Dephosphorylation of RNA Polymerase I by Fcp1p Is Required for Efficient rRNA Synthesis*

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Differently phosphorylated forms of RNA polymerase (Pol) II are required to guide the enzyme through the transcription cycle. Here, we show that a phosphorylation/dephosphorylation cycle is also important for RNA polymerase I-dependent synthesis of rRNA precursors. A key component of the Pol II transcription system is Fcp1p, a phosphatase that dephosphorylates the C-terminal domain of the largest Pol II subunit. Fcp1p stimulates transcription elongation and is required for Pol II recycling after transcription termination. We found that Fcp1p is also part of the RNA Pol I transcription apparatus. Fcp1p is required for efficient rDNA transcription in vivo, and also, recombinant Fcp1p stimulates rRNA synthesis both in promoter-dependent and in nonspecific transcription assays in vitro. We demonstrate that Fcp1 activity is not involved in the formation of the initiation-active form of Pol I (the Pol I-Rrn3p complex) and propose that dephosphorylation of Pol I by Fcp1p facilitates chain elongation during rRNA synthesis.

In growing cells transcription of the ribosomal genes accounts for up to 60% of the newly synthesized RNA. Special adjustments of the RNA polymerase I-containing transcription machinery are required to maintain the high level of transcription during logarithmic growth and to quickly down-regulate rDNA gene expression in slowly growing cells to keep energy consumption as low as possible. One crucial step in control of rDNA transcription is the regulated recruitment of RNA polymerase I (Pol I) to the ribosomal gene promoters, which requires the coordinated interaction of Pol I and several transcription factors (reviewed in Ref. 1).

In yeast, reconstitution of transcription in vitro depends on several multisubunit complexes (2). Upstream activating factor (UAF) consists of six subunits (Rrn5, Rrn9, Rrn10, UAF30, and histones H3 and H4) (3–5) and binds to the rDNA ~60–155 bp upstream of the transcription start site. The TATA-binding protein binds UAF through the Rrn9 subunit (6, 7) and apparently connects promoter-bound UAF to the core factor that contains three different polypeptides (Rrn6, Rrn7, and Rrn11) (8–10). To achieve initiation competence, promoter-bound Pol I has to form a complex with Rrn3p and to interact with the C-terminal domain of the core factor component Rrn6p (11, 12).

The reversible interaction between Pol I and Rrn3p plays a crucial role for the formation of preinitiation complexes (13). First, growth-dependent rDNA transcription is dependent on the reversible association between Rrn3p and Pol I; the formation of active Rrn3p-Pol I complexes is strongly reduced in stationary cells. Second, Rrn3p leaves the Pol I complex during one round of transcription (13).

Formation and dissociation of the Rrn3p-Pol I complex is conserved in evolution. Mammalian Rrn3p/TIFIA is a growth-regulated factor in rDNA transcription (14) and was shown to associate with Pol I only in growing but not in quiescent or down-regulated cells (15–18). Together, these findings suggest that reversible post-translational modification(s) of either Pol I or Rrn3p mediate their stable interaction. Both components were found to be differently phosphorylated throughout the transcription cycle, and phosphorylation is required for complex formation.

In yeast, phosphorylation of Pol I is required for binding Rrn3p. Furthermore, phosphorylation of Pol I acquires different patterns, because the initiation-active, Rrn3p-bound Pol I exhibits a different phosphorylated state than the bulk of Pol I, which is probably involved in transcription elongation (19).

In contrast to the yeast system, the association of mammalian Rrn3p/TIFIA with Pol I is dependent on the phosphorylated form of Rrn3p (17, 18). The activity of mammalian Rrn3p/TIFIA is modulated by mitogen-activated protein kinases, thus connecting regulation of rDNA transcription to cellular growth and proliferation (20).

Pol I and Rrn3p/TIFIA are not the only targets for (de)phosphorylation to modulate transcriptional activity. Distinct phosphorylation sites in either upstream binding factor or promoter selectivity factor have been reported to mediate cell cycle dependent or growth-dependent regulation of rRNA synthesis (21–25). Accordingly, phosphorylation of mammalian UBF appears to play also a key role in mammalian target of rapamycin and extracellular signal-regulated kinase-dependent activation of rRNA synthesis (26, 27).

In contrast, not much is known about the enzymes that modify the phosphorylation pattern of Pol I. One possible can-
discuss the general role of Fcp1 in transcription by Pol I.

To generate fraction PA600 (protein concentration, 2.5–3.0 μg/ml) of the yeast strain YMK18 (FCP1 WT) mat a ade2–1 can1–100 ural3–1 trp1–1 his3–11,15 leu2–3,112 ssd1–2 fcp1D::LEU2 pFK4

In vitro transcription reactions were performed as described elsewhere (37). Chromatin immunoprecipitation was performed as described (28).

In Vitro Transcription—Promoter-dependent and nonspecific transcription reactions were performed as described elsewhere (37). Alkaline Phosphatase Treatment—Alkaline phosphatase covalently attached to agarose beads (Sigma) was washed two times with 20 mM Hepes (pH 7.8) and 1 mM MgCl₂. 40 μl of fraction PA600 were incubated with 20-μl beads (14 units) of phosphatase. The beads were pelleted twice, and the supernatants were carefully removed with capillary pipette tips. In control reactions, phosphatase was inhibited either by heat denaturation (15 min, 95 °C) or by the addition of a mixture of phosphatase inhibitors (final concentrations: 10 mM NaF, 5 mM sodium orthovanadate, and 0.5 mM EDTA). 4 μl of the supernatant were tested for promoter-dependent transcription, and 2 μl were tested for nonspecific transcription.

Analysis of in Vivo rRNA Synthesis—Isolation of RNA from wild type and temperature-sensitive strains and subsequent primer extension analysis were performed as described in Ref. 28.

Generation of Recombinant Fcp1p—Active recombinant Fcp1p and mutated, inactive Fcp1p (D180E) were obtained from insect cells that carry recombinant bacmids obtained from pMK87 and pMK88 for expression of wild type Fcp1 and the Fcp1 with the D180E mutation (32).

Immunoprecipitation of HA-tagged Pol I—Immunoprecipitation of HA-tagged Pol I and co-immunoprecipitation of Fcp1p from yeast strains GPY2 and OG39–6d were performed as described (40).

Gel Filtration of Fraction PA600 on Superose 6—50 μl of fraction PA600 were analyzed on a Superose 6 column as described (40).

Fcp1p Phosphatase Treatment of Pol I-containing Fractions—0.1 μl of fraction B2000 (37) and 1 μl of purified Pol I-A or increasing amounts (1–6 μl) of Rrn3p-depleted fraction PA600 (19) were preincubated for 10 min at room temperature with various amounts of recombinant Fcp1p or D180E, as indicated in the figure legends, and tested for nonspecific or promoter-dependent transcription. After Fcp1p preincubation, the Rrn3p-depleted fraction PA600 was reconstituted with bacterial lysate and tested for nonspecific transcription as described (19).

Interaction of Pol I-A with Bacterially Expressed Rrn3p—Interaction studies were performed as described (19).

Chromatin IP/PCR Analysis—Temperature-sensitive mutant strains were grown at 24 °C to an A₆₀₀ between 0.8 and 1.5 and further incubated for 2 h at 24 °C or shifted to nonpermissive temperature (37 °C). As a control, wild type strain w303-TAP-Rrn3 was grown to an A₆₀₀ between 0.8 and 1.5, washed in medium lacking Trp/ura, and further incubated for 8 h in the depletion media. Chromatin immunoprecipitation was performed as described.
(41). Dilutions of 1:100 (input) or 1 to 1:256 (ChIP) were analyzed by PCR using the following oligonucleotides: promoter fragment: Chip1, 5'-H11032-GTG TGA GGA AAA GTA GTT GGG AGG TA-3' and Chip promoter reverse, 5'-H11032-TAA ACG CAA AAG AAA CAC ACT CTG GG-3'; 5SR N A fragment: Chip3, 5'-H11032-TGG GAT TTA GCA TAG GAA GCC AAG AA-3', and Chip 5 S fragment reverse, 5'-H11032-CTG ACC GAG TAG TGT AGT GGG TGA-3'; termination fragment: ON 10140f, 5'-H11032-CGG GGT ATT GTA AGC AGT AGA GTA-3' and Chip termination fragment, 5'-H11032-CGG GCA AAT CCT TTC ACG CTC GGG-3'; and control fragment (nontranscribed chromosomal DNA): III-L, 5'-H11032-ACT TTG GCT TTC CGC TCG TG 3' and 5'-H11032-GAA AGT CTT CTA GAG TTA CAG G-3' (42).

RESULTS

Dephosphorylation of Isolated Pol I Complexes Can Either Activate or Inhibit Transcription—We previously reported that dephosphorylation of Pol I by alkaline phosphatase inhibits initiation of transcription by blocking the formation of the Rrn3p-Pol I complex. Closer examination, however, has now revealed that there is a time-dependent modulation of Pol I activity in vitro by alkaline phosphatase. Long periods of dephosphorylation result in inhibition, whereas short periods result in stimulation of transcription, as shown in the time course experiments of Fig. 1. Fraction PA600, which is enriched in a nucleolar substructure that is highly active in Pol I-dependent transcription (40), was treated without (Fig. 1A, upper panel) or with (Fig. 1A, lower panel) immobilized alkaline phosphatase. Aliquots were removed at various time points and assayed for their capacity to initiate and elongate transcripts in an assay for promoter-dependent transcription (Fig. 1A). Transcription activity rose shortly after starting the dephosphorylation but dropped significantly after 20 min of phosphatase treatment.

A stimulatory effect of the dephosphorylation reaction was also observed when nonspecific rRNA synthesis on single-stranded or nicked DNA templates was measured after phosphatase treatment of Pol I (Fig. 1B). However, the declines in promoter-dependent activity and in nonspecific RNA synthesis followed different kinetics. Removal of phosphate groups at later time points has a stronger negative impact on accurate transcription than on nonspecific rRNA synthesis, indicating that some phosphate groups function predominantly in transcription initiation. When purified Pol I was incubated with alkaline phosphatase, the kinetics of activating and inactivating nonspecific transcription resembled that of phosphatase-treated fraction PA600 (data not shown). This result suggests that the change in nonspecific transcription is due to changes...
in the phosphorylation pattern of Pol I itself. Western blot analysis revealed no significant degradation of the Pol I subunits during the incubation periods (Fig. 1C). Of the five phosphorylated Pol I subunits (A190, A43, 34.5, A23, and A19), subunit 34.5 exhibited a faster migrating band on SDS-PAGE 30 min after phosphatase treatment, which is probably due to the removal of phosphates. Subunit A43 migrated as doublet indicating two differently phosphorylated forms.

These results indicated that several phosphorylation sites in the Pol I transcription apparatus are differentially accessible to alkaline phosphatase. We conclude that removal of distinct phosphates is necessary for either efficient initiation or elongation by Pol I, whereas other sites have to be phosphorylated to allow rRNA synthesis. To gain more insights into how dephosphorylation of the Pol I machinery stimulates rDNA transcription, we searched for enzymes that change the phosphorylation pattern of Pol I or Pol I-associated factors.

The Phosphatase Fcp1p Associates with the Pol I Transcription Apparatus—We recently reported that the basal Pol II transcription factor TFIIH also participates in Pol I transcription (28). Because one of the TFIIH subunits, Kin28, contains a protein kinase activity, we asked whether the essential Pol II phosphatase, Fcp1p, is also involved in rRNA synthesis. We previously described the isolation of a nucleolar substructure active in Pol I-dependent rRNA synthesis and processing (40). This nucleolar substructure was significantly enriched in Pol I and all transcription factors required for accurate transcription but lacked Pol II and most Pol II-specific transcription factors. Interestingly, Fcp1p was also present when this nucleolar substructure was isolated from logarithmically growing yeast cells (fraction PA600) (Fig. 2A). However, the presence of Fcp1p in the nucleolar substructure depended on functional Pol I engaged in rDNA transcription. Purification of the same nucleolar substructure from a strain that lacks the second largest Pol I subunit (A135) and has Pol II-driven rRNA synthesis revealed that most of the Pol I subunits and Fcp1p were not present. This suggests that Fcp1p is incorporated into the nucleolar substructure through a direct or indirect interaction with Pol I (Fig. 2A).

We further analyzed the presence of Fcp1p in Pol I-containing complexes by co-immunoprecipitation and affinity purification experiments using HA and His6 tags of the Pol I subunit A43, as well as in gel permeation chromatography (Fig. 2, B and C). A small but noticeable amount of Fcp1p co-immunopurified with Pol I using low stringency conditions that had been previously established (40) when Pol I was immunoprecipitated via its HA tag on subunit A43 (Fig. 2B, lane 3). Significantly less Fcp1p was bound to the beads in control experiments when HA-tagged A43 was not present (Fig. 2B, lane 4). Furthermore, a small but distinct proportion of Fcp1p co-purified with Pol I on nickel-agarose using the His6 tag of A43 for affinity purification (data not shown). Finally, when the Pol I-containing fraction PA600 was separated on a Superose 6 column, a distinct fraction of the Fcp1p co-migrated with Pol I in a large complex (Fig. 2C, fraction 18).

However, even under the low stringent conditions applied during all our interaction studies, only a minor population of Fcp1p was found in the same fractions as the Pol I machinery. For instance, in the gel filtration experiment bulk Fcp1 and Pol I do not co-migrate. Most of Fcp1p starts to elute with fraction 26 and peaks in fraction 29, whereas Pol I peaks in fraction 24/25 (data not shown). The fact that only a minor proportion of Fcp1p interacts with Pol I indicates an either labile or transient interaction between the phosphatase and the transcription apparatus. It is possible that Fcp1 either acts catalytically rather than in stoichiometric fashion or that only a minor

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Fcp1p is part of the Pol I transcription machinery. A, Fcp1p co-purifies with a nucleolar substructure isolated from wild type cells, but not if the nucleolar subcomplex is derived from cells lacking Pol I. Fraction PA600, which is enriched in a nucleolar substructure (40), was generated either from growing wild type cells or from strain OG39–6d (ΔA135), which lacks the Pol I-specific subunit A135 and in which RNA synthesis is mediated by Pol II (54). B, immunoprecipitation of HA-tagged A43. Equal amounts of whole cell extracts (WCE) (12.5 mg/ml; 25 mg protein) derived from strains GPY2, which contains a HA- and His6-tagged A43 subunit, and OG39–6d (ΔA135), in which A135 is disrupted, were immunoprecipitated with antibodies directed against the HA tag. 20 μg of WCE (lanes 1 and 2), and 10% of the immunoprecipitations (lanes 3 and 4) were analyzed by Western blotting. C, analysis of fraction PA600 by gel filtration using Superose 6. 50 μl of fraction PA600 were applied to a Superose 6 column (SMART; Amersham Biosciences) and processed with a flow rate of 12.5 μl/min in buffer BU300. 50-μl fractions were collected, and 25 μl of each were analyzed by Western blotting. D, Chromatin IP/PCR at different dilutions were carried out using a strain expressing ProtA-tagged Fcp1. Proteins bound to chromatin were immunoprecipitated using IgG-Sepharose. Increasing dilutions of the immunoprecipitated samples were analyzed by PCR using primers specific for the rDNA promoter and the termination region, as well as of a control fragment (nontranscribed chromosomal DNA, III-L) (42).
A proportion of the Pol I machinery is a target for Fcp1 activity. However, in any case, if the association between Fcp1 and the transcription apparatus takes place at the site of transcription, Fcp1 should be detected at the rDNA genes in growing yeast cells. Therefore we performed chromatin IP/PCR experiments and found that Fcp1 is accumulated at the transcribed rDNA region (Fig. 2D) compared with a nontranscribed control DNA fragment. Taken together, these experiments strongly suggest that Fcp1p interacts physically with at least part of the Pol I-containing transcription machinery.

**Fcp1p Is Required for rDNA Transcription in Vivo**—Having established a physical connection of Fcp1p with the Pol I transcription apparatus, we next tested whether Fcp1p is necessary for the synthesis of rRNA. Yeast strains carrying the temperature-sensitive alleles fcp1-1 and fcp1-4 (32) were shifted to the nonpermissive temperature, and the amount of newly synthesized 35S pre-rRNA was compared with stable 25 S rRNA at various time points after the shift. Pre-rRNA synthesis in the fcp1-1 and fcp1-4 mutant strains was reduced with similar kinetics and to almost the same extent as in mutants carrying either a temperature-sensitive allele of the Pol I subunit A43 (Fig. 3) or of the Pol I transcription factor Rrn3 (data not shown). In contrast, rRNA synthesis was only slightly reduced in wild type strains or strains that carry a temperature-sensi-
A Phosphatase Involved in Both Pol I and II Transcription

Fig. 4. Fcp1p increases efficiency of Pol I-dependent transcription in vitro. Representative experiments are depicted. A, Fcp1p stimulates transcription initiation. Fraction PA600 derived from a strain containing chromosomally ProtA-tagged Rrn3p was depleted of Rrn3p-ProtA using IgG-Sepharose beads (upper panel). The supernatant, which lacked Rrn3p (compare Western blot depicted in lanes 1 and 2) and consequently failed to initiate transcription (lanes 3 and 4), was used to assay the effect of Fcp1p on promoter-specific transcription. Increasing amounts of Rrn3p-depleted fraction PA600 were preincubated for 10 min at room temperature with increasing fraction in nonspecific transcription. 0.1 μl of the Pol I-containing fraction B2000 was preincubated for 10 min at room temperature with increasing amounts of either recombinant wild type or mutant (D180E) Fcp1p and tested for nonspecific transcription. C, purified recombinant Fcp1p but not mutant protein D180E stimulates Pol I that was purified to apparent homogeneity to synthesize nonspecific RNA. Increasing amounts of purified Fcp1p and D180E (0.45, 0.9, and 1.5 μg of each protein, respectively) were incubated with 0.2 μg of purified Pol I-A. The fractions used for the nonspecific transcription assay were analyzed by SDS-PAGE and either silver-stained (Pol I-A) or stained by Coomassie Simply Blue Safe Stain (Invitrogen) (recombinant Fcp1p). 1 μl of purified Pol I-A (0.4 mg/ml) was assayed with increasing amounts of Fcp1p in nonspecific transcription (right panel).

To demonstrate that inhibition of rRNA synthesis is not due to an indirect effect via the impaired Pol II transcription machinery, we performed control experiments using the yeast strain rpb1-1 that carries a temperature-sensitive mutation in the largest Pol II subunit (44). This mutant exhibited fast and pronounced defects in a genome-wide analysis of genes transcribed by Pol II (45). As expected, the amount of newly synthesized rRNA also dropped in the rpb1-1 mutant strain at the nonpermissive temperature. This effect was, however, less significant and had slower kinetics than when there were defects in Fcp1p or the Pol I subunit A43. The simplest explanation of these results implies that Fcp1p is directly involved in Pol I-dependent transcription.

In Fig. 3B, the data of three independent experiments with the strains YMK18 (WT), GPY11–6, YMK20, and rpb1-1, respectively, were normalized to the internal standard, the 25S rRNA, and quantified. Interestingly, the reduction of rRNA synthesis in the fcp1 mutants never reached exactly the same level as in the Pol I mutants, which indicates that a basal, inefficient level of rRNA is still produced if Fcp function is impaired. This finding is in accordance with the suggestion described above that only a fraction of the Pol I machinery might be a target for Fcp1 activity.

The Phosphatase Activity of Fcp1p Increases the Efficiency of Pol I Promoter-dependent Transcription in Vitro and Stimulates Homogeneous Pol I in Nonspecific RNA Synthesis—The requirement of Fcp1p activity for efficient rDNA transcription could also be measured in experiments in vitro. Levels of stimulation were obviously dependent on the quality of the Pol I preparation because stimulation by Fcp1p varied from 2.5- to 7-fold when different Pol I preparations were used. The results of representative experiments are depicted in Fig. 4. Purified recombinant Fcp1p (Fig. 4C) significantly stimulated promoter-dependent transcription in a system that is dependent on addition of Rrn3p (Fig. 4A). When the initiation-active Rrn3p-Pol I complex was immunodepleted from fraction PA600, which contains the transcriptionally active nucleolar substructure (Fig. 4A, lanes 1 and 3), promoter-dependent transcription was greatly reduced (Fig. 4A, lanes 2 and 4). The addition of purified, bacterially expressed Rrn3p partially restored the level of transcription (Fig. 4A, lanes 10–13). Importantly, preincubation of the transcriptionally inactive fraction (PA600ΔRrn3p) with Fcp1p prior to the addition of Rrn3p further stimulated promoter-dependent transcription up to 4-fold (compare Fig. 4A, lanes 6–9 and lanes 10–13 with the quantification on the right). Preincubation of fraction PA600ΔRrn3p with a mutated
form of Fcp1p that lacks phosphatase activity did not stimulate accurate transcription (data not shown).

To study whether or not Fcp1p function requires an initiation complex at the ribosomal gene promoter, we analyzed the effect of Fcp1p on Pol I complexes in promoter-free, nonspecific transcription. Fcp1p-associated phosphatase activity generally increased the efficiency of RNA synthesis by Pol I, even when the template consisted of single-stranded or nicked DNA (Fig. 4B). When this Pol I-containing fraction (fraction B2000) was incubated with fairly homogeneous Fcp1p, the amount of RNA synthesized in the nonspecific assay increased up to 7-fold. This effect was dependent on the phosphatase activity of Fcp1p; stimulation of Pol I activity was not observed when a similar amount of a mutated form of Fcp1p that lacks phosphatase activity (D180E) (32) was used (Fig. 4B).

Fcp1p acts directly on Pol I because enhancement in RNA synthesis was also achieved when almost homogeneous Pol I (Pol I-A) was incubated with the purified recombinant Fcp1p fraction, but not if the purified mutant protein was added (Fig. 4C). The purified protein fractions of Pol I and Fcp1p used for the nonspecific transcription assay as well as the assay results are shown in Fig. 4C. Based on these findings, we conclude that Pol I itself is the primary target of the Fcp1p-mediated dephosphorylation event that stimulates Pol I activity. Phosphate group(s) from one or more Pol I subunits must be removed by Fcp1p, which then allows the enzyme to transcribe more efficiently regardless of whether initiation occurs nonspecifically or at the appropriate rDNA promoter.

**Fcp1p Is Not Involved in Formation of the Preinitiation Complex**—Next, we wanted to identify the molecular mechanism for the Fcp1p requirement in rRNA synthesis. First, we tested the effect of Fcp1p on preinitiation complex formation. Dephosphorylation of Pol I by Fcp1p did not significantly influence the formation or the stability of the initiation-competent Pol I. Recombinant Fcp1p also did not affect the binding of purified Rrn3p to Pol I (Fig. 5A), nor did it dissociate the isolated Pol I-Rrn3p complex (data not shown). In yeast strains carrying the temperature-sensitive fcp1 allele, Rrn3p was still found at the rDNA promoter region in chromatin immunoprecipitation experiments 2 h after shift to the restrictive temperature (Fig. 5B, lanes 6–8). Accordingly, the initiation-active form of Pol I, the Pol I-Rrn3p complex, can be recruited to the promoter independently of Fcp1p function. This is in contrast to yeast strains that down-regulate rRNA synthesis because of limited nutrient availability. Under these conditions no initiation-active Pol I-Rrn3p complex can be formed. Consequently, these yeast cells have much less Rrn3p accumulated at the promoter compared with logarithmically growing yeast (Fig. 5B, lanes 10 and 11). In further experiments we investigated whether Fcp1p is either involved in promoter clearance or in the release of Pol I from the template when transcription is terminated; however, no influence of Fcp1p on either of the two processes could yet be observed. In contrast, binding of single stranded DNA to Fcp1p-treated Pol I was slightly enhanced if compared with mock-treated Pol I (data not shown), which indicated that Fcp1 activity might increase the affinity of Pol I to the template during transcription. Taken together, these data suggest that rather than playing a role in transcription initiation, Fcp1 might be involved in RNA chain elongation.

**DISCUSSION**

**Fcp1p Is Involved in Pol I-dependent Transcription**—Pol I is phosphorylated on many of its subunits in vivo (46, 47). Phosphorylation and dephosphorylation of Pol I likely play complex roles in regulating enzyme activity at various stages of the transcription cycle. This complexity is illustrated by the time-dependent differential effects of alkaline phosphatase treat-

![Figure 5](http://www.jbc.org/Downloaded from)

**Fig. 5.** Fcp1p is not involved in formation of the Pol I-Rrn3p complex at the rDNA promoter but increases the ability of Pol I to bind single-stranded DNA. A, Fcp1p-treatment of Pol I does not increase the efficiency of Pol I binding to Rrn3p. 5 μl of fraction PA600 was treated with 1.8 μg of either recombinant wild type or mutant Fcp1p (D180E) for 10 min at 25°C. 5 μl of bacterially expressed ProtA-tagged Rrn3p was added, Rrn3p was affinity-purified using its ProtA tag, and co-purifying proteins were analyzed by Western blotting. B, Rrn3p binds to the rDNA promoter even in temperature-sensitive fcp1 mutant strains at the restrictive temperature. Chromatin IP/PCR at different dilutions were carried out using either FCP1 or fcp1-1 strains containing chromosomally TAP-tagged Rrn3p. Before (lanes 2–4) and 2 h after shift to restrictive temperature (37°C) (lanes 6–8), proteins bound to chromatin were immunoprecipitated using IgG-Sepharose. Increasing dilutions of the immunoprecipitated samples were analyzed by PCR using primers specific for the rDNA promoter and the termination region, as well as for the 5 S rDNA fragment, which served as an internal control because it is not transcribed by Pol I. A control experiment is shown in lanes 9–11. To down-regulate rDNA transcription, the cells were starved in a medium depleted of amino acids and subsequently analyzed in chromatin IP/PCR.
defective in Pol II transcription than fcp1 mutant strains (32, 45).

Third, Fcp1p can stimulate the activity of Pol I in both specific and nonspecific in vitro transcription assays. Stimulation by Fcp1p requires its phosphatase activity because it is not observed with a catalytically inactive, mutant Fcp1p protein.

Although similarities between the functions of Fcp1p in Pol I and Pol II transcription exist, our data clearly suggest that there are fundamental differences. Dephosphorylation of Pol II by Fcp1p at the end of one transcription cycle is necessary to make it usable for a subsequent round of transcription (32, 33, 51). In contrast, we found no indication so far that Fcp1p is involved in reinitiation of Pol I transcription (data not shown).

On the other hand, it was recently suggested that Fcp1p could stimulate Pol II to elongate its transcripts in a manner that is independent of its phosphatase activity (52). Our data now suggest that Fcp1p functions in Pol I-dependent transcription in a somewhat similar way as was reported for Pol II transcription. However, the phosphatase activity is required for this function; Fcp1p increases Pol I activity on single-stranded DNA, which might help to enhance chain elongation by Pol I.

Because TFIIH possesses kinase activity, it is tempting to speculate that Fcp1p is the antagonist of TFIIH in the Pol I-dependent transcription cycle. However, several lines of evidence contradict this assumption: (i) In contrast to the situation for Pol II promoter-dependent transcription, the kinase activity of TFIIH was not found to be required to reconstitute Pol I promoter-dependent transcription in vitro (28). (ii) Effects of TFIIH on nonspecific transcription by Pol I have not been observed so far. In contrast, Fcp1p stimulates nonspecific transcription by Pol I. (iii) Preliminary data suggest that the enzymatic activities of Fcp1p and TFIIH affect different Pol I subunits.2 (iv) Consistent with this, it was recently reported that, even in the Pol II transcription system, Fcp1p and the TFIIH subunit Kin28 might act at different stages of the transcription cycle; Fcp1p predominantly removed phosphates from Ser2 of the Pol II C-terminal domain during elongation, whereas Kin28 phosphates Ser2 during initiation (53). Thus, it appears that TFIIH and Fcp1p utilize different targets in the Pol I machinery, thereby modulating transcription in independent ways.

Fcp1p Plays No Role in the Formation of the Initiation-active Pol I-Rrn3p Complex—At which stages can the dephosphorylation reaction by Fcp1p influence Pol I transcription? Our data are not consistent with a function of Fcp1p during transcription initiation. Formation of the initiation-active form of Pol I, the Rrn3p-Pol I complex, requires a distinct phosphorylation state of its components. Although dephosphorylation of Pol I by alkaline phosphatase inhibits Rrn3p-Pol I complex formation (19), it is unlikely that Fcp1p acts in a similar way. Fcp1p stimulates rather than inhibits transcription. Furthermore, Fcp1p-dependent dephosphorylation of Pol I affects nonspecific RNA synthesis (Fig. 4B) but still allows formation of the initiation-active Rrn3p-Pol I complex from purified Rrn3p and Pol I (Fig. 5). Fcp1p might also modulate the phosphorylation pattern of the Rrn3p-Pol I complex after the formation of the preinitiation complex at the promotor has occurred. Because Rrn3p leaves Pol I during one round of transcription (13, 18), Fcp1p activity might result in dissociation of the Rrn3p-Pol I complex, allowing the elongation-active Pol I to clear the promotor and continue RNA synthesis. However, our results indicate that Fcp1p neither affects formation nor dissociation of the free Rrn3p-Pol I complex, and it remains to be determined whether Fcp1p dissociates the Pol I-Rrn3p complex if the complex is bound to the promotor.

Based on our results, it is likely that Fcp1p interacts only with a subpopulation of cellular Pol I. First we found only a substoichiometric (and weak) interaction between Fcp1p and the Pol I machinery (Fig. 2); second, although greatly reduced, a basal level of RNA synthesis could still be measured in Fcp1p-deficient strains if compared with strain-deficient in Pol I (Fig. 3); and third, ChIP/PCR experiments revealed that even in Fcp1p-deficient strains a significant amount of Pol I was still accumulated at the rDNA (data not shown). It is possible that only a minor fraction of cellular Pol I requires Fcp1p activity for rRNA synthesis, whereas the majority of Pol I is properly phosphorylated to initiate and elongate transcription without Fcp1p. Future experiments are required to determine at which stage in the rDNA transcription cycle Fcp1p functions and which kind of Pol I is the target. Our experiments indicate that phosphorylation of subunit ABC23 is reduced after incubation of Pol I with Fcp1p. We are presently trying to identify the crucial phosphorylation site and to analyze both initiation and elongation-competent Pol I active in response to the Fcp1p-dependent dephosphorylation of ABC23.

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Dephosphorylation of RNA Polymerase I by Fcp1p Is Required for Efficient rRNA Synthesis

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