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Cks1 Enhances Transcription Efficiency at the *GAL1* Locus by Linking the Paf1 Complex to the 19S Proteasome

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Cks1 was originally identified based on genetic interactions with *CDC28*, the gene that encodes Cdk1 in the budding yeast *Saccharomyces cerevisiae*. Subsequent work has shown that Cks1 binds Cdc28 and modulates its activity against certain substrates. However, the Cks1/Cdc28 complex also has a role in transcriptional chromatin remodeling not related to kinase activity. In order to elucidate protein networks associated with Cks1 transcriptional functions, proteomic analysis was performed on immunoaffinity-purified Cks1, identifying a physical interaction with the Paf1 complex. Specifically, we found that the Paf1 complex component Rtf1 interacts directly with Cks1 and that this interaction is essential for efficient recruitment of Cks1 to chromatin in the context of *GAL1* gene induction. We further found that Cks1 in this capacity serves as an adaptor allowing Rtf1 to recruit 19S proteasome particles, shown to be required for efficient RNA production from some rapidly inducible genes such as *GAL1*.

Cks1 (cyclin-dependent kinase subunit 1), first identified in yeasts based on genetic and physical interaction with Cdc28 and Cdc2, the budding and fission yeast orthologs of Cdk1, has been shown to be involved in cell cycle regulation (1, 2). Loss of Cks1 function impedes cell cycle progression due to inadequate expression of Cdc20, a positive regulator of mitosis (3). Recruitment of Cks1 to the promoter of *CDC20* is correlated with the periodic expression of *CDC20* mRNA, suggesting a direct role in *CDC20* transcription (3). The observation that Cks1 interacts with the proteasome (4) implies that the transcriptional function of Cks1 may involve the proteasome. Indeed, both Cks1 and the proteasome bind to the promoter of *CDC20* coordinately with its expression (3). Proteolysis is the primary function carried out by the 26S proteasome, composed of 19S regulatory and 20S proteolytic core particles (5), and degradation of RNA polymerase (Pol) II and some transcription factors has been shown to be an integral part of the transcription cycle (6, 7). However, nonproteolytic roles of the proteasome are also central to the transcription process (8, 9). In particular, the 19S regulatory particle acts as a distinct unit on chromatin during transcription regulation, independent of protein degradation functions (10). It has been suggested that the complete 26S proteasome is recruited to chromatin initially, but whether the proteolytic or nonproteolytic functions will be executed is context dependent (11).

The 19S proteasome affects many aspects of transcription, including (i) modulation of association between transcription factors and chromatin (12–14), (ii) regulation of interaction among transcription factors (15, 16), (iii) modification of histones (15, 17–21), and (iv) enhancement of transcription efficiency (22, 23). The interaction between Cks1 and the proteasome led us to investigate the contribution of Cks1 to aspects of transcription that require the function of the 19S proteasome. To this end, we chose the *GAL1* gene, encoding galactokinase, as the readout system for study of Cks1 function because of its robust inducibility, a signature shared by most 19S proteasome-targeted genes (24), and because induction can be easily manipulated. We have previously shown that Cks1 is required for recruitment of the 19S proteasome to the *GAL1* locus (25), leading to induction-specific nucleosome eviction required for efficient transcriptional elongation (26) and possibly other functions. Presumably the ATPase activities associated with the 19S proteasome provide energy required for this aspect of chromatin remodeling. However, how Cks1 is recruited to genes and executes regulatory roles during transcription remains to be elucidated. Therefore, to gain more insight into Cks1-mediated transcriptional functions, we have carried out analyses to identify Cks1-interacting proteins.

In the present study, a proteomic analysis of Cks1 immunoprecipitates identified components of the Paf1 complex (RNA polymerase II-associated factor 1). The Paf1 complex, consisting of Cdc73, Ctr9, Leo1, Paf1, and Rtf1, is thought to be a platform for assembling factors that facilitate RNA polymerase II movement along chromatin during transcriptional elongation, although each component has specific roles during these processes (27). Recent findings indicate that the Paf1 complex is also involved in Pol I transcription and RNA processing and export (28). Notably, the Paf1 complex is required for H2B ubiquitylation (29, 30), and the ubiquitylation of histone H2B is required for the recruitment of the 19S proteasome to *GAL* genes (17). Nevertheless, biochemical evidence linking the Paf1 complex to the 19S proteasome is lacking. Here we report that Cks1 serves as a mediator for recruitment of the 19S proteasome to the Paf1 complex, thereby promoting transcriptional elongation by providing energy for nucleosome eviction.

MATERIALS AND METHODS

Yeast strains. All *Saccharomyces cerevisiae* strains were derived from 15Daub (31). Genotypes were generated by standard yeast genetic methods (32) and are listed in Table 1.

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TABLE 1 *S. cerevisiae* strains used in this study

Strain	Genotype	Reference or source
15D a	a *bar1 ura3 ade1 his2 leu2-3 trp1*	31
15D α	α *bar1 ura3 ade1 his2 leu2-3 trp1*	31
Δcks1	15D α *cks1*Δ::*KAN*^{R}	25
Cks1-Flag	15D a *CKS1-Flag*	25
Cks1-Flag, Rtf1-HA	15D a *CKS1-Flag RTF1-HA*	This study
Cks1-Flag, Rtf1-Myc	15D a *CKS1-Flag RTF1-Myc*	This study
Δcdc73	15D α *cdc73*Δ::*HYG*^{R}	This study
Δctr9	15D α *ctr9*Δ::*HYG*^{R}	This study
Δleo1	15D α leo1Δ::*HYG*^{R}	This study
Δpaf1	15D α *paf1*Δ::*HYG*^{R}	This study
Δrtf1	15D α *rtf1*Δ::*HYG*^{R}	This study
Cks1-Flag, Δcdc73	15D a *CKS1-Flag cdc73*Δ::*HYG*^{R}	This study
Cks1-Flag, Δctr9	15D a *CKS1-Flag ctr9*Δ::*HYG*^{R}	This study
Cks1-Flag, Δleo1	15D a *CKS1-Flag leo1*Δ::*HYG*^{R}	This study
Cks1-Flag, Δpaf1	15D a *CKS1-Flag paf1*Δ::*HYG*^{R}	This study
Cks1-Flag, Δrtf1	15D a *CKS1-Flag rtf1*Δ::*HYG*^{R}	This study
Rtf1-Flag	15D α *RTF1-Flag*	This study
Rtf1-Flag, Δcks1	15D α *RTF1-Flag cks1*Δ::*KAN*^{R}	This study
Rtf1-HA	15D a *RTF1-HA*	This study
Rpt1-Flag	15D a *RPT1-Flag*	This study
Rpt1-Flag, Rtf1-HA	15D a *RPT1-Flag RTF1-HA*	This study
Rpt1-Flag, Δrtf1	15D a *RPT1-Flag rtf1*Δ::*HYG*^{R}	This study

Yeast growth. Regular growth medium for our yeast stains was yeast extract-peptone (YEP) with 2% dextrose (YEPD). For *GAL1* induction experiments, yeast cells were grown in YEP medium with 2% raffinose, galactose (Gal) with a final concentration of 2% was added, and cells were incubated for 45 min. Mating pheromone arrest synchrony experiments were performed as described previously (33).

Antibodies. The following antibodies were obtained from commercial sources: anti-Flag (Sigma), anti-glutathione *S*-transferase (anti-GST; Millipore), antihemagglutinin (anti-HA; Roche), anti-His (Millipore), anti-Myc (Sigma), and anti-Rpt1 (Abcam). Anti-Cdc28 antibodies were described previously (34).

Western blotting and coimmunoprecipitation. Western blotting and coimmunoprecipitations were carried out as previously described (4).

Purification of proteins for mass spectrometry analysis. Flag-Cks1 and untagged strains were grown to an optical density (OD) of 2.0, typically in 1 liter of YEPD medium. Cells were harvested and washed once with ice-cold water. The cell pellet was drop frozen in liquid nitrogen and ground to a fine powder using a Retsch grinder chilled with liquid nitrogen. The ground powder was collected in a 50-ml screw-cap tube and thawed in one pellet volume of 50 mM Tris (pH 7.5), 150 mM NaCl, 10% glycerol, and 5 mM $MgCl_2$ (buffer A). The thawed cell lysate was centrifuged for 20 min at 17,000 rpm, and the pellet was discarded. A 3-ml aliquot of the supernatant was mixed with 100 μl of anti-Flag M2 agarose beads (Sigma, St. Louis, MO) for 2 h on a rocker at 4°C. The beads were then collected, transferred to 2-ml microcentrifuge tubes, and washed with 50 volumes total of buffer A plus 0.2% Triton X-100. Specifically bound proteins were eluted for 3 h at 4°C with three bead volumes of elution buffer containing 25 mM Tris (pH 7.5), 150 mM NaCl, 15% glycerol, 5 mM $MgCl_2$, and 100 μg/ml of Flag peptide. Eluates from both Flag-Cks1 and untagged strains were prepared for analysis by mass spectrometry.

Mass spectrometry analysis. Mass spectrometry analysis was performed as previously described (35, 36). Proteins were considered candidate Cks1-interacting proteins only if they were identified in Cks1 affinity purifications and not in control purifications from untagged wild-type yeast strains.

TABLE 2 Cks1-interacting proteins

Protein	Function	No. of peptides	No. of spectra	% sequence coverage
Cdc28	CDK	7	11	20.8
Clb1	B-type cyclin	4	5	12.7
Clb2	B-type cyclin	3	5	10.8
Clb3	B-type cyclin	4	4	15.9
Clb4	B-type cyclin	1	1	2.4
Clb5	B-type cyclin	5	8	12.4
Cln1	G1 cyclin	1	1	1.5
Cln2	G1 cyclin	2	2	5.5
Sic1	CDK inhibitor	10	16	27.5
Hhf1,2	Histone H4	3	3	34
Hht1,2	Histone H3	1	2	23.5
Paf1	Paf1 complex	3	3	9.9
Rtf1	Paf1 complex	2	2	4.7
Cdc73	Paf1 complex	2	5	7.1
Ctr9	Paf1 complex	6	6	9.6

ChIP. Chromatin immunoprecipitation (ChIP) samples were prepared as previously described (37). Primer locations and sequences for *GAL1* have been described previously (26).

RNA purification and RT-PCR. Total RNA was purified using the RNeasy kit (Qiagen) per the manufacturer's instructions. Real-time PCR (RT-PCR) operations and primer sequences were described previously (26). Data analysis was performed using MJ Opticon Monitor analysis software, version 3.0.

Protein purification. *Escherichia coli* BL21 cells expressing GST or GST fusion proteins were grown to an OD of 0.5 at 600 nm. The induction conditions were 0.2 mM isopropyl-β-D-thiogalactopyranoside (IPTG) for 12 h at 27°C. Cells were centrifuged and washed once with phosphate-buffered saline (PBS). They were then resuspended in 1/20 culture volume of buffer containing 50 mM Tris-HCl (pH 7.8), 500 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM dithiothreitol (DTT), and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) and then lysed by sonication on ice. Lysates were precleared at 35,000 × *g* for 15 min and then incubated with glutathione-Sepharose beads (Pharmacia Biotech) at 4°C. The GST fusion protein-bound beads were washed five times with 50 mM Tris-HCl (pH 7.8), 150 mM NaCl, 1 mM EDTA, 1% NP-40, 1 mM DTT, and 0.5 mM PMSF. For 6×His-tagged proteins, cells were lysed in lysis buffer (PBS, 1% Triton X-100, 5 mM imidizole [pH 8.0], 0.1% PMSF), followed by sonication on ice. Lysates were precleared at 35,000 × *g* for 15 min and then incubated with nickel-nitrilotriacetic acid (Ni-NTA) agarose (Qiagen) at 4°C and washed according to the manufacturer's instructions. All binding assays were performed at 4°C for 4 h. Samples were resolved by 4 to 20% SDS-PAGE. Western blots were developed using antibodies as indicated below.

Reproducibility and significance. All experiments were carried out at least two times. Error bars, where shown, correspond to standard deviations (SD). *P* values were determined using Student's *t* test.

RESULTS

Cks1 interacts with the Paf1 complex. To identify Cks1-interacting proteins, immunoprecipitates of Cks1 were subjected to high-resolution mass spectrometric analysis (multidimensional protein identification technology [MuDPIT]) (35, 36). Specifically, proteins bound to Flag-Cks1 immunoprecipitated using anti-Flag resin were compared to proteins bound to the resin when incubated with extract from a nontagged strain. Among Flag-Cks1-specific interactions detected (Table 2), components of the Paf1 transcriptional elongation complex were of particular interest from a transcriptional perspective. To confirm the interaction,

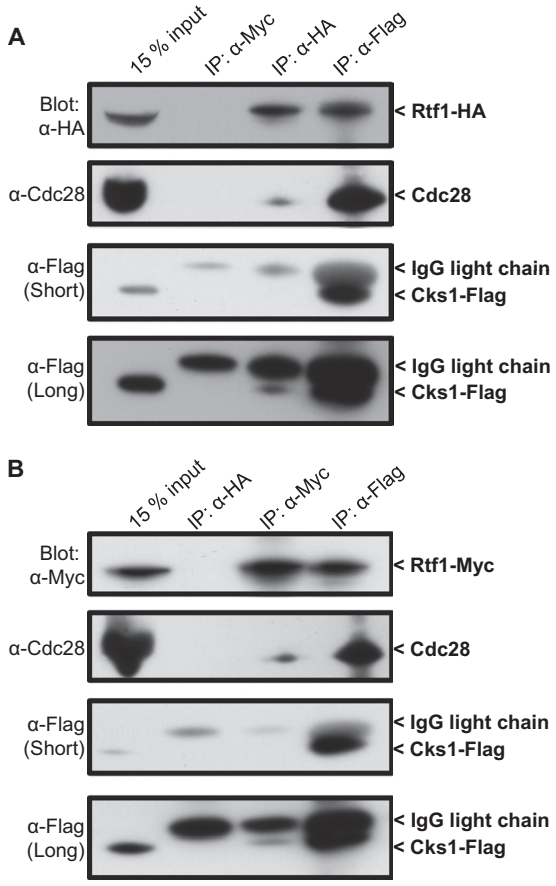

FIG 1 Rtf1 binds Cks1/Cdc28 *in vivo*. (A) Cells harboring Flag-tagged Cks1 and HA-tagged Rtf1 were lysed and immunoprecipitated (IP) using anti-Myc (as a negative control), anti-HA, or anti-Flag antibodies. Immunoprecipitates were analyzed by SDS-PAGE and Western blotting using the indicated antibodies. The middle image shows a short exposure, and the bottom blot shows a long exposure. (B) Cells harboring Flag-tagged Cks1 and Myc-tagged Rtf1 were lysed and immunoprecipitated using anti-HA (as a negative control), anti-Myc, or anti-Flag antibodies. Immunoprecipitates were analyzed by SDS-PAGE and Western blotting using the indicated antibodies. The middle blot shows a short exposure, and the bottom blot shows a long exposure. Note that Cks1-Flag runs immediately below the IgG light chain in immunoprecipitates.

Cks1 was tagged with the Flag epitope and one component of the Paf1 complex, Rtf1, was tagged with the HA (Fig. 1A) or Myc (Fig. 1B) epitope. To maintain endogenous expression levels, both proteins were tagged at their carboxyl termini by insertion of sequences into the respective chromosomal loci. Immunoprecipitation of Cks1 and Rtf1 was performed, and in each case, the heterologous protein was detected by Western blotting, revealing an interaction and thus confirming the proteomic analysis. In addition, Cdc28 (Cdk1), a binding partner of Cks1, was detected in all immune complexes. Note that whereas Cks1 immune complexes were highly enriched for Rtf1, a relatively smaller proportion of Rtf1 immune complexes contained Cks1 and Cdc28. This suggests that a significant fraction of the Cks1 pool in the cell is associated with Rtf1, whereas a much smaller fraction of the Rtf1 pool is bound to Cks1. It has been reported that Rtf1 binds nonspecifically to anti-Flag resin (38, 39), possibly producing an artifactual result for the Flag-Cks1 pulldown component of the experiment. However, when extract containing HA-Rtf1 but not Flag-Cks1 was incubated with anti-Flag resin, HA-Rtf1 was not detected in the eluate, whereas it was detected in parallel anti-HA immunoprecipitates (see Fig. S1 in the supplemental material). Therefore, under the experimental conditions employed in the current study, Rtf1 does not bind to anti-Flag resin at a level that is detectable.

***GAL1* induction is defective in an *rtf1* deletion mutant.** We have previously shown that Cks1 regulates transcription of *GAL1* (25). Because of the physical interaction between Cks1 and Rtf1, we hypothesized that Cks1 is involved in *GAL1* transcription through interaction with the Paf1 complex. Thus, mutants with deletions of each component in the Paf1 complex were generated and induction of *GAL1* was measured 45 min after addition of galactose by quantitative real-time PCR analysis. Previously, it has been shown that deletion of some genes encoding Paf1 complex subunits confers defective galactose-dependent transcription at some loci (40, 41). However, a complete analysis of all component genes has not been reported, and strain background differences appear to affect Paf1 complex mutant phenotypes (our unpublished observations), justifying this partial repetition of previous work. As shown in Fig. 2A, the induction of *GAL1* is attenuated in several of the mutants, but most severely in the *rtf1* deletion mutant. To exclude the trivial possibility that the defect in *GAL1* transcription in the Paf1 complex mutants is due to altered expression of Cks1, endogenous Cks1 was Flag tagged in the various deletion mutant strains and Western blotting was carried out to compare the levels of Cks1 protein expression. Whereas the *leo1* and *cdc73* mutants indeed showed reduced expression of Cks1, there was no detectable alteration of Cks1 expression in the *rtf1* mutant (Fig. 2B). Therefore, the defect in *GAL1* induction in the *rtf1* mutant is not due to the impairment of Cks1 expression but most likely is attributable to a direct contribution of Rtf1 to *GAL1* transcription.

Rtf1 is required for recruitment of Cks1 onto the *GAL1* ORF. Because failure to express Cks1 was ruled out as the mechanism accounting for the deficiency of *GAL1* induction in the *rtf1* mutant, we investigated whether recruitment of Cks1 to the promoter or the open reading frame (ORF) regions of the *GAL1* gene was defective. Note that recruitment of Cks1 to the *GAL1* gene has been shown to be essential for efficient transcriptional induction (25). Therefore, ChIP was carried out using anti-Flag antibodies to determine the occupancy of Flag-Cks1 on *GAL1* chromatin during a 45-min time course. Four primer sets designed to amplify the upstream activating sequence (UAS)/promoter, 5′ ORF region, middle ORF region, or 3′ ORF region of the *GAL1* gene are shown schematically in Fig. 2C (upper portion). In contrast to wild-type cells and the *paf1* deletion mutant, the *rtf1* mutant showed highly compromised Cks1 binding along the entire *GAL1* ORF (Fig. 2C). Thus, a failure to efficiently recruit Cks1 to the *GAL1* ORF could account for the defect in *GAL1* induction observed in the *rtf1* mutant.

The failure to accumulate *GAL1* mRNA observed in the *rtf1* mutant could be due to a defect in transcription initiation or in a downstream function, such as elongation, 3′ end processing, or mRNA transport. We therefore carried out an RNA polymerase II ChIP in parallel with the Flag-Cks1 ChIP described above (see Fig. S2 in the supplemental material). Clearly, there is no defect in RNA polymerase II occupancy as a function of *GAL1* induction in the *rtf1* mutant, ruling out a defect in transcription initiation.

To determine if Cks1 and Rtf1 are required mutually for load-

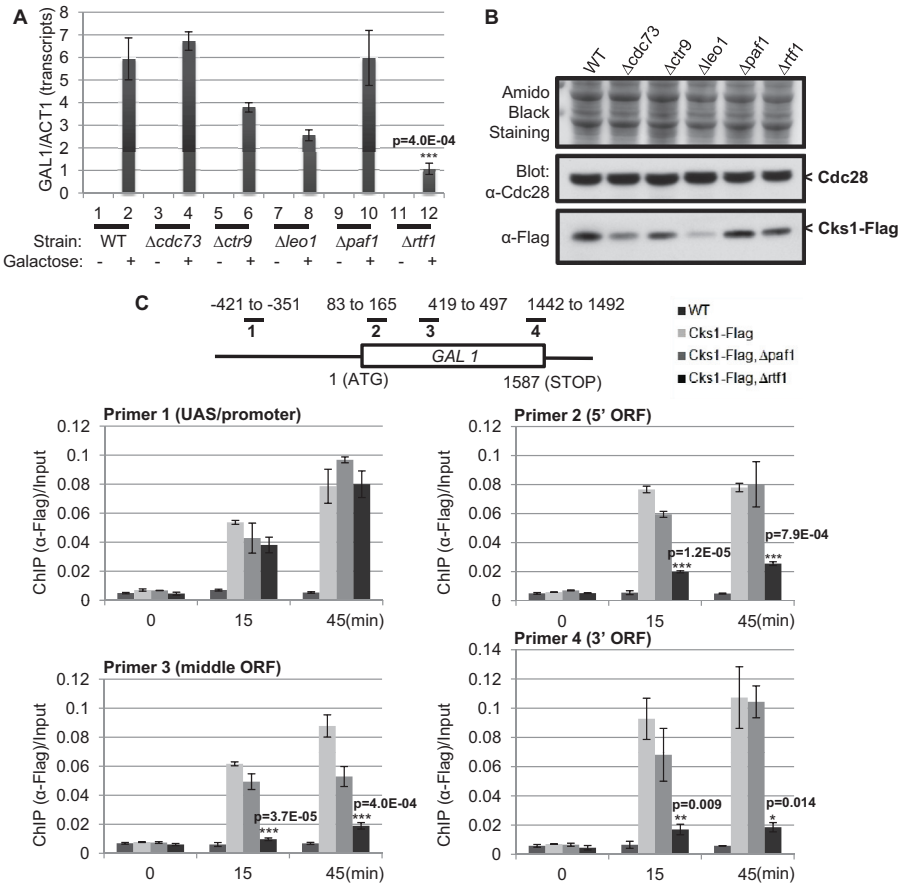

FIG 2 Rtf1 is required for effective recruitment of Cks1 to the *GAL1* ORF. (A) Each component of the Paf1 complex was deleted separately. Wild-type (WT) and mutant cells were cultured in raffinose (−) for 2 h, and 2% galactose (+) was added for 45 min. *GAL1* transcripts were quantified and normalized to actin (*ACT1*) mRNA. (B) Expression level of Flag-tagged Cks1 in WT and various Paf1 complex deletion mutant strains. Samples were analyzed by SDS-PAGE and Western blotting. Amido black staining (top) and Cdc28 (middle) served as loading controls. (C) Recruitment of Flag-tagged Cks1 to the *GAL1* ORF in the absence of Paf1 or Rtf1. Upon galactose induction for 15 or 45 min, chromatin immunoprecipitation using anti-Flag antibodies was performed in WT, Flag-tagged Cks1, and *paf1* and *rtf1* deletion mutant strains. Cks1-associated chromatin fragments were isolated, amplified using the indicated primers, and normalized to the amount of input DNA prior to immunoprecipitation. All error bars represent SD. Statistical analysis was carried out comparing WT and Δ*rtf1* in panels A and C. *P* values were determined using Student's *t* test.

ing onto chromatin during transcription, ChIP analysis was conducted to compare the recruitment of Rtf1 to the *GAL1* gene in the *cks1* deletion mutant versus the wild-type strain. As previously shown (25), *GAL1* expression is reduced in the absence of Cks1 (Fig. 3A). However, levels of Rtf1 (Fig. 3B) and binding of Rtf1 to the *GAL1* gene are comparable in the *csk1* mutant and wild-type strain (Fig. 3C). Taken together, these results indicate that whereas binding of Cks1 to the *GAL1* ORF in the context of transcriptional induction is dependent on Rtf1, binding of Rtf1 is independent of Cks1.

Interaction between Cks1 and Rtf is direct. The binding detected between Cks1 and Rtf1 in Fig. 1 could be direct or mediated by other proteins. In order to distinguish between these mechanisms, Cks1 (GST tagged) and Rtf1 (Flag tagged) were expressed in *E. coli*. GST alone or GST-tagged Cks1 was immobilized on glutathione agarose beads and incubated with extracts containing Flag-Rtf1. The beads were washed, and eluted proteins were analyzed by SDS-PAGE and Western blotting. Rtf1 was captured on beads containing GST-Cks1 but not those containing GST (Fig. 4A), indicating a direct interaction. Through a series of domain mapping experiments analogous to the experiment described above, except that fragments of Rtf1 were tested for ability to bind Cks1 (see Fig. S3 in the supplemental material), the Cks1 binding domain of Rtf11 was identified as residing between residues 234 and 373 (Fig. 4B). In this case, the central fragment of Rtf1 was tagged with the 6×His epitope rather than the Flag epitope used to mark the full-length protein in Fig. 4A. This is a region shown genetically to be essential for a number of the transcriptional functions attributed to Rtf1 (42).

Cks1 is essential for recruiting the 19S proteasome particle to Rtf1. Since Rtf1 is required for efficient recruitment of Cks1 to *GAL1* (Fig. 2C and 3C) and Cks1 is required for recruitment of the 19S proteasome particle (25), we speculated that Cks1 might serve as an adaptor to allow binding the 19S proteasome particle to Rtf1. To test this idea, we first carried out an immunoprecipitation experiment to determine whether Rtf1 and the 19S proteasome interact *in vivo*. Rtf1 was tagged using the HA epitope, and Rpt1, an ATPase component of the 19S proteasome base, was tagged using the Flag epitope. When lysates were immunoprecipitated using anti-Myc (negative control), anti-Flag, and anti-HA anti-

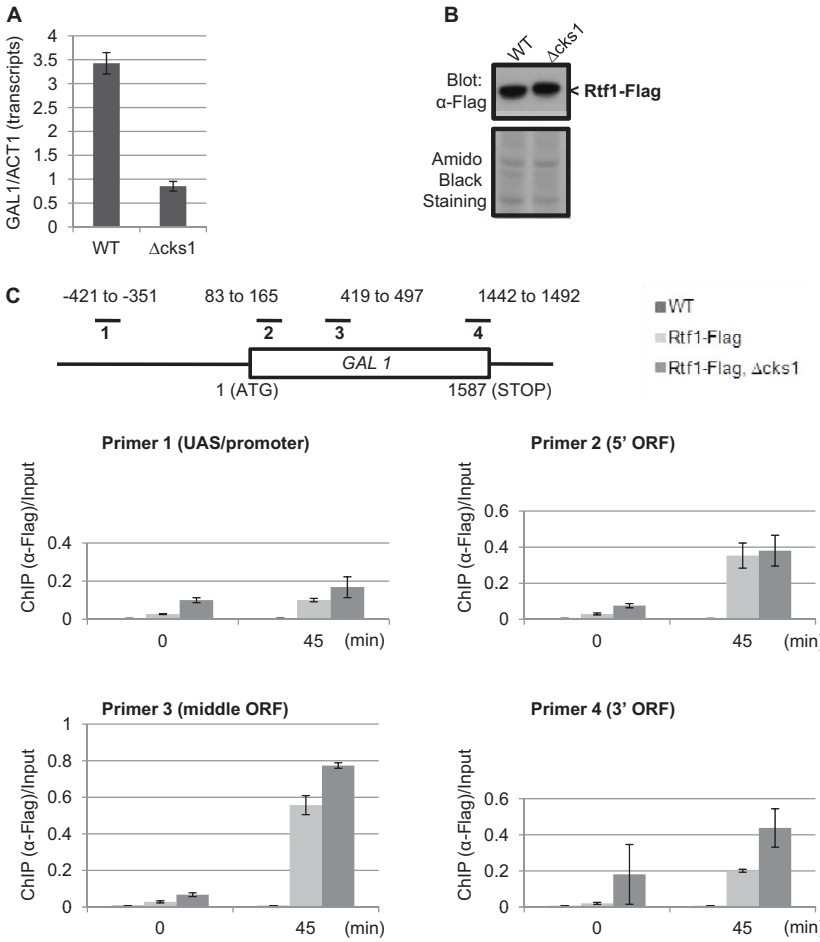

FIG 3 Cks1 is not required for the recruitment of Rtf1 to the *GAL1* ORF. (A) Wild-type or *cks1* deletion mutant cells were cultured in raffinose for 2 h, and 2% galactose was added for 45 min. *GAL1* transcripts were quantified and normalized to actin (*ACT1*) mRNA. (B) Expression level of Flag-tagged Rtf1 in wild-type and *cks1* deletion mutant strains. Samples were analyzed by SDS-PAGE and Western blotting. Amido black staining (bottom) was used as a loading control. (C) Recruitment of Flag-tagged Rtf1 to the *GAL1* ORF in the absence of Cks1. Before and after galactose induction for 45 min, chromatin immunoprecipitation using anti-Flag antibodies was performed on wild-type untagged (control) wild-type Rtf1-Flag and *cks1* Rtf1-Flag strains. Rtf1-associated chromatin fragments were isolated, amplified using the indicated primers, and normalized to the amount of input DNA prior to immunoprecipitation. Error bars represent SD.

bodies (Fig. 4C) a strong band at the position of Rpt1 was detected in the lane corresponding to the anti-Flag immunoprecipitate, as expected (upper and lower portions). In the lane corresponding to the HA-Rtf1 pulldown, a weak band at the mobility corresponding to Rpt1 is detected over background. This minimal signal is not surprising, since only a very small fraction of 19S proteasome particles in the cell is likely to be involved in transcription rather than proteolysis, the primary function of proteasomes. It was not possible to determine whether Rtf1 was coimmunoprecipitated with Flag-Rpt1 because of the high background contributed by anti-Flag IgG at the relevant position on the blot. To examine whether Cks1 is essential for the interaction between Rtf1 and the 19S proteasome, we devised a modified pulldown experiment. The Cks1-interacting region of Rtf1, residues 234 to 373, containing a 6×His tag produced in *E. coli*, was immobilized on Ni-NTA beads, which were then incubated with wild-type yeast extract or extract from a *cks1*Δ strain. When proteins were eluted and subjected to SDS-PAGE and Western blotting, Rpt1 from wild-type but not *cks1*Δ extract was captured on the Rtf1 beads (Fig. 4D). Therefore, the 19S proteasome particle cannot bind directly to Rtf1 but can bind in the presence of Cks1. To confirm that Cks1 is necessary and sufficient to mediate this interaction, we performed an *in vitro* reconstitution experiment (Fig. 4E). Again, the Cks1-interacting region of Rtf1 was preloaded onto Ni-NTA beads, after which the beads were incubated with GST alone or GST-tagged Cks1. Lysates from a *cks1*Δ strain were then incubated with the beads supplemented with GST-Cks1 or GST alone. Capture of 19S proteasome particles was again determined by Western blotting using anti-Rpt1 antibody. 19S particles were only captured by beads that had been preincubated with GST-Cks1 (Fig. 4E), confirming that Cks1 can serve as a mediator for recruiting 19S particles to Rtf1. If this relationship between Rtf1, Cks1, and the 19S proteasome particle exists *in vivo*, then Rtf1 should contribute to recruitment of the 19S proteasome to chromatin. We therefore used ChIP analysis to compare recruitment of the 19S particle to the *GAL1* ORF in wild-type versus *rtf1* deletion strains. There was a significant decrease in Rpt1 binding to the *GAL1* ORF in the *rtf1* mutant after 45 min of induction (Fig. 5B), similar to the reduction observed for Cks1 binding (Fig. 2C). Deletion of *rtf1* did not affect the level of Rpt1 (Fig. 5A), thereby excluding changes in

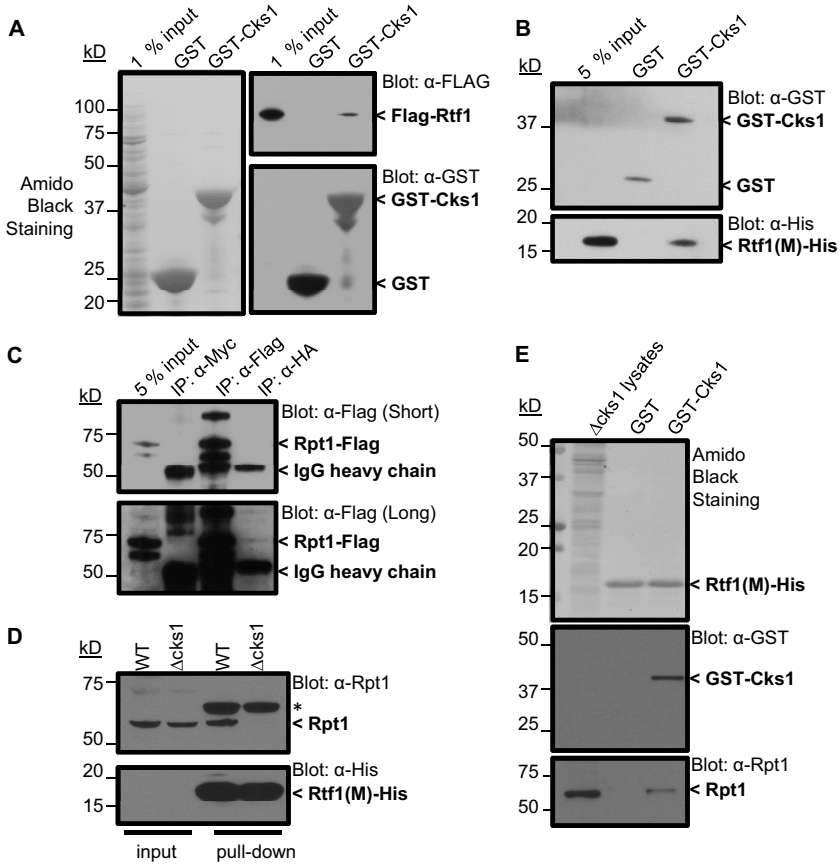

FIG 4 Cks1 serves as an adaptor for recruiting the 19S proteasome to Rtf1. (A) Rtf1 binds directly to Cks1. GST or GST-Cks1 immobilized on glutathione resin was incubated with crude extract from *E. coli* expressing Flag-tagged Rtf1 (full length). Rtf1, GST, and GST-Cks1 were detected by Western blotting using the indicated antibodies. (B) A central domain of Rtf1 is essential for binding Cks1. Immobilized GST or GST-Cks1 was incubated with His-tagged Rtf1 central domain (234 to 373 amino acids) purified from *E. coli* extracts by Ni-NTA chromatography. Rtf1, GST, and GST-Cks1 were detected by Western blotting using the indicated antibodies. (C) Rtf1 associates with the 19S proteasome *in vivo*. Cells expressing Flag-tagged Rpt1 and HA-tagged Rtf1 were lysed and immunoprecipitated using anti-Myc (negative control), anti-HA, or anti-Flag antibodies. Samples were analyzed by SDS-PAGE and Western blotting. (D) The association between Rpt1 and the 19S proteasome requires Cks1. Lysates from wild-type (WT) or Δ*cks1* cells were incubated His-tagged Rtf1 central domain (234 to 373 amino acids) immobilized on Ni-NTA resin. Eluted proteins were analyzed by SDS-PAGE and Western blotting for the indicated proteins. (E) Cks1 is necessary and sufficient for binding of the 19S proteasome to Rtf1. Lysates from Δ*cks1* cells were incubated with His-tagged Rtf1 central domain (234 to 373 amino acids) immobilized on Ni-NTA resin, which was then incubated with GST or GST-Cks1. Bound Rpt1 was detected by SDS-PAGE, followed by Western blotting. Approximate molecular masses are indicated to the left of each blot.

Rpt1 expression as an explanation for reduced recruitment. These data are consistent with Cks1 serving as an adaptor that facilitates Rtf1-mediated recruitment of the 19S proteasome particle to actively transcribed chromatin in order to carry out transcriptional functions (Fig. 5C).

DISCUSSION

Transcriptional elongation is a highly regulated process involving both positive and negative factors (43). In this context, the Paf1 complex serves as a multifunctional platform for recruiting factors that alter chromatin dynamics (28). Nevertheless, the molecular mechanisms downstream of the Paf1 complex have not been completely elucidated. Our proteomic analysis of Cks1-interacting proteins identified 4 out of 5 components of the Paf1 complex (Table 2), implying a potential role for Cks1 in transcriptional functions of the Paf1 complex. In the current study, we demonstrated that Cks1 serves as an adaptor that allows Rtf1, a component of the Paf1 complex, to recruit the 19S proteasome particle to target genes, necessary for efficient transcriptional elongation and transcript processing (22, 23). Although we have not directly determined the specific role of Rtf1 in this context at the *GAL1* locus in our strain background, the fact that *GAL1* message accumulation was impaired in the *rtf1*Δ mutant, but occupancy of RNA polymerase II on the *GAL1* ORF was not, argues against a role in transcriptional activation or elongation and favors a role in mRNA processing. On the other hand, the facts that both Cks1 and the 19S proteasome particle have a role in nucleosome eviction (26) and that Rtf1 is required for efficient Cks1 and 19S proteasome particle recruitment are consistent with a role in transcriptional elongation. More direct experiments will be required to distinguish between these functions.

Rtf1-dependent recruitment of Cks1 and the 19S proteasome to the *GAL1* gene during transcriptional induction. The Paf1 complex was identified based on its physical association with RNA polymerase II (44). Using the *GAL1* gene as a model system, it was previously shown that deletion of genes encoding Ctr9 or Paf1, components of the Paf1 complex, led to reduced expression by impairing nucleosome eviction as well as decreasing association of

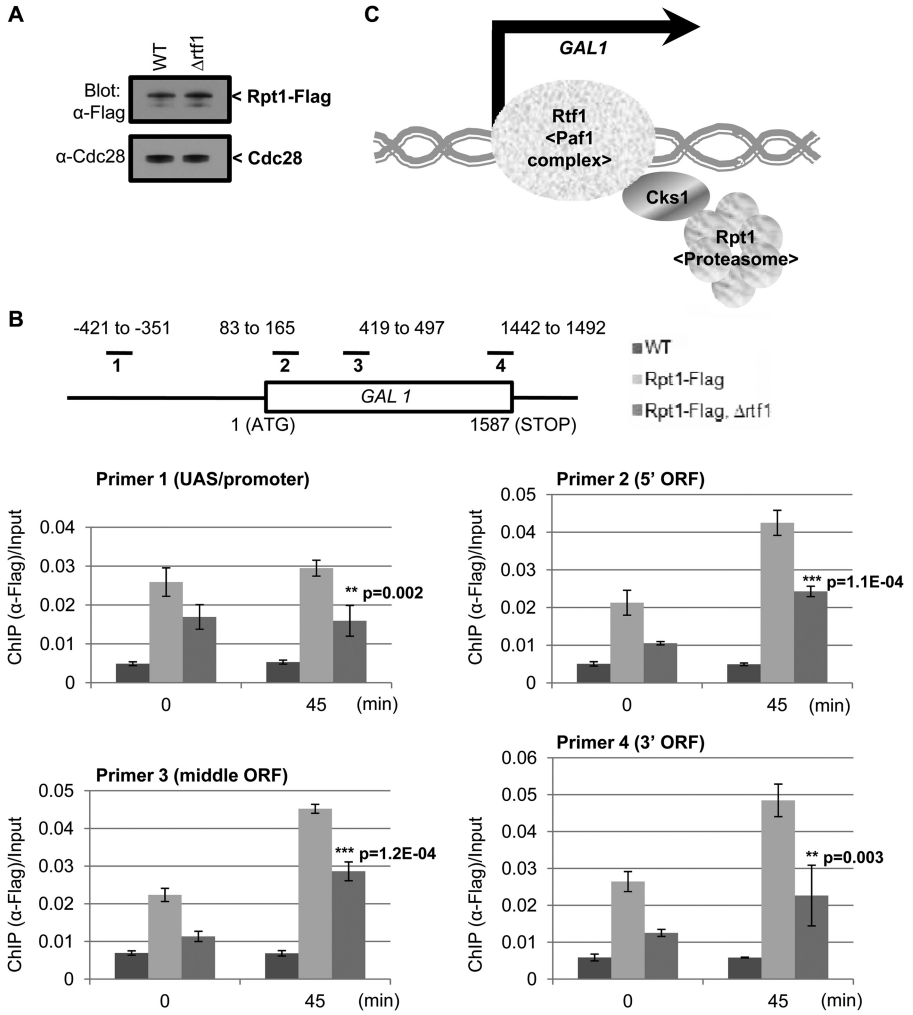

FIG 5 Rtf1 is required for efficient recruitment of the 19S proteasome to the *GAL1* ORF. (A) Expression level of Flag-tagged Rpt1 in wild-type (WT) and *rtf1* deletion mutant strains. Samples were analyzed by SDS-PAGE and Western blotting. Cdc28 (Cdk1) was used as a loading control. (B) Recruitment of Flag-tagged Rpt1 to the *GAL1* ORF in the absence of Rtf1. Before and after galactose induction for 45 min, chromatin immunoprecipitation using anti-Flag antibodies was performed in wild-type untagged protein (control), wild-type Rpt1-Flag, and *rtf1* Rpt1-Flag. Rpt1-associated chromatin fragments were isolated, amplified using the indicated primers, and normalized to the amount of input DNA prior to immunoprecipitation. All error bars represent SD. Statistical analysis was carried out comparing WT and Δ*rtf1*. *P* values determined using Student's *t* test. (C) Model for Cks1-mediated transcription. We propose that Cks1 functions as an adaptor for recruiting the 19S proteasome to Rtf1 in order to promote efficient transcription elongation.

RNA Pol II (40). Yet the molecular mechanism whereby the Paf1 complex promotes nucleosome eviction has remained poorly understood. We have previously shown that Cks1 and the 19S proteasome particle are required for induction-dependent nucleosome eviction at the *GAL1* gene (26). In the present study, we demonstrate that Cks1 promotes Rtf1-dependent recruitment of the 19S proteasome particle to the *GAL1* gene, possibly providing an explanation for Paf1 complex-mediated nucleosome eviction. Interestingly, the recruitment of Cks1 was affected within the ORF region but not the UAS region of the *GAL1* gene in the *rtf1* deletion mutant (Fig. 2C). However, 19S proteasome particle loading was defective in both the UAS and ORF regions (Fig. 5B). This suggests that different mechanisms of proteasome recruitment might be operative with the UAS versus ORF regions. Indeed, it has been shown that recruitment of the 19S proteasome particle to the UAS region of the *GAL1* gene for transcriptional activation functions is dependent on the transcription factor Gal4 and the chromatin-remodeling complex SAGA (16). Therefore, it appears that Cks1 is only important for recruitment of the 19S proteasome particle for elongation functions of Rtf1. This is also consistent with previous reports suggesting that the 19S proteasome particle has distinct functions in transactivation and elongation (22, 23).

General applicability of the mechanisms proposed in this study. Cks1 has been shown to regulate expression of two inducible genes, *CDC20* (3) and *PHO5* (26). Other inducible cell cycle regulated genes (45, 46) and *PHO5* (47) have also been reported to be regulated by the Paf1 complex. These data suggest that Rtf1-Cks1-19S proteasome particle axis comes into play particularly when rapid gene induction requires energy for nucleosome eviction. Presumably the ATPases of the 19S particle are mobilized for this function. Interestingly, Rtf1 seemed to be the most critical member of the Paf1 complex in *GAL1* expression in our genetic background (Fig. 2A). This is consistent with a recent study in which a fragment of Rtf1 could support H2B ubiquitylation even

in the absence of other Paf1 complex members (48). In addition, Rtf1 is essential for the association of the Paf1 complex with chromatin and RNA Pol II in yeast (49). Therefore, Rtf1 appears to be central to the Paf1 complex and in some cases can carry out functions independently. Indeed, we observed no defect in *GAL1* induction in the *paf1* deletion mutant but a strong defect in the *rtf1* deletion mutant. It should be pointed out, though, that in another study, the *paf1* mutant was defective in *GAL1* induction (40), most likely attributable to differences in genetic background.

Possible parallels between Cks protein transcriptional functions in yeast and mammalian cells and possible roles in cancer. We discovered novel physical and functional interactions between Cks1 and a component of the Paf1 complex in yeast. Several observations suggest that a similar functional relationship may exist in mammals. First, Cks1 (50) and the Paf1 complex (28) share significant structural similarity with their counterparts in mammals. Second, Cks paralogs, Cks1 and Cks2, are required for efficient expression of *CDK1*, *CCNA2*, and *CCNB1* (encoding Cdk1, cyclin A2, and cyclin B1) (51, 52). A similar relationship exists between the Paf1 complex and expression of the same three genes (53). Moreover, in some ductal breast carcinomas, the expression of Cdk1, cyclin B1, and cyclin A2, as well as Cks proteins, is coordinately upregulated (54). However, although Cks protein overexpression is a characteristic of many forms of cancer (55–73) and Cks1 deletion has protective effects in some mouse cancer models and cell line studies (74–76), it is not clear that Cks-mediated oncogenicity is related to transcriptional functions. As Cks1 is a component of the SCF^{Skp2} ubiquitin ligase, it has been suggested that oncogenic functions of Cks1 might be mediated by affecting the stability of the Cdk inhibitor $p27^{Kip1}$, an SCF^{Skp2} target (77). Yet disruption of Skp2 has only a modest effect on Myc-mediated lymphomagenesis in a mouse model, whereas deletion of Cks1 significantly attenuates the disease (78). On the other hand, it is more likely that oncogenic functions of Cks proteins are linked to override of cell cycle checkpoints and oncogene-induced stress barriers associated with Cks protein overexpression (76). A thorough understanding of the Cks1-proteasome-Paf1 interaction in humans, should it exist, will allow the determination of whether Cks protein-mediated transcription functions contribute to oncogenesis.

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