCl2, 10 units of DNase added; lane 4, 10 mM MgCl2, 1 mM each NTP, and 10 units of DNase added; lane 5, 10 mM MgCl2, 10 mM MnCl2, 1 mM each NTP, and 10 units of DNase added. B, chromatin extracts from wild type W303 (lanes 1–4) and the Fcp1 temperature-sensitive fcp1-4 strain (lanes 5–8) were incubated in the presence of 10 mM MgCl2, 10 mM MnCl2, 10 units of DNase, and 1 mM each NTP. Reactions were incubated at the indicated temperature for 30 min. Lanes 1 and 5 contain untreated controls. Samples were separated on a 7.5% SDS-PAGE prior to blotting. Western signals were analyzed using NIH Image, and the amount (in %) of RNA-PPIII relative to the untreated control (lane 1) is indicated below the lanes. Loading variations were normalized using the Rpb3 signal, whereas the levels of the CTD were also normalized using the 8WG16 signal. This was necessary because incubation of the crude extract also led to varying degrees of Rpb1 proteolysis (explaining why only slight increases in the responsiveness to 8WG16 antibody were observed).

**Fig. 2.** Fcp1, nucleic acids, RNAPII0, and Elongator in the crude chromatin fraction. Crude chromatin was partially digested with DNase and RNAse and subjected to gel filtration through TSK G4000SW (5). Fractions were assayed for nucleic acid content (DNA/ RNA). Western-blotted, and probed for the presence of RNAPII, Elongator (Elp3), and Fcp1. Please note that the quality of Western blots with crude chromatin fractions is severely reduced because of the large amount of DNA and RNA in the fractions.

eluting with hyper-phosphorylated RNAPII as well as with hypo-phosphorylated RNAPII (Fig. 2). No Fcp1 was observed in the size range otherwise expected for the protein (~100 kDa) (data not shown). Similar to Fcp1, Elongator (Elp3) also eluted with elongating RNAPII, peaking in fraction 28. These results are consistent with the idea that Fcp1 interacts with elongating RNAPII holoenzyme in chromatin.

**CTD De-phosphorylation with Purified Proteins**—In order to investigate CTD de-phosphorylation in a more defined system, we now purified RNAPII, TFIIH, CTDK1, and Fcp1 to near homogeneity and used these proteins to enable a study of RNAPII CTD de-phosphorylation.

We first tested the substrate specificity of yeast Fcp1 in vitro by incubating the enzyme with RNAPII that had been hyper-phosphorylated by either TFIIH or CTDK1 (Fig. 3). TFIIH-mediated phosphorylation is exclusively on serine 5 of the CTD repeat (see Ref. 36; data not shown), whereas CTDK1-mediated phosphorylation occurs primarily on serine 2 but also to a smaller extent on serine 5 (see Ref. 37; data not shown). Most interestingly, Fcp1 only had slight activity against CTDK1-phosphorylated RNAPII (predominantly serine 2-phosphorylated), whereas it efficiently removed TFIIH-mediated phosphorylated RNAPII CTD (see Ref. 37; data not shown). These results were somewhat surprising as previous chromatin immunoprecipitation experiments had supported the idea that yeast Fcp1 removes serine 2 phosphorylation but does not seem to be important for the removal of serine 5 phosphorylation in genes in vivo (17). On the other hand, FCP1 (and serine 5 phosphorylation) is essential for yeast viability, whereas phosphorylation of serine 2 is not, pointing to a potentially important cellular role of Fcp1 besides de-phosphorylation of serine 2. As a consequence of the results of Fig. 3, we used TFIIH-phosphorylated RNAPII to investigate the substrate preference and mechanism of action of Fcp1 in the subsequent experiments.

To generate substrates for the de-phosphorylation reactions, biotinylated, single-strand tailed DNA templates whose transcribed region starts with a U/C-less cassette (Fig. 4A) were used in transcription reactions with RNAPII purified to virtual homogeneity or with RNAPII that had first hyper-phosphorylated by TFIIH in the presence of radioactively labeled ATP. Under the conditions used, a significant proportion of the
input DNA template was utilized for transcription, and RNA-PII shifted the migration of the DNA probe in a nucleotide-dependent manner (proof-of-principle reactions using labeled DNA template in “cold” transcription reactions are shown in Fig. 4B). Ternary complexes could be efficiently separated from free label, TFIIH, as well as nontranscribing RNA-PII by the use of streptavidin beads (reactions in which TFIIH-phosphorylated (radiolabeled) RNA-PII was used in cold transcription reactions on an unlabeled DNA template is shown in Fig. 4C). By the use of these procedures (see “Experimental Procedures” for more details), it was thus possible to generate different substrates for comparing the substrate specificities of Fcp1, namely (i) hyper-phosphorylated, nontranscribing RNA-PII (free RNA-PII), and (ii) hyper-phosphorylated RNA-PII in a ternary complex (“elongating RNA-PII”) (Fig. 5A).

When these RNA-PII substrates were used in phosphatase reactions with virtually homogeneous Fcp1 (see Fig. 5B for the purity of the components in the de-phosphorylation reaction), we observed that free RNA-PII0 was a much better substrate for de-phosphorylation than elongating RNA-PII (Fig. 5C). 200 ng of free RNA-PII0 was more than 90% dephosphorylated when incubated with 200–400 ng of recombinant Fcp1, whereas little, if any, de-phosphorylation was observed with a similar amount of RNA-PII0 ternary complexes. We obtained virtually identical results with long plasmid transcription templates (data not shown), showing that these results were not simply due to the short DNA ends of the oligonucleotides perturbing the phosphatase activity of Fcp1. Remarkably, a labeled phosphoprotein of the same molecular mass as Fcp1 (∼100 kDa) was observed whose appearance correlated precisely with the disappearance of RNA-PII phosphorylation (Fig. 5C, lanes 2–5). Indeed, when the migration of the radiolabel resulting from the de-phosphorylation reaction was compared with the migration of Fcp1, these precisely coincided (Fig. 5D). We note that the phosphatase experiments were performed with proteins purified to near homogeneity and, more importantly, in the absence of free radioactive label, effectively ruling out the possibility that the labeled protein originated through phosphorylation by a kinase. Rather, this result suggests that de-phosphorylation of the CTD entails phosphoryl transfer directly to the Fcp1 protein. These results thus point to the likely enzymatic reaction mechanism of Fcp1 (see “Discussion”).

In contrast to Fcp1, which clearly preferred free RNA-PII0 to elongating RNA-PII0 as a substrate, elongating RNA-PIIA was a much better substrate than free RNA-PIIA for the kinase activity of TFIIH (Fig. 6). These data suggest that the substrate specificity of TFIIH kinase and Fcp1 phosphatase may help to drive the transcription cycle; TFIIH-mediated RNA-PII phosphorylation is stimulated by the polymerase being in a ternary complex as a result of transcriptional initiation, whereas Fcp1-mediated CTD de-phosphorylation of serine 5 is made possible by the disruption of ternary complexes subsequent to the termination of transcription.

Genetic Interaction between FCP1 and Elongator—The data in Fig. 2 suggest that Fcp1 and Elongator may both associate with the hyper-phosphorylated, elongating form of RNA-PII. This raised the possibility that Elongator might affect the dephosphorylation activity of Fcp1. However, no effect of adding purified Elongator to Fcp1 de-phosphorylation reactions in vitro was observed (data not shown). Failing to observe a functional interaction between Fcp1 and Elongator in vitro, we investigated potential genetic interactions between FCP1 and ELP genes (Fig. 7). As FCP1 is an essential gene, we knocked out the ELP genes in an fcp1Δ strain, which carried wild type FCP1 on a URA3-marked plasmid, as well as either wild type FCP1 or different temperature-sensitive fcp1 alleles on a TRP1-marked plasmid. The growth of the resulting strains in the presence of 5-fluoroorotic acid (to kill cells depending on the
URA3 plasmid for survival) was then compared at the permissive temperature (Fig. 7). Strikingly, cells lacking ELP1, ELP2, or ELP3 were unable to survive if the function of Fcp1 was compromised by a temperature-sensitive mutation. These results demonstrate a strong genetic interaction between Fcp1 and Elongator in vivo.

To generally evaluate the function of Elongator by the use of genetics, we finally used a method for systematic construction of double mutants, termed synthetic genetic array analysis (35), in which elp mutations were crossed to a miniarray of 384 mutant strains, each carrying a unique gene deletion corresponding to a protein known or suspected to function in some aspect of transcription and/or chromatin modification (a list of these transcription-related genes are available on request).3 The elp deletions were first introduced into a haploid starting strain of mating type MAT/a/H9251 and then crossed to the miniarray of gene-deletion mutants of the opposite mating type, MAT/H. Sporulation of the resulting diploid cells led to the formation of double-mutant meiotic progeny. This resulted in an ordered array of double-mutant haploid strains whose growth rate was monitored by visual inspection and image analysis of colony size. Inviable or slow growing double-mutant meiotic progeny identifies functional relationships between genes.

Components of the Elongator complex were found to genetically interact with four nonessential members of the transcriptional initiation factor, Mediator (Med2, Med3, Gal11 and Srb2) (38), as well as with Swi4 and Swi6, the components of SBF, which activate cell cycle-dependent gene expression (39) (Fig. 8). Deletion of Elongator subunits also resulted in growth defects when combined with deletions of subunits of the histone acetyltransferase complex SAGA (Spt3, Spt7, Spt8, and Ngg1) (40), as well as with four members of the Paf1 complex (Paf1, Cdc73, Rtf1, and Ctr9) (41–43), and a transcriptional elongation complex is required for the activity of two histone methyltransferases, Set1 and Set2 (3, 4, 7). Synthetic growth defects were also observed with other complexes involved in histone modifications, including members of the histone H2B ubiquitination machinery (Bre1 and Lge1) (44) and components of the Rpd3-containing histone deacetylase complex (Rpd3, Sin3, Pho23, Sup30, and Sin3) (45–47). Because deletions of Elongator subunits cause synthetic growth phenotypes when com-

3 N. J. Krogan and J. F. Greenblatt, unpublished data.
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Fig. 7. Genetic interaction between FCP1 and Elongator. elp1Δ, elp2Δ, and elp3Δ, respectively, were introduced into an fcp1Δ strain carrying a CEN/ARS/URA plasmid expressing wild type Fep1, as well as a CEN/ARS/STR plasmid expressing the FCP1 allele indicated in the diagram. Strains were grown overnight in synthetic complete medium lacking tryptophan and containing 5-fluoroorotic acid, and grown for 4 days at 30 °C.

DISCUSSION

The experiments described here were prompted by our inability to purify large amounts of hyper-phosphorylated elongating RNAPII holoenzyme for biochemical studies. The vast majority of RNAPII0 contained in ternary complexes isolated with sheared yeast chromatin is thus de-phosphorylated in the first steps of the purification procedures, despite the use of various phosphatase inhibitors. The results of the present study support the idea that Fcp1 phosphatase associates with elongating RNAPII0 and that the serine 5 position of the polymerase CTD only becomes a substrate for the catalytic activity of Fcp1 upon disruption of the ternary DNA-RNA-RNAPII0 complex. Like Fcp1, Elongator also associates with elongating RNAPII0, and our data show that FCP1 and Elongator are genetically interact.

Fcp1, Elongator, and RNAPII0 co-elute with DNA and RNA from gel filtration columns run at a high salt concentration, which disrupt the vast majority of conventional protein-DNA interactions but not ternary RNAPII0-DNA-RNA complexes (5, 48). These data suggest that Fcp1 interacts with Elongating RNAPII holoenzyme, providing biochemical support for the idea that Fcp1 travels with elongating RNAPII in vivo. Data indicating complex formation by direct, stable Fcp1-RNAPII interaction were published while this paper was under revision (49).

Because of the evidence pointing to an association of Fcp1 with elongating RNAPII, and because it was repeatedly observed that RNAPII becomes de-phosphorylated when released from ternary complexes, we established a biochemical system with purified proteins to investigate Fcp1-mediated RNAPII de-phosphorylation in vitro. By using this system, we observed that Fcp1 can dephosphorylate serine 5 but not serine 2 of the RNAPII CTD. This result was surprising, given that purified human Fcp1 has been shown previously to dephosphorylate these substrates with similar efficiency (50). Moreover, yeast FCP1 affects the serine 2 phosphorylation levels, whereas it has little, if any, effect on serine 5 phosphorylation levels in genes in vivo (17). However, in agreement with the serine 5 data obtained from the chromatin immunoprecipitation experiments (17), our data show that Fcp1 only de-phosphorylates free RNAPII. Thus, we found that yeast Fcp1 has a strong preference for free RNAPII0 over elongating RNAPII0 in phosphatase assays with highly purified proteins. This discrimination is much more pronounced than previously reported for the human CTD phosphatase (51). Most importantly, the significantly reduced activity of Fcp1 observed with RNAPII-DNA ternary complexes is not simply due to the CTD being less inaccessible for enzymes in this assay system, as TFIIH kinase showed the opposite substrate preference, namely a pronounced preference for ternary complexes over free RNAPII. To our knowledge, this substrate preference of TFIIH has not been described previously. These results thus uncover two mecha-
nisms that might help to drive the transcription cycle. First, TFIIH preferentially phosphorylates RNAPII when it is incorporated in a ternary complex (enabling promoter clearance), and second, Fcp1 helps de-phosphorylate RNAPII at serine 5 only after it has been released from the ternary complex (during termination), in all likelihood so that the polymerase can be re-cycled for a new round of transcription. We envision that Fcp1 and the newly identified serine 5-specific CTD phosphatase, Ssu72 (52), together ensure that RNAPII is completely de-phosphorylated after termination to ensure rapid polymerase recycling. In apparent agreement with this idea, ssu72 mutation can be suppressed by Fcp1 overexpression (53).

By using the purified Fcp1 assay system, we also observed that a protein migrating at the position of the Fcp1 protein became phosphorylated as RNAPII became de-phosphorylated. We believe that this phosphoprotein must be Fcp1. First, TFIIH and free label from the CTD phosphorylation reaction was carefully removed prior to the experiments by studying Fcp1-mediated de-phosphorylation, and accumulation of the “Fcp1 label” did indeed nicely correlate with the loss of CTD label. Moreover, in the de-phosphorylation reactions using ternary complexes as substrate (where little or no CTD de-phosphorylation was observed), the same proteins (labeled RNAPII and Fcp1) were present in the reaction mixture, yet no labeling of the Fcp1-sized protein transfer took place. Finally, other proteins of the superfamily of Asp-X-Asp-(Thr/Val) phosphatases, phosphorytases, and phosphotransferases, and phosphatases to which Fcp1 belongs are also known to perform phosphoryl transfer via two successive transfers with the enzyme playing the role as an intermediate covalent phosphoryl receptor (54, 55). For example, human phosphomannomutase forms a phosphoenzyme when incubated with its substrate, where the phosphorylated residue is the first aspartate in the Asp-14-Asp-X-(Thr/Val) motif (55). Other experimental support for the idea that CTD-de-phosphorylation by Fcp1 occurs via a phosphorylated Fcp1 intermediate was published by Cramer and co-workers (49) while this manuscript was under review. These research-ers solved the crystal structure of an Fcp1-related phosphatase in the presence of a phosphate mimetic, beryllium fluoride, and showed that the compound indeed bound this phosphatase via its Asp-X-Asp-(Thr/Val) motif.

It is surprising that a large, stable population of labeled (Fcp1) protein accumulates as the CTD becomes dephosphorylated in our reactions. Presumably, under normal conditions, Fcp1 is only transiently phosphorylated. Factors that enhance the catalytic activity of Fcp1, such as the Fcp1 stimulatory factor identified by the Kane laboratory (56), might thus affect not only the ability of Fcp1 to recognize and accept phosphoryl groups from RNAPII10 but also (alternatively or additionally) the turnover of the phosphorylated Fcp1 intermediate. The absence of such (possibly yeast-specific) accessory factors from our defined system might also help explain why yeast Fcp1, in contrast to human Fcp1, was unable to dephosphorylate serine 2-phosphorylated RNAPII in our experiments.

Our data show that Elongator and Fcp1 both associate with elongating RNAPII holoenzyme. In agreement with the idea that Elongator and Fcp1 might functionally interact in this context, cells expressing a compromised (temperature-sensitive) version of Fcp1 rely on the function of Elongator for survival. This suggests that the function of Fcp1 and Elongator overlap. This result complements previous genetic results showing that Elongator is required for survival of cells lacking the gene CTK1, encoding the catalytic subunit of the CTD kinase Ctdk1 (57). Taken together, these results could thus indicate a role for Elongator, Fcp1, and Ctdk1 in maintaining the integrity of elongation complexes. Elongator also genetically interacts with RPB9 (58), as well as with SPT16 (59) and GCN5 (gen5 elp3 has severe growth defects and severe reductions of acetylation in chromatin (60, 61)). To complement these data, we also performed an extensive synthetic genetic array screen with elp mutants, which showed genetic interactions between Elongator and a large number of transcription-related factors, such as Mediator, Pafl complex, Rad6 ubiquitin ligase, SAGA complex, Rpd3-Sin3 complex, Nap1 and histones. Although genetic effects can be indirect, all these data, together with the previous biochemical data showing a functional interaction between Elongator and RNAPII (5, 21, 22), as well as recent data indicating that Elongator is present at active genes in vivo (24, 25), thus further indicate a relationship between Elongator, RNAPII, and chromatin modification.

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