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Functional Relationships of Srb10-Srb11 Kinase, Carboxy-Terminal Domain Kinase CTDK-I, and Transcriptional Corepressor Ssn6-Tup1

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The Srb10-Srb11 protein kinase of *Saccharomyces cerevisiae* is a cyclin-dependent kinase (cdk)-cyclin pair which has been found associated with the carboxy-terminal domain (CTD) of RNA polymerase II holoenzyme forms. Previous genetic findings implicated the Srb10-Srb11 kinase in transcriptional repression. Here we use synthetic promoters and LexA fusion proteins to test the requirement for Srb10-Srb11 in repression by Ssn6-Tup1, a global corepressor. We show that *srb10*Δ and *srb11*Δ mutations reduce repression by DNA-bound LexA-Ssn6 and LexA-Tup1. A point mutation in a conserved subdomain of the kinase similarly reduced repression, indicating that the catalytic activity is required. These findings establish a functional link between Ssn6-Tup1 and the Srb10-Srb11 kinase in vivo. We also explored the relationship between Srb10-Srb11 and CTD kinase I (CTDK-I), another member of the cdk-cyclin family that has been implicated in CTD phosphorylation. We show that mutation of *CTK1*, encoding the cdk subunit, causes defects in transcriptional repression by LexA-Tup1 and in transcriptional activation. Analysis of the mutant phenotypes and the genetic interactions of *srb10*Δ and *ctk1*Δ suggests that the two kinases have related but distinct roles in transcriptional control. These genetic findings, together with previous biochemical evidence, suggest that one mechanism of repression by Ssn6-Tup1 involves functional interaction with RNA polymerase II holoenzyme.

RNA polymerase II holoenzyme forms purified from the yeast *Saccharomyces cerevisiae* contain a mediator complex, which functions in transcriptional activation (3, 12, 18, 22, 23, 53). Components of mediator/holoenzyme forms include Srb proteins, Gal11, Sin4, Rgr1, Rox3, and general transcription factors (16, 18, 22, 23, 29, 30). Genetic evidence suggests that the mediator/holoenzyme plays a role not only in transcriptional activation but also in repression (for a review, see reference 4). Mutations in the genes encoding Srb8 to Srb11, Gal11, Sin4, Rgr1, and Rox3 appear to relieve negative regulation of diversely regulated genes (6, 9, 13, 20, 25, 39, 44, 49, 52, 60, 61).

Two of these proteins, Srb10 and Srb11, constitute a cyclin-dependent kinase (cdk)-cyclin pair (25, 30). The connection to RNA polymerase II was first established by the isolation of *srb10* and *srb11* alleles as suppressors of truncations in the carboxy-terminal repeat domain (CTD) of the largest subunit of polymerase (30). The Srb10-Srb11 kinase was found associated with an RNA polymerase II holoenzyme form and was shown to affect phosphorylation of the CTD in vitro (18, 30). Mutations in *SRB10* and *SRB11* also reduced the activation of *GAL* promoters (25, 30). Mutations in both genes had previously been isolated in genetic selections for specific effects on gene regulation. Alleles called *ssn3* and *ssn8* were identified as suppressors of a defect in the Snf1 protein kinase and were shown to affect glucose repression of the *SUC2* gene (5, 25, 60). A related selection for suppressors affecting the Snf1-dependent expression of gluconeogenic genes yielded the *gig* mutations (2). A search for mutations that allow the expression of meiotic genes in vegetatively growing cells yielded *ume3* and *ume5* (8, 51, 52). The kinase subunit was also identified by mutations (*are1*) that impair α2 repression, the repression of **a**-specific genes in *MAT*α cells (61). Both glucose repression of *SUC2* and α2 repression depend on the Ssn6(Cyc8)-Tup1 complex, a global corepressor (21, 33, 46, 47, 56, 62). Thus, these genetic findings implicate the Srb10-Srb11 kinase in transcriptional repression and also raise the possibility of a direct role in the response to Ssn6-Tup1.

The Ssn6-Tup1 corepressor is recruited to many different promoters by specific DNA-binding regulatory proteins (1, 21, 24, 32, 34, 48, 55, 59, 65). Tup1 plays a primary role in repression (58), while Ssn6 mediates most, although not all, contacts with DNA-binding proteins (24, 48, 59). The mechanisms responsible for repression are not yet understood. Several lines of evidence indicate that Ssn6-Tup1 is required for the formation of chromatin structures that may be inhibitory to the binding of transcription factors (reviewed in reference 40). Positioned nucleosomes are observed at Ssn6-Tup1-repressed promoters in the wild type but not in *ssn6* or *tup1* mutants (7). In addition, Tup1 interacts in vitro with histones H3 and H4, and mutations in the histones that reduce this interaction also reduce repression by Ssn6-Tup1 in vivo (11). These findings suggest that Ssn6-Tup1 functions by establishing or maintaining repressive chromatin. However, other evidence suggests that the corepressor directly contacts components of the transcription machinery; Ssn6-Tup1-dependent repression was reconstituted in an in vitro transcription system without chromatin assembly (19, 36), and α2 repression can be achieved in the apparent absence of positioned nucleosomes (37).

In this study, we present evidence that the Srb10-Srb11 kinase plays a role in repression by Ssn6-Tup1 in vivo. We have used simple synthetic reporters and LexA fusion proteins to test the requirement for Srb10-Srb11 in repression by Ssn6-Tup1. Previous studies showed that LexA-Ssn6 and LexA-Tup1 fusion proteins, when bound to a promoter via LexA operators, repress transcription; LexA-Ssn6 requires Tup1 for repression (21), whereas LexA-Tup1 functions independently of Ssn6 (58). We show that mutations in the Srb10-Srb11

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TABLE 1. Strains of *S. cerevisiae* used in this study

Strain[a]	Genotype[b]	Source
FY86	*MAT*α *ura3-52 leu2-Δ1 his3-Δ200*	F. Winston
FY250	*MAT*α *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63*	F. Winston
FY251	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63*	F. Winston
MCY3639	*MAT*α *ura3-52 leu2-3,112 his3-Δ200 lys2-801 mig1Δ2::LEU2*	This laboratory
MCY3644	Identical to MCY3647, except *srb11Δ::LEU2*	This laboratory
MCY3647	*MAT*α *ura3-52 leu2-3,112 his3-Δ200 lys2-801*	This laboratory
MCY3655	Identical to FY250, except *srb11Δ::LEU2*	This work
MCY3657	*MAT*α *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 srb10Δ::HIS3*	This work
MCY3658	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 ctk1ΔE::URA3*	This work
MCY3659	*MAT***a** *ura3-52 leu2-3,112* or *-Δ1 his3-Δ200 lys2-801*	This work
MCY3660	*MAT*α *ura3-52 leu2-3,112* or *-Δ1 his3-Δ200 mig1Δ2::LEU2 ctk1ΔE::URA3*	This work
MCY3661	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63*	This work
MCY3662	*MAT***a** *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 srb10Δ::HIS3*	This work
MCY3663	*MAT*α *ura3-52 leu2-Δ1 his3-Δ200 trp1-Δ63 ctk1ΔE::URA3*	This work
MCY3664	Identical to MCY3663, except *ctk1ΔE::ura3::LEU2*	This work
MCY3667	*MAT*α *ura3-52 leu2-Δ1 his3-Δ200 srb10Δ::HIS3 ctk1ΔE::URA3*	This work
MCY3668	Identical to MCY3912, except *srb10Δ::HIS3*	This work
MCY3694	Identical to FY250, except *srb10-D290A*	This work
MCY3912	*MAT***a** *ura3-52 leu2-3,112 his3-Δ200 lys2-801 trp1-Δ1 ade2-101*	This laboratory
CTY10-5d	*MAT***a** *ade2-101 leu2-Δ1 his3-Δ200 trp1-Δ901 gal4 gal80 URA3::lexAop-lacZ*	R. Sternglanz

[a] All strains except CTY10-5d are derived from S288C.
[b] *srb10Δ::HIS3* and *srb11Δ::LEU2* are the same as *ssn3Δ1::HIS3* and *ssn8Δ1::LEU2*, respectively (25).

kinase substantially impair transcriptional repression by DNA-bound LexA-Ssn6 and LexA-Tup1.

The *srb10*Δ and *srb11*Δ mutations cause modest defects in the repression of natural promoters, indicating that the repression mechanism involving the Srb10-Srb11 kinase is only one of the mechanisms that contribute to repression. Because Srb10-Srb11 has been implicated in CTD phosphorylation, we have explored the functional relationship between Srb10-Srb11 and CTD kinase I (CTDK-I), also a member of the cdk-cyclin family. CTDK-I exhibits CTD kinase activity in vitro and affects CTD phosphorylation in vivo (26, 50). In this study, we determined the effects of mutation of *CTK1*, encoding the cdk subunit, on transcriptional repression and activation and examined the genetic interactions of *srb10*Δ and *ctk1*Δ mutations. The genetic evidence suggests that the two kinases play related but distinct roles in transcriptional control.

MATERIALS AND METHODS

Strains and genetic methods. The *S. cerevisiae* strains used in this work are listed in Table 1. The *Escherichia coli* strains used for propagation of plasmid DNA were XL1-Blue or DH5α. Genetic methods were as described previously (38), except that yeast extract-peptone (YEP), or rich, medium required supplemental tryptophan (40 μg/ml) to allow germination of *ctk1Δ trp1* spores and was used for all rich medium cultures. To introduce the *ctk1ΔE::URA3* allele into the S288C background, a 2.4-kb *Cla*I fragment from plasmid pSZ17 (26) was used to replace (42) one of two allelic copies of *CTK1* in diploid FY251 × FY86 cells, followed by sporulation and tetrad analysis. To generate strain MCY3664 (*ctk1ΔE::ura3::LEU2*), the *Hin*dIII fragment from plasmid pWJ460 (41) carrying a *ura3::LEU2* allele was used to transform strain MCY3663.

Construction of the *srb10-D290A* mutant. A DNA fragment encoding the C-terminal part of Srb10-D290A was generated by PCR with Vent DNA polymerase (New England Biolabs), plasmid pPY24 (25) as a template, and primers K-24 (5′-GGATGTGTTAAAATTGGaGcTcTAGGTTTGGCCAGAAA-3′) and K-17 (5′-GACGGATCCTGAATGTTGCAGACTTGC-3′). K-24 incorporates mismatching nucleotides (lowercase type) that encode the Asp290-to-Ala substitution and create a silent diagnostic *Sac*I site (underlined); K-17 is complementary to a region 3′ to the *SRB10* gene and contains a *Bam*HI site (underlined). The fragment was gel purified and used as a primer in the second round of PCR with the second primer K-16 (5′-CGGGATCCTAATGTATAA TGGCAAGGATAGAGC-3′) and pPY24 as a template; K-16 is at the 5′ end of the gene and contains a *Bam*HI site (underlined). The resulting mutant fragment was digested with *Bam*HI and cloned into the vector pBTM116 (a gift of Stan Fields, University of Washington, Seattle, Wash.), which expresses LexA from a weak version of the *ADH1* promoter, to generate pSK74. The LexA-Srb10-D290A protein expressed from pSK74 interacts with GAD-Srb11 in the two-hybrid system but, in contrast to the wild-type LexA-Srb10, confers a dominant flocculent phenotype. The *Bam*HI fragment from pSK74 was cloned into the integrating *URA3* vector pRS306, yielding pSK90. pSK90 was digested with *Hpa*I and used to transform strain FY250. Because the mutant fragment contains no yeast promoter sequence and the unique *Hpa*I site is downstream of the mutated site, only the wild-type copy of *SRB10* was expressed from the resulting gene duplication, and all of the transformants were nonflocculent. Three transformants were subjected to selection against *URA3* on plates containing 5-fluoroorotic acid. Two independent flocculent Ura$^-$ isolates were colony purified. To confirm the presence of the *srb10-D290A* allele at the correct chromosomal location, genomic DNA was used as a template in PCRs directed by primers K-41 (5′-AGGCCGCCTAGTTTTGAC-3′) and K-42 (5′-GGGCTGTAATCCTATC AG-3′). K-41 anneals to the chromosome 5′ to the PCR-amplified region; K-42 anneals within the amplified region 3′ to the mutation. The fragments resulting from the mutants were the same size as those from a wild-type control and contained the diagnostic *Sac*I site.

Plasmid construction. Plasmid pBM2762 (35) was modified to carry one LexA operator 5′ to UAS_{LEU2}, yielding reporter plasmid pMT27 (54). pSK101 was constructed by inserting the *Bam*HI-*Sal*I *MIG1* fragment from pLexA-MIG1 (55) between the *Bam*HI and *Sal*I sites of the vector pBTM116. The *CTK1* and *CTK2* coding regions were amplified from genomic DNA of FY250 by PCR. The synthetic primers were as follows: for *CTK1*, 5′-GCGCGGATCCTAATGTCC TACAATAATGGC-3′ and 5′-GCGCGGATCCTTATTTATCATCATC-3′; for *CTK2*, 5′-GCGCGGATCCTAATGCCTAGCACGTTTGAATC-3′ and 5′-GC GCGGATCCTATGCATGTCTTGTAGAAC-3′ (*Bam*HI sites and ATG are underlined). To generate fusions to the Gal4 activation domain (GAD) and the DNA-binding domain (amino acids 1 to 87) of LexA ($LexA_{87}$), the PCR fragments were digested with *Bam*HI and cloned into the *Bam*HI site of pSH2-1 (17) and pACTII (28), respectively. The resulting plasmids, pSK63, pSK64, and pSK65, express $LexA_{87}$-Ctk2, GAD-Ctk2, and $LexA_{87}$-Ctk1, respectively. Expression of $LexA_{87}$-Ctk1 and $LexA_{87}$-Ctk2 was confirmed by immunoblot analysis; $LexA_{87}$-Ctk1 complements *ctk1*Δ for the slow-growth and cold-sensitive phenotypes and interacts with GAD-Ctk2 in a two-hybrid assay. $LexA_{87}$-Srb10, GAD-Srb10, and GAD-Srb11 were expressed from pSK39, pSK40, and pSK36, respectively (25). pSK45 is pSK40 in which the sequences encoding GAD are deleted.

Enzyme assays. For reporter repression assays, cells were grown to mid-log phase in selective synthetic complete (SC) medium containing 2% glucose. Because the mutant strains used here are flocculent, the density of cell cultures was determined after the addition of EDTA to 5 or 10 mM to disperse cell clumps. β-Galactosidase activity was assayed in permeabilized cells or in protein extracts (38) and expressed in Miller units or in arbitrary units (1 unit = 1,000 × optical density at 420 nm [OD_{420}] per min per mg of protein), respectively. Glucose-repressed cells were obtained by growth to mid-log phase in selective synthetic complete (SC) or rich medium containing 2% glucose; for derepression, repressed cells were shifted for 3 h to medium containing 0.05% glucose. Invertase activity was assayed in whole cells (60). For two-hybrid interaction assays, filter lift assays of β-galactosidase activity in transformants were performed (63) after growth on selective SC plates containing 2% glucose.

Immunoblot analysis. Cells were grown to the mid-log phase in selective SC medium containing 2% glucose. Protein extracts were prepared as described previously (63). Alternatively, cell pellets were directly resuspended in sample buffer (1×) containing 2% sodium dodecyl sulfate and 2% β-mercaptoethanol (0.1 ml of sample buffer per equivalent of 1 ml of culture at an OD_{600} of 1.0), boiled for 5 min, and cleared by centrifugation at 12,000 × *g* for 1 min. Proteins were separated on sodium dodecyl sulfate–8% polyacrylamide gels and analyzed by immunoblotting with rabbit polyclonal LexA antibodies (gift of C. Denis,

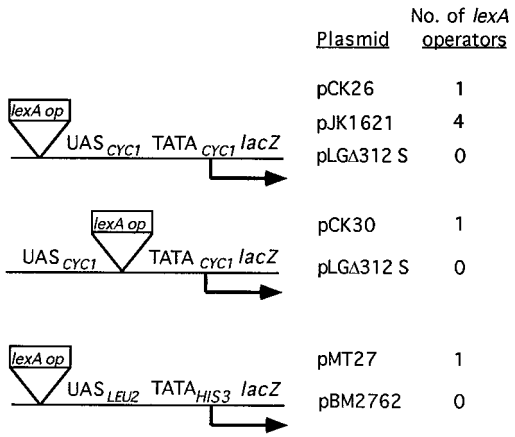

FIG. 1. Reporter plasmids used in repression assays. The *CYC1-lacZ* reporter plasmid pLGΔ312S (15) has no LexA operator, and its derivatives pCK26 and pCK30 (21) carry one LexA operator located 5′ or 3′ to UAS_{CYC1}, respectively; pJK1621 carries four LexA operators 5′ to UAS_{CYC1}. The UAS_{LEU2}-*HIS3-lacZ* reporter plasmid pBM2762 (35) has no operator, and its derivative pMT27 carries one LexA operator 5′ to UAS_{LEU2}. Representations are not to scale.

University of New Hampshire) and enhanced chemiluminescence (ECL reagents; Amersham).

RESULTS

Transcriptional repression by LexA-Tup1 requires the Srb10-Srb11 kinase. We first tested whether transcriptional repression by LexA-Tup1 requires the Srb10-Srb11 kinase. Wild-type and isogenic *srb10*Δ and *srb11*Δ mutants were cotransformed with a plasmid carrying genes expressing LexA-Tup1 (58) and *CYC1-lacZ* reporters (15, 21) containing no or one LexA operator located 5′ to the *CYC1* upstream activation sequence (UAS) (Fig. 1). Transformants were assayed for β-galactosidase activity after growth in glucose. In wild-type cells, LexA-Tup1 repressed transcription 14-fold, as calculated by comparing the expression of the reporter containing a LexA operator to that of the reporter lacking an operator (Table 2). In the *srb10*Δ and *srb11*Δ mutants, however, LexA-Tup1 repressed transcription only 1.7- and 1.3-fold, which was not significantly different from the results with the LexA controls. Thus, repression was reduced 8- to 10-fold in the mutants. Immunoblot analysis indicated that LexA-Tup1 was expressed at the same level in the mutants and wild type (Fig. 2A and data not shown). The use of a reporter with a larger number of LexA operators (four) (pJK1621; Fig. 1) did not improve repression or relieve the dependence on Srb10-Srb11 (data not shown).

To demonstrate that efficient repression requires the catalytic activity of Srb10, we constructed a mutation that causes Asp290 in subdomain VII to be replaced with Ala, here designated *srb10-D290A*. This mutation encodes the same amino acid substitution as the allele *srb10-3*, which was reported by Liao et al. (30) to inactivate the kinase without affecting its incorporation into the RNA polymerase II holoenzyme. In the *srb10-D290A* mutant, LexA-Tup1 repressed transcription of the reporter with one LexA operator only 2.8-fold, which is comparable to the LexA control value, 2.2-fold (Table 2). Immunoblot analysis detected the same levels of LexA-Tup1 in the mutant and wild type (data not shown).

We also performed repression assays by using a *CYC1-lacZ* reporter with a LexA operator inserted between the UAS and TATA sequence (pCK30; Fig. 1). In this case, LexA-Tup1 repressed transcription 59-fold (Table 2). Repression by LexA-Tup1 in an isogenic *srb10*Δ mutant was 6.2-fold, which is only slightly higher than that of the LexA control (3.4-fold).

TABLE 2. Mutations in *SRB10*, *SRB11*, and *CTK1* affect repression of *CYC1-lacZ* by LexA-Tup1[a]

Protein	Genotype	β-Gal activity[b]		Fold repression[c]
		−LexAop	+LexAop	
		5′ to UAS		
LexA	WT	162	95	1.7
	*srb10*Δ	277	207	1.3
	*srb11*Δ	270	200	1.4
	srb10-D290A	281	129	2.2
	*ctk1*Δ	247	157	1.6
LexA-Tup1	WT	102	7.5	14
	*srb10*Δ	182	105	1.7
	*srb11*Δ	152	115	1.3
	srb10-D290A	240	87	2.8
	*ctk1*Δ	60	18	3.3
		3′ to UAS		
LexA	WT	167	36	4.6
	*srb10*Δ	321	94	3.4
LexA-Tup1	WT	124	2.1	59
	*srb10*Δ	261	42	6.2

[a] Isogenic wild-type (WT) and mutant strains were cotransformed with plasmid YCp91 or pLexA-Tup1 (58) expressing LexA and LexA-Tup1, respectively, and *CYC1-lacZ* reporter plasmids (Fig. 1) with no LexA operator (−LexAop) or one LexA operator (+LexAop) located 5′ or 3′ to UAS_{CYC1}, as indicated.

[b] β-Galactosidase (β-Gal) activity was assayed in protein extracts, and values are averages for 4 to 10 independent transformants. Standard errors were typically 10 to 15%.

[c] Fold repression was calculated as the ratio of the values obtained for reporters with and without a LexA operator. The host strains were FY250, MCY3655, MCY3661, MCY3662, MCY3664, and MCY3694.

Thus, repression by LexA-Tup1 requires the catalytic activity of the Srb10-Srb11 kinase. Deletion of either subunit of the kinase reduces repression 8- to 10-fold, which is close to the limit of sensitivity in this assay. Moreover, repression is dependent on Srb10-Srb11 when LexA-Tup1 is tethered to sites located either 5′ or 3′ to the UAS.

Repression of a UAS_{LEU2}-*HIS3-lacZ* reporter by LexA-Ssn6 requires Srb10. To show that this Srb10-Srb11 dependence is not specific to the *CYC1-lacZ* reporter, we next examined the repression of a reporter with different UAS and TATA elements. We used a pair of reporters in which UAS_{LEU2} drives the expression of a *HIS3-lacZ* fusion from the $TATA_{HIS3}$ sequence (35), with no or one LexA operator inserted 5′ to the UAS (Fig. 1). In addition, to confirm that Srb10-Srb11 dependence is not specific to the LexA-Tup1 fusion protein, we used $LexA_{87}$-Ssn6 (21) as the DNA-bound protein, which represses by recruiting the native Tup1. Repression was assayed in wild-type and isogenic *srb10*Δ mutant cells. In the wild type, DNA-bound $LexA_{87}$-Ssn6 caused 6.7-fold repression of the reporter containing a LexA operator relative to that of the reporter lacking an operator (Table 3). In the *srb10*Δ mutant, repression was reduced to levels comparable to those for the LexA control (1.4-fold). Immunoblot analysis confirmed that the repression defect is not caused by lower levels of $LexA_{87}$-Ssn6 (Fig. 2B).

Repression of *CYC1-lacZ* by LexA-Mig1 is partially dependent on Srb10 and Srb11. We next examined repression by $LexA_{87}$-Mig1. Mig1 is one of two DNA-binding proteins that function with Ssn6-Tup1 to repress *SUC2* (21, 32, 34, 55, 59,

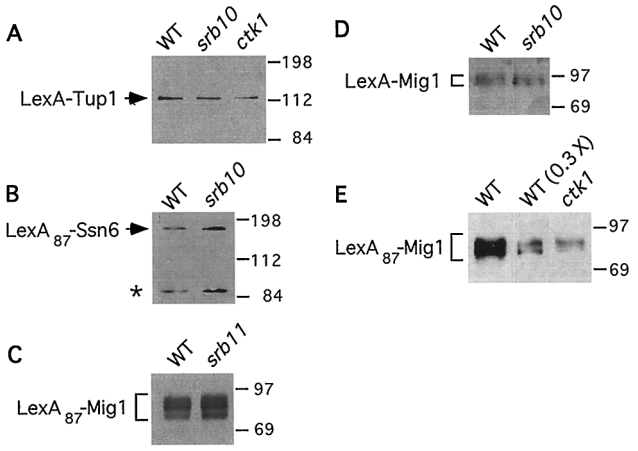

FIG. 2. Immunoblot analysis of LexA fusion proteins. Protein extracts (5 μg [A] or 20 μg [B and C]) or boiled cells (D and E) [equivalent of 0.2 ml of culture at an OD_{600} of 1.0, except lane WT(0.3×)] were analyzed by immunoblotting with LexA antibodies. (A) LexA-Tup1 protein in wild-type (WT) and *srb10*Δ transformants carrying pLexA-Tup1 and pCK26. These transformants were assayed in Table 2. Two *srb11*Δ transformants were also analyzed and expressed LexA-Tup1 at levels equal to those of the wild type (data not shown). (B) $LexA_{87}$-Ssn6 protein in wild-type and *srb10*Δ transformants carrying pCK23 and pMT27. These transformants were assayed, and the results are shown in Table 3. The asterisk marks a degradation product. (C) $LexA_{87}$-Mig1 protein in wild-type and *srb11*Δ transformants carrying pLexA-Mig1 (Table 4, experiment A). (D) LexA-Mig1 protein in wild-type and *srb10*Δ transformants carrying pSK101 and pCK26. These transformants were assayed, and the results are shown in Table 4 (experiment B). (E) $LexA_{87}$-Mig1 protein in wild-type and *ctk1*Δ transformants carrying pLexA-Mig1 and pCK26. These transformants were assayed, and the results are shown in Table 4 (experiment C). The lane labeled WT(0.3×) was loaded with threefold less protein than the other lanes. In panels C to E, the multiple bands correspond to different phosphorylation states (55).

62), and this $LexA_{87}$-Mig1 fusion protein is an Ssn6-Tup1-dependent repressor (55). $LexA_{87}$-Mig1 repressed *CYC1-lacZ* expression 16-fold in the wild type but only 5.9-fold in an *srb11*Δ mutant (Table 4, experiment A). The latter value, however, is still significantly greater than that for the $LexA_{87}$ control (1.3-fold). $LexA_{87}$-Mig1 protein levels in the mutant and wild type were comparable; moreover, the repression defect in the *srb11*Δ mutant was not associated with a major change in phosphorylation of $LexA_{87}$-Mig1 (Fig. 2C) (55).

The fact that this dependence was only partial could result from the massive overexpression of $LexA_{87}$-Mig1 from the strong *ADH1* promoter of the vector pSH2-1. To address this possibility, we tested a different LexA-Mig1 fusion protein, which was expressed at a much lower level (data not shown) from a shorter version of the *ADH1* promoter present in the vector pBTM116. Again, the Srb10 dependence was partial (Table 4, experiment B). The LexA-Mig1 protein levels were comparable in the wild type and *srb10*Δ mutant (Fig. 2D).

TABLE 3. Effect of *srb10*Δ on repression of UAS_{LEU2}-*HIS3-lacZ* by $LexA_{87}$-Ssn6[a]

Protein	Genotype	β-Gal activity[b]		Fold repression
		−LexAop	+LexAop	
LexA	WT	292	232	1.3
	*srb10*Δ	330	232	1.4
$LexA_{87}$-Ssn6	WT	370	55	6.7
	*srb10*Δ	436	253	1.7

[a] Isogenic strains MCY3912 (wild type [WT]) and MCY3668 (*srb10*Δ) were cotransformed with plasmid pBTM116 or pCK23 (21) expressing LexA and $LexA_{87}$-Ssn6, respectively, and UAS_{LEU2}-*HIS3-lacZ* reporter plasmids with no or one LexA operator 5′ to UAS_{LEU2} (Fig. 1).

[b] β-Galactosidase (β-Gal) activity was assayed in permeabilized cells. Values are averages for three to five independent transformants. Standard errors were <17%.

CTDK-I contributes to repression of *SUC2*. For all natural promoters tested, the repression defects caused by *srb10* and *srb11* mutations are modest. Thus, the repression mechanism involving the Srb10-Srb11 kinase is only one of the mechanisms that contribute to repression. We considered the possibility that another, related kinase functions redundantly, or partly so, with Srb10-Srb11. CTDK-I is similar to Srb10-Srb11 in that two of the subunits are cdk and cyclin homologs (Ctk1 and Ctk2, respectively) and CTDK-I exhibits CTD kinase activity (26, 50). In addition, *ctk1* and *ctk2* mutants apparently resembled *srb10* and *srb11* mutants with respect to their flocculent, cold-sensitive, and slow-growth phenotypes and their failure or reduced ability to sporulate, although the characterized mutants were in different genetic backgrounds (25, 26, 30, 50, 52). Thus, CTDK-I seemed a likely candidate.

To test whether CTDK-I is functionally related to Srb10-Srb11, we first introduced a *ctk1*Δ allele (26) into the S288C genetic background, which we have used for previous studies of *srb10*Δ (25) (see Materials and Methods). The resulting mutants exhibited slow growth, cold sensitivity, and inability of homozygous diploids to sporulate, as reported previously (26, 50); however, in the S288C background, *ctk1*Δ mutants were not flocculent, although microscopic examination revealed clustered cells. We also found that these mutants were impaired in growth on galactose. The *srb10*Δ mutants in the S288C background are similar with respect to slow growth and impaired utilization of galactose, but they are highly flocculent, less cold sensitive, and able to sporulate (25).

We next examined the *ctk1*Δ mutants for repression of the *SUC2* (invertase) gene in response to glucose, which requires Ssn6-Tup1 (5, 46, 56). The *ctk1*Δ mutation did not substantially relieve repression; however, *ctk1*Δ impaired positive regulation of *SUC2* a fewfold, which could mask an effect on repression (Fig. 3). We therefore turned to a more sensitive assay to detect relief of glucose repression, based on a *SUC2-HIS3* fusion in which *SUC2* regulatory sequences mediate glucose repression of *HIS3* expression (57). When cells are grown on glucose, the *SUC2* promoter is repressed and *HIS3* is not expressed; however, when cells are grown on sucrose or when cells are defective in glucose repression, the fusion confers a His^+ phenotype. A *CEN-TRP1* plasmid carrying this reporter was introduced into isogenic wild-type, *ctk1*Δ, and *srb11*Δ strains, all with the chromosomal *HIS3* locus deleted. Both *ctk1*Δ and *srb11*Δ strains grew on glucose in the absence of histidine, whereas the wild type did not (Fig. 4). In the control experiment, all the strains grew on sucrose without histidine. Thus, *ctk1*Δ and *srb11*Δ relieve glucose repression of the *SUC2* promoter in this assay.

Both *srb10*Δ and *srb11*Δ synergize strongly with the *mig1*Δ mutation to relieve repression of *SUC2* (60). In a *mig1*Δ mutant, the recruitment of Ssn6-Tup1 to the *SUC2* promoter is impaired but the Mig2 protein partially substitutes for Mig1 (32); however, the resulting repression is highly dependent on Srb10-Srb11. We therefore tested *ctk1*Δ for synergy with *mig1*Δ. In *ctk1*Δ *mig1*Δ double mutants, constructed by genetic crossing, the repressed invertase activity was about fourfold higher than in *mig1*Δ single-mutant segregants (Fig. 3). We also examined the regulation of an integrated *SUC2-LEU2-lacZ* fusion, under control of the *SUC2* regulatory region and the *LEU2* promoter (45), and the β-galactosidase activity correlated well with invertase activity (Fig. 3). Although the syn-

TABLE 4. Effects of *srb10*Δ, *srb11*Δ, and *ctk1*Δ on repression of *CYC1-lacZ* by LexA-Mig1[a]

Protein	Genotype	β-Gal activity			Fold repression	
		−LexAop	+1 LexAop	+4 LexAop	1op	4op
Expt A[b]						
$LexA_{87}$	WT	76	78	ND	1.0	ND
	*srb11*Δ	199	148	ND	1.3	ND
$LexA_{87}$-Mig1	WT	106	6.6	ND	16	ND
	*srb11*Δ	124	21	ND	5.9	ND
Expt B[c]						
LexA	WT	91	66	63	1.4	1.4
	*srb10*Δ	105	74	73	1.4	1.4
LexA-Mig1	WT	111	19	16	5.8	6.9
	*srb10*Δ	86	35	21	2.5	4.1
Expt C[d]						
$LexA_{87}$	WT	77	51	46	1.5	1.7
	*ctk1*Δ	106	72	58	1.5	1.8
$LexA_{87}$-Mig1	WT	99	6.3	2.8	16	35
	*ctk1*Δ	144	26	14	5.5	10

[a] Pairs of isogenic strains were cotransformed with plasmids expressing fusion proteins and *CYC1-lacZ* reporter plasmids carrying zero, one, or four LexA operators 5′ to the UAS (Fig. 1). Fold repression for both one-operator (1op) and four-operator (4op) reporters is relative to the operator-less reporter. ND, not determined.
[b] The strains were MCY3647 and MCY3644, and the plasmids were pSH2-1 or pLexA-Mig1. β-Galactosidase (β-Gal) activity was assayed in permeabilized cells. Values are averages for three independent transformants. Standard errors were <20%.
[c] The strains were MCY3661 and MCY3662, and the plasmids were pBTM116 and pSK101. β-Galactosidase activity was assayed in protein extracts. Values are averages for five transformants. Standard errors were <12%.
[d] Same as described for experiment B, except that the strains were MCY3661 and MCY3664 and the plasmids were pSH2-1 or pLexA-Mig1. Standard errors were <14%.

ergistic effect of *srb10*Δ is more pronounced (about 10-fold [60]), the effect of *ctk1*Δ on repression may be underestimated due to the defect in *SUC2* activation. Together, these results strongly suggest that CTDK-I contributes to the transcriptional repression of *SUC2*.

Effect of *ctk1*Δ on repression by LexA-Tup1. To test whether CTDK-I contributes to *SUC2* repression via the Ssn6-Tup1 corepressor, we examined the effect of *ctk1*Δ on the ability of LexA-Tup1 to repress transcription of a *lexAop-CYC1-lacZ* reporter (pCK26; Fig. 1). LexA-Tup1 repressed transcription only 3.3-fold in the mutant, compared to 14-fold in the isogenic wild type (Table 2). LexA-Tup1 expression in the mutant was confirmed by immunoblot analysis (Fig. 2A).

We also tested the effect of *ctk1*Δ on repression by LexA-

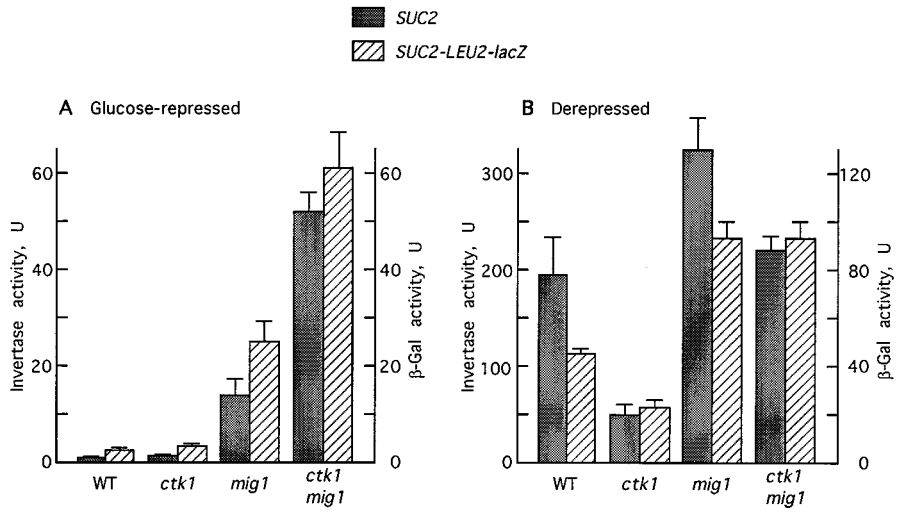

FIG. 3. Combined effect of *ctk1*Δ and *mig1*Δ on the regulation of *SUC2* and *SUC2-LEU2-lacZ*. Invertase assays were performed in glucose-repressed and derepressed segregants of the MCY3639 × MCY3658 cross. Segregants bearing *SUC2-LEU2-lacZ* were derived from the cross of MCY3660 (*ctk1*Δ *mig1*Δ) with a transformant of MCY3659 (wild type [WT]) carrying integrated pLS11 (45). β-Galactosidase (β-Gal) activity was assayed in permeabilized cells. Values are averages for at least three segregants. (A) Glucose-repressed cultures. (B) Derepressed cultures.

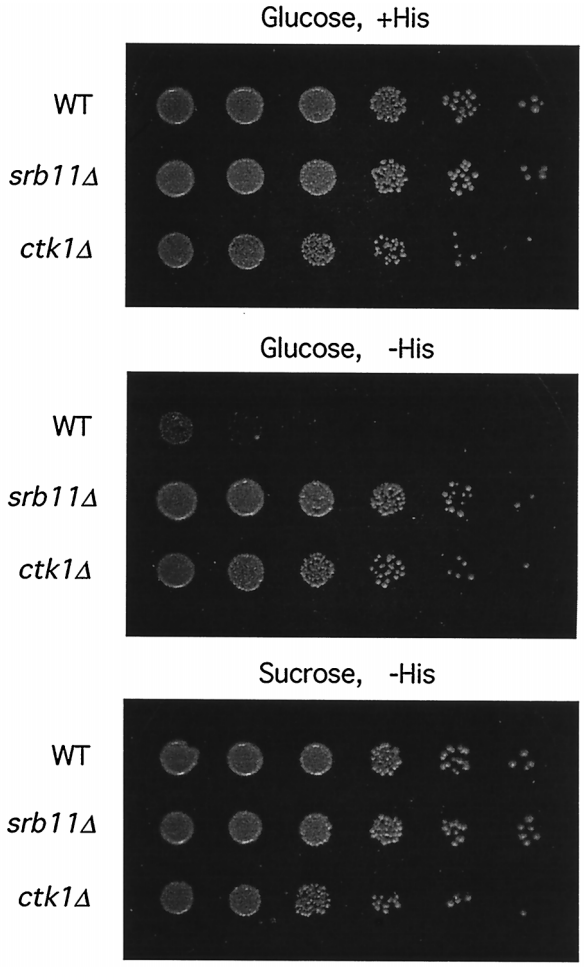

FIG. 4. *ctk1*Δ relieves glucose repression of a *SUC2-HIS3* reporter. The *trp1*Δ *his3*Δ strains FY250 (WT), MCY3663 (*ctk1*Δ), and MCY3655 (*srb11*Δ) were transformed with the *CEN-TRP1* plasmid pYSH (57). Serial sixfold dilutions of cell suspensions (diluted in 10 mM Tris-HCl [pH 7.0] containing 10 mM EDTA to disperse flocculated cells) were spotted on SC-Trp or SC-Trp-His containing 2% glucose or sucrose, as indicated. The plates were photographed after 4 days at 30°C. For either mutant, the number of colonies on glucose-His was approximately equal to that in the corresponding dilution on sucrose-His, suggesting that possible cross-feeding and stochastic effects did not occur.

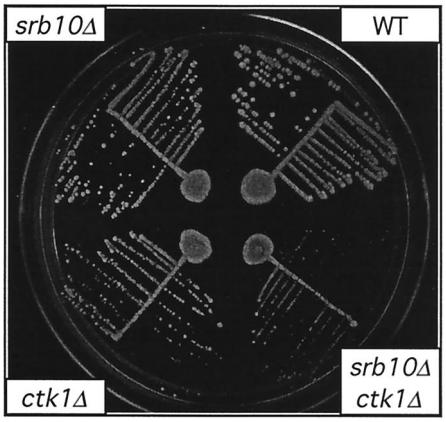

FIG. 5. Growth of *srb10*Δ *ctk1*Δ double mutants. A diploid heterozygous for *srb10*Δ*::HIS3* and *ctk1*Δ*E::URA3* (MCY3657 × MCY3658) was sporulated and subjected to tetrad analysis. Double-mutant segregants were identified by their His^+ Ura^+ phenotype and corresponded to slowly growing spore clones (data not shown). Representative segregants were streaked on rich medium containing 2% glucose. The plate was photographed after 2 days at 30°C. The single-mutant colonies are smaller than those of the wild type (WT) during the early stages of growth.

Mig1. Repression by LexA-Mig1 in the mutant was reduced to the LexA control levels, but we could not detect the fusion protein (data not shown). In the case of $LexA_{87}$-Mig1, expressed from a stronger version of the *ADH1* promoter, repression was threefold lower in the mutant (Table 4, experiment C) but the $LexA_{87}$-Mig1 protein level was also at least threefold lower than in the wild type (Fig. 2E). Therefore, it is not clear that *ctk1*Δ has any effect on repression by LexA-Mig1.

Ctk1 and Srb10 are not functionally interchangeable. The similarities between the *ctk1*Δ and *srb10*Δ mutant phenotypes suggested that Ctk1 and Srb10 are functionally related and raised the possibility that they are interchangeable cdk subunits; the differences in phenotype could conceivably result from different levels of expression of the two proteins. To address this issue, we first tested whether overexpression of Srb10 can suppress *ctk1*Δ for the *SUC2* repression defect. We transformed a *mig1*Δ *ctk1*Δ double mutant (MCY3660) with a multicopy plasmid expressing a functional $LexA_{87}$-Srb10 protein from the strong *ADH1* promoter (pSK39) (25). Invertase activity in glucose-repressed transformants was not significantly different from that in transformants expressing LexA (average values, 81 and 87 U, respectively). In another experiment, overexpression of Srb10 from the *ADH1* promoter (pSK45) in a *ctk1*Δ mutant did not improve growth on galactose relative to that of vector controls. Thus, these assays provide no evidence that elevated levels of Srb10 can compensate for the absence of Ctk1.

Taking a different approach, we next used the two-hybrid system to test for heterotypic interaction between the kinase and cyclin subunits of the two complexes, using the *lexAop-GAL1-lacZ* reporter in strain CTY10-5d. No interaction was detected between $LexA_{87}$-Ctk1 and GAD-Srb11 or between $LexA_{87}$-Ctk2 and GAD-Srb10; in control experiments, $LexA_{87}$-Srb10 interacted with GAD-Srb11 and $LexA_{87}$-Ctk1 interacted with GAD-Ctk2 (see Materials and Methods). This lack of heterotypic interaction is consistent with the sequence divergence between Srb10 and Ctk1; for example, the sequence corresponding to the PSTAIRE motif, which is required for specific interaction with cyclins, is SQSACRE in Srb10 and PITSIRE in Ctk1.

These two-hybrid experiments also revealed another difference between the subunits of the two kinases. In contrast to $LexA_{87}$-Srb10 and $LexA_{87}$-Srb11 (25), $LexA_{87}$ fusions to Ctk1 and Ctk2 showed no ability to activate transcription of reporters (data not shown).

Evidence for independent function of the Srb10-Srb11 and CTDK-I kinases. To explore the functional relationship between the CTDK-I and Srb10-Srb11 kinases, we examined the genetic interactions between the *ctk1*Δ and *srb10*Δ mutations. If the two kinases function independently, the double mutant should exhibit a more pronounced phenotypic defect than either single mutant. We constructed such double mutants by genetic crossing, and we found that the *srb10*Δ *ctk1*Δ segregants were viable but grew more slowly than the single mutants (Fig. 5). The double mutants exhibited a substantially longer doubling time in rich medium (6.1 h) than did either single mutant (3.4 h for *ctk1*Δ; 2.4 h for *srb10*Δ) or the wild type (1.8 h). These findings indicate that CTDK-I and Srb10-Srb11 have independent inputs into some cellular processes. Moreover, the viability of the double mutants indicates that the two

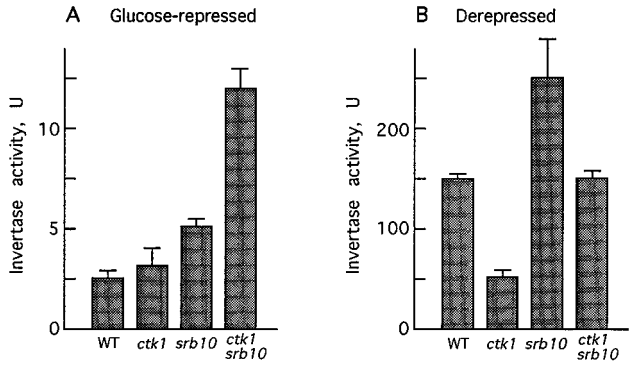

FIG. 6. Combined effect of *srb10*Δ and *ctk1*Δ on *SUC2* expression. Segregants from the cross described in the legend to Fig. 5 were assayed for invertase activity after growth under glucose-repressing conditions (A) or after a shift to derepressing conditions (B). WT, wild type.

kinases cannot together be responsible for an essential function.

We next tested whether the *SUC2* repression defect of the *srb10*Δ *ctk1*Δ double mutant exceeds the defect of either single mutant. Under glucose-repressing conditions, invertase activity in the *srb10*Δ *ctk1*Δ double mutant was 2.4- and 3.8-fold higher than in *srb10*Δ and *ctk1*Δ single mutants, respectively (Fig. 6A). However, the fold regulation of *SUC2* was not substantially different in the double mutant than in the *ctk1*Δ mutant, which is defective in derepression (Fig. 6B). Derepressed invertase activity was closer to the wild-type level in the double mutant.

The Srb10-Srb11 kinase has been implicated not only in repression but also in transcriptional activation, and both *srb10*Δ and *srb11*Δ mutants are defective in induction of *GAL* genes (25, 30). The growth defect of *ctk1*Δ mutants on galactose suggested that CTDK-I plays a similar role, so we tested whether *ctk1*Δ similarly impairs the induction of an integrated *GAL1-lacZ* fusion (64). Wild-type and mutant strains were assayed for β-galactosidase activity after growth in galactose (Table 5). Activity in the *srb10*Δ and *ctk1*Δ mutants was 2.5- and 8.0-fold lower, respectively, than in the wild type. To test whether Srb10-Srb11 and CTDK-I contribute independently to the induction of *GAL1-lacZ*, we examined the *srb10*Δ *ctk1*Δ double mutants. The defect was more pronounced in the double mutants, and activity was decreased 18-fold relative to that of the wild type, suggesting that the two kinases function independently.

TABLE 5. Effects of *ctk1*Δ and *srb10*Δ on *GAL1-lacZ* expression[a]

Relevant genotype	β-Gal activity[b]	Fold induction defect
Wild type	2,210	1.0
*srb10*Δ	871	2.5
*ctk1*Δ	275	8.0
*srb10*Δ *ctk1*Δ	122	18

[a] Strains carrying an integrated *GAL1-lacZ* fusion gene were derived from the cross of MCY3667 (*srb10*Δ *ctk1*Δ) with MCY3659 (wild type), which had been transformed with plasmid pRY171 (64). Cells were grown in rich medium with 2% galactose (inducing conditions).

[b] β-Galactosidase (β-Gal) activity was assayed in permeabilized cells. Values are averages for at least three segregants of each genotype. Standard errors were <15%.

DISCUSSION

In this study, we have examined the role of the Srb10-Srb11 kinase in transcriptional repression by the Ssn6-Tup1 corepressor. We show that *srb10*Δ and *srb11*Δ mutations reduce the repression of synthetic reporters by DNA-bound LexA-Ssn6 and LexA-Tup1. Repression was reduced 8- to 10-fold when LexA-Tup1 was tethered to sites either 5′ or 3′ to the UAS of the reporter. Moreover, using a point mutation that inactivates the kinase, we showed that repression requires the catalytic activity of Srb10. The Srb10-Srb11 kinase has been found associated with an RNA polymerase II holoenzyme form (18, 30). Together with biochemical evidence that Ssn6-Tup1-dependent repression acts on the transcription apparatus (19, 36), these genetic data suggest that one mechanism of repression involves functional interaction between Ssn6-Tup1 and RNA polymerase II holoenzyme.

Repression of the native *SUC2* promoter by Ssn6-Tup1 is only partially dependent on the Srb10-Srb11 kinase. Mutation of *SSN6* or *TUP1* abolishes the repression of *SUC2*, yielding a greater than 100-fold increase in expression relative to the level of expression of the wild type. In contrast, mutation of *SRB10* or *SRB11* causes a modest effect (a fewfold alone and 10-fold when combined with *mig1*, which partially impairs the recruitment of Ssn6-Tup1 [25, 60]). We show here that the absence of a stronger phenotype in these mutants cannot be attributed to a redundant function provided by CTDK-I, a related cdk-cyclin which has been implicated in CTD phosphorylation. Mutation of *CTK1* causes a similarly modest effect on repression, and the combination of *srb10*Δ and *ctk1*Δ does not reproduce the effect of *ssn6*Δ or *tup1*Δ. Mutations in various RNA polymerase II holoenzyme components also cause a partial loss of repression at Ssn6-Tup1-dependent promoters (6, 44, 49, 60, 61). These results are consistent with models in which Ssn6-Tup1 effects repression by at least two mechanisms, one involving functional interaction with RNA polymerase II holoenzyme and another most probably involving chromatin (7, 11, 40). In the assays presented here, repression by LexA-Mig1 was less dependent on Srb10-Srb11 than was repression by LexA-Tup1 and LexA-Ssn6, suggesting that the relative contributions of Srb10-Srb11-dependent and -independent repression mechanisms vary with the particulars of the recruitment of Ssn6-Tup1. The spatial relationship of the Ssn6-Tup1 corepressor to chromatin, RNA polymerase II holoenzyme, and other transcription factors should more closely approximate the natural configuration when Ssn6-Tup1 is recruited by LexA-Mig1 than when the corepressor is tethered by direct fusion to LexA.

We have explored the relationship of the CTDK-I kinase to Srb10-Srb11, and we present genetic evidence that these two kinases have related but distinct roles in transcriptional control. First, we compared the effects of mutations in the cognate genes in the S288C genetic background and showed that *ctk1*Δ and *srb10*Δ cause similar but not identical phenotypes. Both mutants exhibit slow growth and impaired utilization of galactose. However, the mutant phenotypes differ with respect to flocculence, degree of cold sensitivity, and sporulation proficiency. We also examined the *ctk1*Δ mutant for defects in transcriptional regulation of *SUC2*. Like *srb10*Δ, the *ctk1*Δ mutation alone causes mild defects in glucose repression of *SUC2* but acts synergistically with *mig1*Δ to relieve repression. We present evidence that repression by LexA-Tup1 is reduced in the *ctk1*Δ mutant. A difference between the two mutants is that the *ctk1*Δ mutant exhibits a significant defect in derepression of *SUC2* whereas the *srb10*Δ mutant derepresses invertase activity to somewhat higher levels than does the wild type.

Finally, we showed that *ctk1*Δ impairs induction of the *GAL1* promoter, as previously shown for *srb10*Δ (25, 30). The many shared mutant phenotypes indicate that the Ctk1 and Srb10 proteins function in some of the same cellular processes, including both transcriptional repression and activation. The mutant phenotypes are not identical, however, strongly suggesting that CTDK-I and Srb10-Srb11 have related but distinct functions.

Genetic studies further support the view that Ctk1 and Srb10 are not functionally interchangeable. First, overexpression of Srb10 did not suppress defects caused by the loss of Ctk1. Second, in two-hybrid assays, Ctk1 did not interact with the Srb11 cyclin and Srb10 did not interact with the Ctk2 cyclin. These studies also showed that Srb10 and Srb11 differ from Ctk1 and Ctk2 with respect to the ability of the corresponding LexA fusions to activate transcription of reporters.

Evidence that the CTDK-I and Srb10-Srb11 kinases contribute separately to transcriptional control was provided by analysis of *ctk1*Δ *srb10*Δ double mutants. These double mutants are viable, indicating that the two kinases are not together responsible for any essential function. Moreover, the double mutants exhibited more severe phenotypes than the single mutants with respect to slow growth (on glucose) and induction of *GAL1*. Thus, strains lacking both kinases are more defective for certain transcriptional regulatory responses than are cells lacking only one of the kinases. In contrast, derepressed invertase activity was closer to the wild-type level in the double mutant than in either single mutant. These findings support the view that the two kinases have some related, overlapping roles but are in other respects functionally distinct.

Biochemical studies have also revealed both similarities and differences between the two kinases. The Srb10-Srb11 kinase is associated with the CTD in some RNA polymerase II holoenzyme forms (18, 30), whereas CTDK-I has not been detected in purified holoenzyme (50). However, CTDK-I may interact transiently or may be a component of an alternative form of the holoenzyme that has not yet been characterized. Both kinases have been implicated in phosphorylation of the CTD. Phosphorylation of the CTD accompanies the transition from initiation to elongation (reviewed in reference 10) and therefore represents a potentially important step for transcriptional control. Although the general transcription factor TFIIH is responsible for the major phosphorylation of the CTD (14), CTDK-I phosphorylates the CTD in vitro and phosphorylation is reduced in *ctk1*Δ mutants in vivo (26, 50). Recent work suggests that CTDK-I stimulates the elongation efficiency of RNA polymerase II (27). Srb10-Srb11 also phosphorylates the CTD, or modulates the activity of another CTD kinase, since phosphorylation of the CTD was 10-fold reduced in assays of holoenzyme purified from an *srb10-3* mutant (30).

The evidence that Srb10-Srb11 and CTDK-I affect repression by Ssn6-Tup1 establishes a functional connection between the corepressor and RNA polymerase II holoenzyme. A simple model is that Ssn6-Tup1 affects CTD phosphorylation by these kinases. However, it also remains possible that Ssn6-Tup1 affects the activity of these kinases in phosphorylation of other proteins, such as general transcription factors or components of the mediator/holoenzyme, and thereby represses transcription. Alternatively, Srb10-Srb11 and CTDK-I may modulate transcriptional repression and activation by phosphorylating repressors and activators. The activator Gal4 is known to be phosphorylated as a consequence of transcriptional activation (31, 43). Both Ssn6 and Tup1 are phosphorylated in vivo (36, 47), and it is possible that the Srb10-Srb11 and CTDK-I kinases potentiate transcriptional repression by phosphorylating the Ssn6-Tup1 corepressor.

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