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Roles for the *Saccharomyces cerevisiae SDS3*, *CBK1* and *HYM1* Genes in Transcriptional Repression by *SIN3*

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Manuscript received August 4, 1999
Accepted for publication October 12, 1999

ABSTRACT

The *Saccharomyces cerevisiae* Sin3 transcriptional repressor is part of a large multiprotein complex that includes the Rpd3 histone deacetylase. A LexA-Sin3 fusion protein represses transcription of promoters with LexA binding sites. To identify genes involved in repression by Sin3, we conducted a screen for mutations that reduce repression by LexA-Sin3. One of the mutations identified that reduces LexA-Sin3 repression is in the *RPD3* gene, consistent with the known roles of Rpd3 in transcriptional repression. Mutations in *CBK1* and *HYM1* reduce repression by LexA-Sin3 and also cause defects in cell separation and altered colony morphology. *cbk1* and *hym1* mutations affect some but not all genes regulated by *SIN3* and *RPD3*, but the effect on transcription is much weaker. Genetic analysis suggests that *CBK1* and *HYM1* function in the same pathway, but this genetic pathway is separable from that of *SIN3* and *RPD3.* The remaining gene from this screen described in this report is *SDS3*, previously identified in a screen for mutations that increase silencing at *HML*, *HMR*, and telomere-linked genes, a phenotype also seen in *sin3* and *rpd3* mutants. Genetic analysis demonstrates that *SDS3* functions in the same genetic pathway as *SIN3* and *RPD3*, and coimmunoprecipitation experiments show that Sds3 is physically present in the Sin3 complex.

EUKARYOTIC DNA is associated with histone proteins and packaged into chromatin, and transcription of specific genes can be affected by the chromatin structure at the promoter (for reviews see Kingston *et al.* 1996; Wolffe and Pruss 1996; Kadonaga 1998; Struhl 1998). Each of the histones contains an evolutionarily conserved aminoterminal tail that is subject to reversible post-translational modifications such as acetylation, phosphorylation, and ubiquitination. Changes in the acetylation states of lysines on the tails of histones are correlated with gene expression, with transcriptionally active genes having hyperacetylated nucleosomes and transcriptionally inactive genes hypoacetylated nucleosomes (for reviews see Grunstein 1997; Hampsey 1997; Struhl 1998).

Sin3 and Rpd3 are components of a transcriptional repression complex in yeast (Kadosh and Struhl 1997; Kasten *et al.* 1997) that is conserved in vertebrates (Pazin and Kadonaga 1997; Wolffe 1997). Sin3 cannot bind to DNA itself; however, the complex is targeted to specific promoters through interactions with sequence-specific DNA-binding proteins (Alland *et al.* 1997; Hassig *et al.* 1997; Heinzel *et al.* 1997; Kadosh and Struhl 1997; Laherty *et al.* 1997; Nagy *et al.* 1997; Pazin and Kadonaga 1997; Zhang *et al.* 1997). The fact that *RPD3* encodes a histone deacetylase (Taunton *et al.* 1996; Kadosh and Struhl 1998a) provides a mechanism for transcriptional repression, with Sin3 bringing the Rpd3 histone deacetylase to specific promoters. *In vivo*, the presence of the Sin3/Rpd3 complex at a promoter leads to decreased acetylation of histones H3 and H4 that is highly localized over one to two nucleosomes (Kadosh and Struhl 1998b; Rundlett *et al.* 1998). The Sap30 protein is also present in the Sin3 complex, and *sap30* mutations cause similar phenotypes as *sin3* and *rpd3* (Zhang *et al.* 1998; Sun and Hampsey 1999).

SIN3 was first identified as a negative regulator of *HO* expression (Nasmyth *et al.* 1987; Sternberg *et al.* 1987). *SIN3* has since been identified in multiple screens as a negative regulator of numerous genes, including *TRK2* (Vidal *et al.* 1991), *IME2* (Bowdish and Mitchell 1993), *SPO13* (Strich *et al.* 1989), and *INO1* (Hudak *et al.* 1994). Transcriptional activation of certain genes, such as *STE6* (Vidal *et al.* 1991) and middle sporulation genes (Hepworth *et al.* 1998), is reduced in a *sin3* mutant, although the effect may be indirect (Wang *et al.* 1994). The genes regulated by *SIN3* are involved in a wide variety of biological processes and share little or no direct regulatory relationship. Regulation of repression by Sin3 must be controlled, at least in part, at the level of recruitment to promoters. However, regulation may also occur by post-translational mechanisms such as protein phosphorylation.

RPD3 was first identified as a negative regulator of

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the low-affinity potassium transporter *TRK2* (Vidal and Gaber 1991). Mutations in *RPD3* affect expression of the same set of genes as *SIN3*, and genetic analysis suggests that *SIN3* and *RPD3* function in the same genetic pathway (Stillman *et al.* 1994). We have described an assay system using a LexA-Sin3 fusion protein that represses transcription of promoters with LexA binding sites (Wang and Stillman 1993). Transcriptional repression by LexA-Sin3 is reduced in an *rpd3* mutant, consistent with the proposed role for histone deacetylases in repression by Sin3 (Kasten *et al.* 1997).

We have used the LexA-Sin3 repression system in a genetic screen to identify mutations that affect repression by Sin3. The focus of the genetic selection was to identify proteins required for repression rather than for recruitment to specific promoters. In this article we describe four genes identified in the screen, *RPD3*, *CBK1*, *HYM1*, and *SDS3*, that affect repression by Sin3. Our analysis suggests that *CBK1* and *HYM1* function in the same genetic pathway. We also show that mutations in these two genes do not affect all *SIN3*-regulated genes identically, suggesting that they may modulate Sin3 repression in a promoter-specific fashion. We show that mutations in *SDS3* affect the same set of genes affected by *SIN3* and *RPD3*, consistent with the results of Vannier *et al.* (1996). Although these workers suggested that *SDS3* may function in a different pathway from *SIN3* and *RPD3*, we show that *SDS3* is in the same genetic pathway as *SIN3* and *RPD3.* Finally, immunoprecipitation experiments show that Sds3 is present in the Sin3 complex.

MATERIALS AND METHODS

Strains: The yeast strains used in this study, listed in Table 1, are all isogenic in the W303 background (Thomas and Rothstein 1989). Standard genetic methods were used for strain construction (Guthrie and Fink 1991). Plasmids M3737, M3780, M1436, and DV66 (Vannier *et al.* 1996) were used to disrupt the *CBK1*, *HYM1*, *SDS3*, and *RPD3* genes, respectively. All gene disruptions were confirmed by Southern analysis. Strains with either the *CYC1-LexA-lacZ* or the *CYC1-LexA-HIS3* reporter integrated at the *URA3* or the *LYS2* loci, respectively, have been described (Wang and Stillman 1993; Kasten *et al.* 1997). A strain with the *IME2-LacZ* integrated reporter was constructed by cleaving plasmid M3536 with StuI and integrating at the *ADE2.* Plasmids pHU10 (*his3::URA3*), M3927 (*ura3::KanMX3*), and M3926 (*leu2::KanMX3*) were used to convert markers (Cross 1997) in disrupted alleles or in integrated reporters. A W303 strain with a *trk1::HIS3* disruption was generously provided by Rick Gaber, and this marker was converted to *trk1::ADE2* using pRS402 (Brachmann *et al.* 1998) by marker replacement (Vidal and Gaber 1994). The W303 strain DY5699 was made by disrupting the *MET15* gene with plasmid pAD4 (Brachmann *et al.* 1998). Strain DY5870 with a 13 × Myc epitope tag at the C terminus of *SDS3* was constructed by transforming strain DY5699 with a PCR product generated with oligonucleotides F671 (5′ GAATTAACAGGTCAGCCTCCGGCTCCTTTCAGACTAAG GTCTCAGCGGATCCCCGGGTTAATTAA 3′) and F672 (5′ ATAATACAAAGTTAAAGTGGAAGGTTTGCAGCATAAAAT AAATTAGAATTCGAGCTCGTTTAAAC 3′) using plasmid pFA6a:13Myc:*HIS3MX6* (Longtine *et al.* 1998) as template. His$^+$ transformants were selected, and correct integration was verified.

Media: Cells were grown at 30° in standard media (Sherman 1991). YEPD medium was used unless strains had plasmids, in which case cells were grown in synthetic complete medium with 2% glucose supplemented with adenine, uracil, and amino acids, as appropriate, but lacking essential components to select for plasmids. Medium lacking leucine and histidine containing 20 mm 3-aminotriazole (3-AT) was used to analyze repression of the *CYC1-LexA-HIS3* reporter by LexA-Sin3. Low-potassium medium is the same as synthetic complete medium (Sherman 1991), except that sodium phosphate was substituted for potassium phosphate. High- and low-phosphate media were made as described (Han *et al.* 1988), except that plates were made with 1.2% agarose (BRL).

Plasmids: The plasmids used in this study are listed in Table 2. Plasmid M1436 has been described (Kasten *et al.* 1997). Plasmids M1836, M3958, M1835, and M3957 that express LexA-Sin3 or LexA were constructed in multiple steps, and details are available upon request. Plasmid M3496 was a gift of Yi Wei Jiang. Plasmid M3295, with the *CYC1-LexA-HIS3* reporter, has been described (Kasten *et al.* 1997). Plasmid M3536 (YIp, *ADE2*) was constructed in several steps using the YEp-*IME2-LacZ* plasmid previously described (Wang and Stillman 1993). Plasmids p6HA (M1710) and pJH330 (M2022) were kindly provided by Ira Herskowitz and John Lopes, respectively. Plasmid M3365 contains a 3.5-kb *Eco*RI-*Xba*I fragment with *SIN4* cloned into YIplac204 (Gietz and Sugino 1988). Plasmid M3458 contains a 1.5-kb *Eco*RV to *Afl*II fragment with *RPD3*, cloned as a *Xba*I-*Sac*I fragment (polylinker sites) into YIp352 (Hill *et al.* 1986). Plasmid M3561 expressing a Sin3-HA fusion protein was kindly provided by Kevin Struhl (Kadosh and Struhl 1997). The *cbk1::URA3* disruptor in plasmid M3737 removes nt −90 to +1940 (where the ATG = +1) of the *CBK1* gene. The *hym1::TRP1* disruptor in plasmid M3780 removes nt +253 to +1100 (where the ATG = +1) of the *HYM1* gene. The *rpd3::LEU2* disruptor in plasmid M1436 removes nt −556 to +1291 (where the ATG = +1) of the *RPD3* gene. The *sds3::HIS3* disruptor in plasmid DV66 has been described (Vannier *et al.* 1996) and was the generous gift of David Shore. The pHU10 *his3::URA3* marker converter plasmid has been described (Cross 1997) and was kindly provided by Fred Cross. Plasmids M3926 and M3927, with the *leu2::KanMX3* and *ura3::KanMX3* marker converters, will be described elsewhere. Plasmid pFA6a:13Myc:KanMX6 (M3968) containing a 13 × Myc epitope tag and a KanMX6 selectable marker was provided by Mark Longtine (Longtine *et al.* 1998).

Isolation of mutants: In the first screen, strain DY4442 with plasmid M1459 was mutagenized by treatment with UV light (to 60% viability), and cells were grown in the dark at room temperature for 1 day and then at 30° for an additional 2 days on plates lacking histidine and tryptophan with 20 mm 3-AT. From 10^7 surviving cells, 287 colonies were obtained capable of growth. Genetic backcrosses were conducted to eliminate plasmid-based mutations and to verify that a single genetic locus was responsible for the 3-AT-resistant phenotype. A total of 13 good mutants was identified, and these fell into two complementation groups, *rpd3* with five alleles and *sin4* with eight. Complementing clones were obtained, with either the wild-type *RPD3* or *SIN4* genes, and segregation analysis demonstrated allelism of the original mutations with disruption alleles. As homozygous *rpd3/rpd3* strains are sporulation defective, strains for allelism testing were sporulated with a *URA3-RPD3* plasmid. After tetrad dissection, cells were cured of the plasmid before the phenotype was examined. Finally, disrup-

TABLE 1

Strain list

Strain	Genotype
DY150	*MAT***a** *ade2 can1 his3 leu2 trp1 ura3*
DY1539	*MAT***a** *rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY4442	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 ade2 can1 his3 leu2 trp1 ura3*
DY4627	*MAT***a** *URA3::CYC1 UAS-lexA-lacZ ade2 can1 his3 leu2 lys2 trp1*
DY5699	*MAT***a** *ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
DY5888	*MAT*α *adh4::URA3 ade2 can1 his3 leu2 trp1 ura3*
DY5892	*MAT*α *adh4::URA3 sds3::KanMX ade2 can1 his3 leu2 trp1 ura3*
DY5894	*MAT*α *adh4::URA3 rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY5900	*MAT*α *adh4::URA3 sds3::KanMX rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
DY5870	*MAT***a** *SDS3:Myc13:HIS3MX6 ade2 can1 his3 leu2 lys2 met15 trp1 ura3*
SY170	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ can1 his3 leu2 trp1 ura3*
SY326	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ sds3::URA3 can1 his3 leu2 trp1 ura3*
SY334	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ sds3::URA3 sin3::LEU2 can1 his3 trp1 ura3*
SY337	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ sin3::LEU2 can1 his3 trp1 ura3*
SY338	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ sds3::URA3 rpd3::LEU2 can1 his3 trp1 ura3*
SY380	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ rpd3::LEU2 can1 his3 trp1 ura3*
SY383	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ cbk1::URA3 can1 his3 trp1 leu2 ura3*
SY389	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ sin3::hisG cbk1::URA3 can1 his3 leu2 trp1 ura3*
SY415	*MAT***a** *ura3::TRP1::CYC1 UAS-lexA-lacZ LYS2::CYC1 UAS-lexA-HIS3 sds3::URA3 ade2 can1 his3 leu2 trp1 ura3*
SY426	*MAT***a** *ura3::TRP1::CYC1 UAS-lexA-lacZ CYC1 UAS::lexA::HIS3::LYS2 ade2 can1 his3 leu2 trp1 ura3*
SY482	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ hym1::TRP1 can1 his3 leu2 trp1 ura3*
SY484	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ hym1::TRP1 cbk1::URA3 can1 his3 leu2 trp1 ura3*
SY486	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ hym1::TRP1 sin3::LEU2 can1 his3 leu2 trp1 ura3*
SY488	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 ADE2::IME2-lacZ hym1::TRP1 sin3::LEU2 cbk1::URA3 can1 his3 leu2 trp1 ura3*
SY510	*MAT***a** *URA3::CYC1 UAS-lexA-lacZ hym1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
SY515	*MAT***a** *URA3::CYC1 UAS-lexA-lacZ LYS2::CYC1 UAS-lexA-HIS3 hym1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
SY535	*MAT*α *ura3::TRP1::CYC1 UAS-lexA-lacZ LYS2::CYC1 UAS-lexA-HIS3 cbk1::URA3 ade2 can1 his3 leu2 trp1 ura3*
SY562	*MAT*α *trk1::ADE2 ade2 can1 his3 leu2 trp1 ura3*
SY564	*MAT*α *trk1::ADE2 sin3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
SY566	*MAT*α *trk1::ADE2 sds3::URA3 ade2 can1 his3 leu2 trp1 ura3*
SY568	*MAT*α *trk1::ADE2 sds3::URA3 sin3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
SY599	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 URA3::CYC1 UAS-lexA-lacZ cbk1::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY605	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 hym1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
SY606	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
SY609	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 rpd3::LEU2 cbk1::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY610	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 rpd3::LEU2 hym1::TRP1 ade2 can1 his3 leu2 trp1 ura3*
SY617	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 hym1::TRP1 cbk1::KanMX rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
SY618	*MAT***a** *cbk1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
SY620	*MAT***a** *hym1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
SY623	*MAT***a** *rpd3::LEU2 hym1::TRP1 ade2 can1 his3 leu2 lys2 trp1 ura3*
SY625	*MAT***a** *rpd3::LEU2 hym1::TRP1 cbk1::KanMX ade2 can1 his3 leu2 lys2 trp1 ura3*
SY641	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 URA3::CYC1 UAS-lexA-lacZ ade2 can1 his3 leu2 lys2 trp1 ura3*
SY660	*MAT*α *URA3::CYC1 UAS-lexA-lacZ rpd3::LEU2 ade2 can1 his3 leu2 trp1 ura3*
SY662	*MAT***a** *URA3::CYC1 UAS-lexA-lacZ sds3::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY668	*MAT***a** *URA3::CYC1 UAS-lexA-lacZ rpd3::LEU2 sds3::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY693	*MAT***a** *sds3::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY702	*MAT***a** *rpd3::LEU2 sds3::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY716	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 hym1::TRP1 cbk1::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY717	*MAT*α *LYS2::CYC1 UAS-lexA-HIS3 UAS::lexA::lacZ::URA3 rpd3::KanMX ade2 can1 his3 leu2 trp1 ura3*
SY718	*MAT***a** *LYS2::CYC1 UAS-lexA-HIS3 rpd3::LEU2 cbk1::KanMX ade2 can1 his3 leu2 trp1 ura3*

tion of *RPD3* or *SIN4* had the same effect on LexA-Sin3 repression as the UV-generated alleles.

To prevent the identification of additional alleles of *sin4* and *rpd3* in a second screen, strain SY161 was used that contained an additional copy of the *RPD3* and *SIN4* genes. After UV mutagenesis to 40% viability, 3-AT-resistant colonies were obtained as described above. Following backcrossing and elimination of weak mutants, 133 mutants were subjected to complementation analysis. There are at least 11 complementation groups, and complementation analysis continues for the other mutants. We identified two alleles of *sds3*, seven alleles of *cbk1*, and two alleles of *hym1. CBK1* and *HYM1* were cloned from a YCp50 library using a visual screen for complementation of the defect in colony morphology, and *SDS3* was cloned by

TABLE 2

Plasmid list

Plasmid	Description	Type
M1459	Expresses LexA-Sin3 fusion protein from *ADH1* promoter	YCp, *TRP1*
M1836	Expresses LexA-Sin3 fusion protein from *ADH1* promoter	YEp, *LEU2*
M3958	Expresses LexA-Sin3 fusion protein from *ADH1* promoter	YEp, *ADE2*
M1835	Expresses LexA from *ADH1* promoter	YEp, *LEU2*
M3957	Expresses LexA from *ADH1* promoter	YEp, *ADE2*
M3496	Expresses LexA-Ssn6 fusion protein from *ADH1* promoter	YCp, *TRP1*
M3295	*CYC1-LexA-HIS3* reporter	YIp, *LYS2*
M3536	*IME2-lacZ* reporter	YIp, *ADE2*
p6HA	*STE6-lacZ* reporter	YEp, *URA3*
pJH330	*INO1-lacZ* reporter	YEp, *URA3*
M3365	*SIN4* in YIplac204	YIp, *TRP1*
M3458	*RPD3* in YIp352	YIp, *URA3*
M3561	*SIN3*-3x-HA epitope tag	YEp, *LEU2*
M3737	*cbk1::URA3* disruptor	
M3780	*hym1::TRP1* disruptor	
M1436	*rpd3::LEU2* disruptor	
DV66	*sds3::HIS3* disruptor	
pHU10	*his3::URA3* marker converter	
M3926	*leu2::KanMX3* marker converter	
M3927	*ura3::KanMX3* marker converter	
M3970	pFA6a:13Myc:*HIS3MX6* PCR template	

complementation of its derepression of the *IME2-lacZ* reporter. Homozygous mutations in *cbk1*, *hym1*, and *sds3* were shown to be sporulation defective in diploids, and allelism testing was conducted as described above. Disruption alleles of *cbk1*, *hym1*, and *sds3* had the same phenotypes as the UV-generated alleles, and null alleles were used for all further phenotypic analysis.

Phosphatase assays: Phosphatase overlay assays on colonies and quantitative phosphatase assays with extracts were performed as described (Toh-e *et al.* 1973). To measure *PHO5* derepression grown in liquid, cells were grown overnight in high-phosphate medium, diluted and grown to mid-log, and harvested. To measure *PHO5* derepression while grown on plates, cells were grown on high-phosphate plates for 3 days at 30°, and then were scraped from the plate. Extracts were prepared by glass bead lysis. One unit of acid phosphatase is defined as the amount of enzyme that catalyzes the liberation of 1 μm of *p*-nitrophenol per minute at 37°. Each assay represents a minimum of three independent cultures.

Other methods: Assays for β-galactosidase activity using protein extracts were performed as described (Breeden and Nasmyth 1987). Telomeric silencing was measured using strains with a *URA3* gene integrated near the telomere of chromosome VII (Gottschling *et al.* 1990). Expression of the telomeric reporter was measured by plating serial dilutions of an overnight culture grown in rich media onto SC and SC-Ura plates. Immunoprecipitation and Western blotting were conducted as described (Ausubel *et al.* 1987) using monoclonal antibodies to the HA and Myc peptide epitopes.

RESULTS

Identification of genes required for LexA-Sin3 repression: We used a genetic selection to identify genes required for Sin3 to function as a transcriptional repressor. This selection uses a fusion of Sin3 to the DNA-binding domain of the bacterial LexA protein and a *CYC1-LexA-HIS3* reporter construct. In the presence of the fusion, cells produce insufficient His3 protein to be able to grow in the presence of 20 mm 3-AT, a competitive inhibitor of the *HIS3* gene product imidazole glycerol phosphate dehydratase. We selected for UV-light-generated mutations exhibiting growth on media containing 20 mm 3-AT but lacking histidine and leucine (to select for the LexA-Sin3 plasmid) that relieve repression by LexA-Sin3, and thus our efforts are focused on genes required for repression rather than on genes required to target the Sin3 complex to specific promoters. It is important to note that the endogenous *SIN3* gene product is not required for LexA-Sin3 repression.

Genetic tests demonstrated that the mutations are recessive and that a mutation in the LexA-Sin3 plasmid was not responsible for the histidine prototrophy. A *sin3* mutation derepresses the *PHO5* gene under repressing conditions, as a colony-staining overlay assay shows an increase in acid phosphatase activity (Vidal *et al.* 1991). This assay showed that all of the new mutants also had an increase in acid phosphatase activity. The mutants were backcrossed to a wild-type strain with the *CYC1-LexA-HIS3* reporter, and haploid segregants were tested for growth on −Leu, −His + 3-AT plates as well as for acid phosphatase activity. Segregation analysis demonstrated that a single genetic locus was responsible for both the loss of LexA-Sin3 repression and the *PHO5* derepression, except for strains with two contributing mutations that were excluded from further analysis.

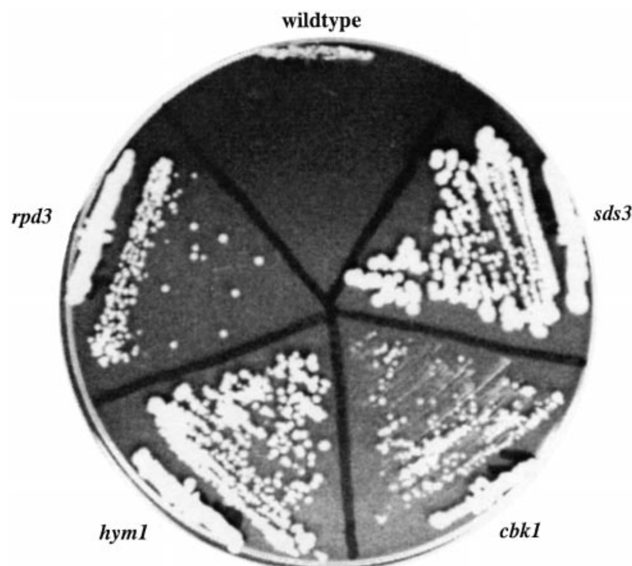

Figure 1.—Mutations in *SDS3*, *CBK1*, *HYM1*, and *RPD3* result in the loss of LexA-Sin3 repression. Strains containing the integrated *CYC1 UAS-LexA-HIS3* reporter and expressing LexA-Sin3 from plasmid M1836 are grown on −Leu −His + 20 mm 3-AT plates for 3 days at 30°. LexA-Sin3 represses expression of the *HIS3* reporter, and wild-type strains were unable to grow. Mutations in *CBK1*, *HYM1*, *RPD3*, and *SDS3* relieve this repression and allow growth. The strains used were SY641 (wild type), SY599 (*cbk1*), SY515 (*hym1*), SY717 (*rpd3*), and SY415 (*sds3*).

Complementation analysis identified at least 11 complementation groups. In this article we describe four of the mutations that we have cloned and genetically characterized. These genes are *RPD3*, *CBK1*, *HYM1*, and *SDS3.*

To demonstrate allelism of the complementing DNA with the original mutation, strains with the appropriate gene disruptions were crossed to strains with the original allele generated by UV mutagenesis. In each case, the diploids were unable to sporulate, as were diploids homozygous for mutations in *SIN3*, so the diploid was transformed with a *URA3*-based plasmid with the wild-type gene. After sporulation and tetrad dissection, cells were cured of the *URA3* plasmid by growth on 5-fluoroorotic acid (5-FOA) medium before phenotypic analysis. Segregation analysis demonstrated that each gene disruption was genetically linked to the appropriate mutation and that the disruptions confer the same phenotypes as the original mutations.

Haploid strains with disruptions for each of these four genes were created, demonstrating that none of these genes is essential for viability. Figure 1 shows how these mutations reduce repression by LexA-Sin3. In the wild-type strain with the *CYC1-LexA-HIS3* reporter, expression of LexA-Sin3 prevents growth on −Leu, −His plates containing 3-AT. Mutations in *RPD3*, *CBK1*, *HYM1*, and *SDS3* allow growth on this medium presumably by reducing repression by LexA-Sin3. A mutation that affects expression or accumulation of Rpd3 or the LexA-Sin3 fusion protein would also decrease repression of the *CYC1-LexA-HIS3* reporter. A Western immunoblot experiment indicated that LexA-Sin3 and Rpd3 protein levels were unaffected (data not shown). This indicates that these mutations reduce repression by affecting Sin3 function. To determine if these genes are specific to *SIN3*, we similarly tested whether these mutations affected repression of the unrelated *SSN6/TUP1* repression complex. Experiments showed that *rpd3*, *cbk1*, *hym1*, and *sds3* disruptions have no effect on repression by LexA-Ssn6 (data not shown). Consequently, we conclude that these mutations specifically reduce repression by LexA-Sin3. In summary, the *RPD3*, *CBK1*, *HYM1*, and *SDS3* genes are all required for efficient repression by LexA-Sin3 and for sporulation in diploids, but are not essential for viability.

***RPD3* is required for LexA-Sin3 repression:** An *rpd3* mutation reduces repression by LexA-Sin3 at the *CYC1-LexA-HIS3* reporter (Figure 1). This result is not surprising, as mutations in *RPD3* and *SIN3* have similar effects on transcriptional regulation, and *rpd3 sin3* double mutants are no more severely affected than either single mutant (Stillman *et al.* 1994). Furthermore, biochemical analysis has shown that Rpd3 functions in a complex with Sin3 (Kadosh and Struhl 1997; Kasten *et al.* 1997). To quantitate the loss of repression, we used the *CYC1-LexA-LacZ* reporter, which has the same *CYC1* promoter driving *LacZ* expression instead of *HIS3.* To determine repression by LexA-Sin3, we compare *LacZ* expression in cells expressing LexA only or the LexA-Sin3 fusion protein. As shown in Table 3, LexA-Sin3 represses transcription by 30-fold, and an *rpd3* mutation reduces this repression by a factor of 7.5. The identification of *rpd3* mutations as relieving repression by LexA-Sin3 demonstrates the validity of our selection strategy.

Mutations in *CBK1* or *HYM1* affect regulation of some *SIN3*-dependent genes: The next two mutations, *cbk1* and *hym1*, will be considered together as they cause similar phenotypes. Both cause a defect in cell separation that can be seen microscopically as large clusters of unseparated cells. The mutations also cause an abnormal colony morphology, with a rough colony surface in contrast to the smooth shimmer of a wild-type colony. In fact, the *CBK1* gene (YNL161w) was given the name *c*ell-wall *b*iosynthesis *k*inase (C. Herbert, personal communication) because *cbk1* mutants display this defect in cell separation, and the protein shows homology to the AGC family of protein kinases (Hunter and Plowman 1997). The *HYM1* gene (YKL189w) is named for its similarity to the *Aspergillus nidulans* gene *hymA.* Mutations in *hymA* affect conidiophore development in *A. nidulans* (Karos and Fischer 1999). Although it was reported that *HYM1* is an essential gene in yeast (Karos and Fischer 1999), we have found that strains with a *hym1* gene disruption are viable and healthy. We attribute the disparity in results to different strain backgrounds.

TABLE 3

Mutations in *CBK1*, *HYM1*, and *RPD3* reduce repression by LexA-Sin3

Strain	Genotype	LacZ activity with LexA	LacZ activity with LexA-Sin3	Repression
A.				
SY641	Wild type	1326 ± 243	44 ± 2	30
SY599	*cbk1*	2339 ± 205	166 ± 16	14
SY605	*hym1*	2079 ± 79	177 ± 49	12
SY606	*rpd3*	324 ± 8	91 ± 15	4
SY609	*cbk1 rpd3*	451 ± 2	119 ± 7	4
SY610	*hym1 rpd3*	391 ± 18	87 ± 18	4
SY617	*cbk1 hym1 rpd3*	507 ± 36	103 ± 7	5
B.				
SY641	Wild type	2775 ± 449	95 ± 27	29
SY599	*cbk1*	2348 ± 488	181 ± 20	13
SY605	*hym1*	2456 ± 180	157 ± 14	16
SY612	*cbk1 hym1*	2041 ± 166	162 ± 14	13

Strains containing the integrated *CYC1 UAS-LexA-LacZ* reporter and expressing either LexA from plasmid M3957 or LexA-Sin3 from plasmid M3958 were grown on medium lacking adenine to maintain the plasmid, and extracts were prepared for β-galactosidase assays. The quantities represent the average of three independent transformants ± SD. Repression is expressed as the ratio of β-galactosidase activity in the presence of LexA divided by β-galactosidase activity in the presence of LexA-Sin3.

To quantitate the observed loss of LexA-Sin3 repression in *CBK1* or *HYM1* mutants (Figure 1), we used the *CYC1-LexA-LacZ* reporter and quantitated *LacZ* activity in strains expressing LexA only or the LexA-Sin3 fusion protein (Table 3). A *cbk1* or a *hym1* mutation results in an ~50% reduction in repression by LexA-Sin3, a much smaller effect on LexA-Sin3 repression than that observed for the *rpd3* mutation. Additionally, the *cbk1 rpd3* and *hym1 rpd3* double mutants show no greater loss of repression than the *rpd3* single mutant, and the *cbk1 hym1* double mutant shows effects similar to either single mutant, suggesting that *CBK1* and *HYM1* function in the same genetic pathway.

Reasoning that the mutations identified in our screen should affect transcriptional regulation of genes affected by *sin3* or *rpd3* mutations, we therefore determined the effect of *cbk1* and *hym1* mutations on expression of certain *SIN3*-dependent genes. *STE6* is an **a**-specific gene required in *MAT***a** cells for the production of **a**-factor, and expression of *STE6* is sharply reduced in *sin3* and *rpd3* mutants (Vidal and Gaber 1991; Vidal *et al.* 1991; Wang *et al.* 1994). Sin3 and Rpd3 are thought to function primarily as transcriptional repressors, and it is believed that reduced *STE6* expression is an indirect effect (Wang *et al.* 1994). Isogenic strains with *cbk1*, *hym1*, and *rpd3* mutations were transformed with a plasmid containing a *STE6-lacZ* reporter, and promoter activity was monitored by quantitating activity of the β-galactosidase enzyme (Figure 2). There was a reduction in *STE6* expression in strains with mutations in *cbk1* and *hym1*, but not to the same extent as seen with the *rpd3* mutant. No synergistic effects with this *STE6-lacZ* reporter were seen in these multiply mutant strains.

The *PHO5* gene encodes an acid phosphatase, and this gene is induced under phosphate starvation. *PHO5* is repressed in wild-type cells in high-phosphate medium, but this repression is lost in *sin3* and *rpd3* mutants.

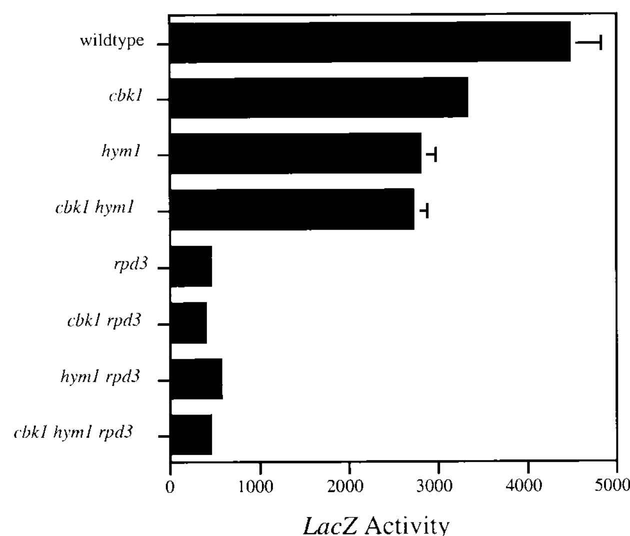

Figure 2.—Mutations in *CBK1*, *HYM1*, and *RPD3* result in a loss of *STE6-LacZ* activation. Strains transformed with plasmid p6HA with the *STE6-LacZ* reporter were grown on medium lacking uracil to maintain the plasmid, and extracts were prepared for β-galactosidase assays. The quantities represent the average of three independent transformants with standard deviations shown as error bars. Activity of *STE6-LacZ* was sharply reduced in *rpd3* strains, and this reduction was not increased in the double and triple mutants. The strains used were DY150 (wild type), SY618 (*cbk1*), SY620 (*hym1*), SY716 (*cbk1 hym1*), DY1539 (*rpd3*), SY718 (*cbk1 rpd3*), SY623 (*hym1 rpd3*), and SY625 (*cbk1 hym1 rpd3*).

TABLE 4

Mutations in *CBK1*, *HYM1*, and *RPD3* result in *PHO5* derepression

Strain	Genotype	Acid phosphatase activity	Derepression
Liquid culture			
SY170	Wild type	0.029 ± 0.005	1.00
SY383	*cbk1*	0.026 ± 0.008	0.90
SY482	*hym1*	0.025 ± 0.003	0.86
SY484	*cbk1 hym1*	0.022 ± 0.001	0.76
SY337	*sin3*	0.102 ± 0.005	3.52
SY389	*cbk1 sin3*	0.15 ± 0.005	5.17
SY486	*hym1 sin3*	0.155 ± 0.005	5.34
SY488	*cbk1 hym1 sin3*	0.155 ± 0.016	5.34
Solid media			
SY426	Wild type	0.018 ± 0.002	1.0
SY535	*cbk1*	0.024 ± 0.002	1.3
SY510	*hym1*	0.027 ± 0.003	1.5

For liquid culture, cells were grown in liquid high-phosphate media to mid-log and were assayed for acid phosphatase activity. The values represent the average of three independent cultures ± SD. For solid media, cells were grown for 3 days on high-phosphate plates. They were then scraped from the plates and acid phosphatase activity was determined from extracts. The values represent the average of seven or eight independent cultures ± SD.

To measure the effects of these mutations on *PHO5* expression, isogenic strains with *cbk1*, *hym1*, and *sin3* mutations were grown in high-phosphate liquid media, and extracts were prepared for quantitative acid phosphatase assays. As shown in Table 4, *PHO5* was not derepressed in *cbk1* or *hym1* mutants, but was derepressed in the *sin3* mutant. We were surprised to find no increase in *PHO5* expression in the *cbk1* and *hym1* mutants because, as noted earlier, these mutants showed an increase in acid phosphatase activity using a colony-staining overlay assay, for which solid media was used. To address this apparent discrepancy, extracts were prepared from cells grown on solid media, and acid phosphatase activity was measured. The results in Table 4 show that *cbk1* and *hym1* mutants have a small but significant and reproducible increase in acid phosphatase activity when cells are grown on high-phosphate plates. This derepression was not additive in the *cbk1 hym1* double mutant (data not shown). It is not easy to explain the difference between the results obtained with the assays from cells grown in liquid or on plates. We do note that cells grown in patches on solid medium would result in a larger fraction of yeast that are in late-log or stationary phase, and this could affect *PHO5* expression. Alternatively, there may be localized depletion of specific nutrients from the solid growth medium, and such effects would not be evident during log phase growth in liquid medium. Using cells grown in liquid medium (Table 4), we did note that there was increased derepression in the *cbk1 sin3* or *hym1 sin3* double mutants compared to the *sin3* single mutant. This additive derepression was observed in combination with either *rpd3* or *sin3*, and in cells grown on plates as well as in liquid (data not shown). An additive effect, *cbk1* or *hym1* with *rpd3* or *sin3*, was not seen in the previous experiments looking at *STE6* expression or repression by LexA-Sin3.

Meiosis-specific genes such as *IME2* and *SPO13* are also negatively regulated by *SIN3* and *RPD3* (Strich *et al.* 1989; Bowdish and Mitchell 1993). *IME2* encodes a kinase required for proper expression of meiotic genes and is expressed normally only in diploid cells preparing to undergo sporulation. Mutations in either *SIN3* or *RPD3* lead to *IME2* expression during vegetative growth, even in the haploid state. To quantitate the level of derepression, we utilized an *IME2-LacZ* reporter integrated at the *ADE2* locus. Haploid cells were grown in rich media to mid-log phase, and extracts were prepared for quantitative β-galactosidase assays. Mutation in either *CBK1* or *HYM1* lead to a weak derepression of the *IME2-LacZ* reporter (Figure 3). As with *PHO5*, we observed a slight additive increase in *IME2-LacZ* expression in the *cbk1 sin3* or *hym1 sin3* double mutants compared to the *sin3* single mutant. No additive increase was seen in the *cbk1 hym1* double mutant.

SIN3 and *RPD3* also repress *INO1* (encoding inositol-1-phosphate synthase) and *TRK2* (low-affinity potassium transporter) expression. Consequently, promoter activity was determined in *cbk1* and *hym1* single mutants, as well as *cbk1 hym1*, *cbk1 rpd3*, and *hym1 rpd3* double mutants using either an *INO1-LacZ* or a *TRK2-LacZ* reporter. An additional growth assay was used to examine mutational effects on *TRK2* expression (the growth assay is described below). Our findings showed no effect of *cbk1* or *hym1* mutations on *INO1* or *TRK2* expression, either alone or when combined with a *rpd3* mutation (data not shown). Furthermore, these mutations (single or in combination with an *rpd3* mutation) did not affect telomeric silencing (data not shown). These observa-

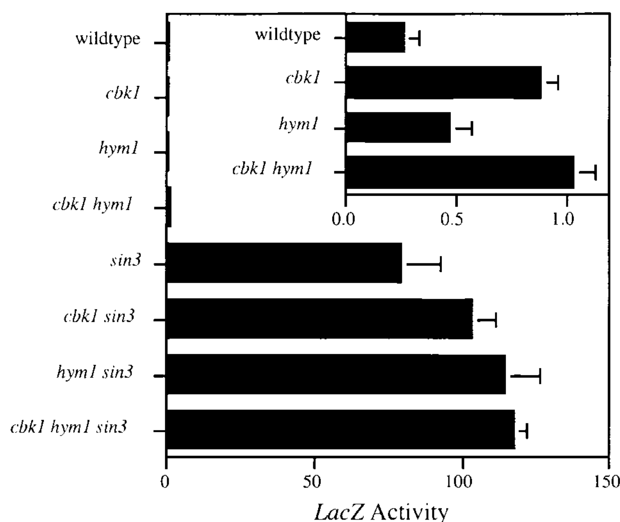

Figure 3.—Mutations in *CBK1*, *HYM1*, and *RPD3* result in derepression of *IME2-lacZ.* Strains containing the integrated *IME2-lacZ* reporter were grown on YEPD medium, and extracts were prepared for β-galactosidase assays. The quantities represent the average of three independent transformants with standard deviations shown as error bars. *IME2* is normally repressed in haploid cells. Strains lacking *CBK1*, *HYM1*, or both show a weak loss of repression. *IME2-LacZ* activity increased dramatically in the *rpd3* strain. The double and triple mutants *cbk1 rpd3*, *hym1 rpd3*, *cbk1 hym1 rpd3* have an additive loss in repression. The inset has an expanded view of the first four strains. The strains used were SY170 (wild type), SY383 (*cbk1*), SY482 (*hym1*), SY484 (*cbk1 hym1*), SY337 (*sin3*), SY389 (*cbk1 sin3*), SY486 (*hym1 rpd3*), and SY488 (*cbk1 hym1 rpd3*).

tions contrast to the increase in silencing at either the silent mating type loci or at genes linked to telomeres as evidenced in *sin3* or *rpd3* mutations (De Rubertis *et al.* 1996; Rundlett *et al.* 1996; Vannier *et al.* 1996).

In summary, strains lacking *CBK1* or *HYM1* show a loss of repression by LexA-Sin3, decreased repression at *PHO5* and *IME2*, and a decrease in *STE6* expression. Thus, these mutations have weak effects on several *SIN3*-dependent promoters, consistent with a role in the function of the Sin3/Rpd3 complex. However, the effects of *cbk1* and *hym1* mutations were quantitatively less severe than *sin3* or *rpd3*, and *cbk1* and *hym1* fail to effect all *SIN3*-dependent promoters. The fact that the *cbk1 hym1* double mutant has no greater effect than the single mutants implies that *CBK1* and *HYM1* function in the same genetic pathway. When *cbk1* or *hym1* are combined with *rpd3* or *sin3* we observe an additive effect only at *PHO5* and *IME2-LacZ.* Finally, strains lacking either *CBK1* or *HYM1* display an additional phenotype, an altered colony morphology due to a defect in cell separation. This defect is not increased in the double mutant, supporting the conclusion that *CBK1* and *HYM1* function in the same genetic pathway. Based on the additive effects seen at *PHO5* and *IME2-LacZ* and the failure of *cbk1* or *hym1* mutations to effect all *SIN3*-dependent genes, we suggest that *CBK1* and *HYM1* are in a common genetic pathway that is distinct from *SIN3* and *RPD3.*

***SDS3* is required for *SIN3*-dependent repression:** A mutation in the SDS3 gene reduces repression by LexA-Sin3 as shown by the histidine prototrophy in strains with the *CYC1-LexA-HIS3* reporter (Figure 1). *SDS3* was originally identified in a screen for mutations that restore silencing at a silencer crippled by both *cis*- and *trans*-mutations (Vannier *et al.* 1996). This screen also identified mutations in *SIN3* (*SDS16*) and *RPD3* (*SDS6*) as restoring silencing. The work of Vannier *et al.* (1996) suggested that *SDS3* function is related to, but genetically distinct from, that of *SIN3* and *RPD3.*

We used strains transformed with plasmids expressing either LexA or the LexA-Sin3 fusion protein and a *CYC1-LexA-LacZ* reporter to quantitatively measure the effects of the *sds3* mutation on repression by LexA-Sin3 (Table 5). Repression was calculated as the ratio of reporter activity in cells expressing LexA only to those expressing LexA-Sin3. The strain with the *sds3* mutation has a loss of LexA-Sin3 repression equivalent to that seen in the *rpd3* strain. The *sds3 rpd3* double mutant shows a loss of repression similar to the two single mutants, suggesting that they function in the same pathway. An *sds3* mutation does not affect repression by the mechanistically distinct LexA-Ssn6 fusion protein, demonstrating specificity toward Sin3 repression. Thus, mutations in

TABLE 5

Mutations in *SDS3* result in a loss of repression by LexA-Sin3

Strain	Genotype	LacZ activity with LexA	LacZ activity with LexA-Sin3	Repression
DY4627	Wild type	1620 ± 410	60 ± 30	27
SY660	*rpd3*	320 ± 90	50 ± 10	6
SY662	*sds3*	470 ± 60	120 ± 10	4
SY668	*rpd3 sds3*	600 ± 20	100 ± 10	6

Strains containing the integrated *CYC1 UAS-LexA-LacZ* reporter and expressing either LexA from plasmid M3957 or LexA-Sin3 from plasmid M3958 were grown on medium lacking adenine to maintain the plasmid, and extracts were prepared for β-galactosidase assays. The quantities represent the average of three independent transformants ± SD. Repression is expressed as the ratio of β-galactosidase activity in the presence of lexA divided by β-galactosidase activity in the presence of LexA-Sin3.

TABLE 6

Mutation of *SDS3* effects regulation of multiple *SIN3*- and *RPD3*-dependent genes and is not additive with *sin3* or *rpd3*

A.

Strain	Genotype	*STE6-lacZ* activity	Expression
DY150	Wild type	4470 ± 350	100%
SY693	*sds3*	620 ± 80	14%
DY1539	*rpd3*	450 ± 15	10%
SY702	*rpd3 sds3*	360 ± 10	8%

B.

Strain	Genotype	Acid phosphatase activity	Derepression
SY170	Wild type	0.022 ± 0.002	1
SY326	*sds3*	0.092 ± 0.006	4
SY380	*rpd3*	0.067 ± 0.006	3
SY338	*rpd3 sds3*	0.071 ± 0.007	3

C.

Strain	Genotype	*IME2-lacZ* activity	Derepression
SY170	Wild type	0.3 ± 0.1	1
SY326	*sds3*	93 ± 13	358
SY380	*rpd3*	79 ± 14	304
SY338	*rpd3 sds3*	101 ± 21	388

D.

Strain	Genotype	*INO1-lacZ* activity	Derepression
DY150	Wild type	26 ± 3	1
SY693	*sds3*	2390 ± 460	92
DY1539	*rpd3*	1570 ± 20	60
SY702	*sds3 rpd3*	2570 ± 230	99

Expression from the *STE6-lacZ* reporter was measured as in Figure 2. Acid phosphatase activity was measured from cells grown in liquid high-phosphate media as in Table 4. Expression from the *IME2-LacZ* reporter was measured as in Figure 3. Strains transformed with plasmid pJH330 with the *INO1-LacZ* reporter were grown on medium lacking uracil to maintain the plasmid, supplemented with 1 mm choline and 0.75 mm inositol to repress *INO1* transcription. Extracts were prepared for β-galactosidase assays, with the values representing the average of three independent transformants ± SD.

SDS3 and *RPD3* cause similar phenotypes, and the double mutants are not additive.

To compare the effects of the *sds3* mutation with that of *rpd3* and *sin3*, we examined expression of a number of *SIN3*-dependent promoters, including *STE6*, *PHO5*, *IME2*, and *INO1.* Promoter activity was determined in *sds3*, *sin3*, and *rpd3* single mutants, as well as in *sds3 sin3* and *sds3 rpd3* double mutants (Table 6 and data not shown). Expression of a *STE6-LacZ* reporter was reduced to similar extents in *sds3* and *rpd3* mutants (Table 6A). The *PHO5* gene was derepressed in both *sds3* and *rpd3* mutants (Table 6B). An *IME2-LacZ* reporter was not expressed in vegetatively grown cells, but was derepressed in both *sds3* and *rpd3* mutants (Table 6C), in agreement with the previous results (Bowdish and Mitchell 1993; Vannier *et al.* 1996). *INO1*, a *SIN3*-dependent gene, is repressed in the presence of inositol and choline. Mutations in *SDS3* and *RPD3* both lead to derepression of an *INO1-LacZ* reporter (Table 6D). There are two important results in this set of experiments on transcriptional regulation. First, an *sds3* mutation has a quantitatively similar effect as *sin3* or *rpd3* mutations. Second, there was no increase in effect in the *sds3 sin3* and *sds3 rpd3* double mutants compared to the single mutants at all five transcriptional reporters.

***SDS3* is in the same genetic pathway as *SIN3* and *RPD3*:** Vannier *et al.* (1996) presented evidence that *SDS3* has similar functions as *SIN3* and *RPD3*, but they also came to the conclusion that *SDS3* was in a different genetic pathway than *SIN3* and *RPD3*, based on two observations. The first was that an *sds3* mutation failed to derepress a *TRK2-LacZ* reporter, while *sin3* and *rpd3* mutations caused an increase in *TRK2-LacZ* expression. The second observation was that *sds3 sin3* and *sds3 rpd3* double mutants displayed an increase in silencing compared to the single mutants, and this additive effect suggested that *SDS3* functioned in a different pathway.

We performed several experiments in an attempt to resolve these apparent discrepancies about the relationship of *SDS3* to *SIN3* and *RPD3.* We first attempted to test the effect of *sds3*, *sin3*, and *rpd3* on expression of the *TRK2-LacZ* reporter present on a multicopy plasmid. We found that this reporter failed to yield reproducible results. In some experiments there was derepression of *TRK2-LacZ* in an *sds3* mutant, while in others this derepression was not observed. While we were always able to demonstrate significant derepression of the *TRK2-LacZ* reporter in *sin3* and *rpd3* mutants, even this was subject to significant fluctuations. Because of the lack of reproducibility with the *TRK2-LacZ* reporter in our hands, we abandoned this reporter in favor of the original growth assay in low-potassium medium for *TRK2* expression (Vidal *et al.* 1990).

Yeast cells contain both high- and low-affinity potassium transporters, encoded by *TRK1* and *TRK2*, respectively. Vidal *et al.* (1990) first isolated *rpd3* as a suppressor mutation that allowed *trk1* mutants, lacking the high-affinity potassium transporter, to grow on media with reduced potassium. The *RPD* gene name stands for *r*educed *p*otassium *d*ependence, and *sin3* was also isolated in this screen as *rpd1.* Strains lacking the high-affinity potassium transporter encoded by *TRK1* must rely on the low-affinity transporter, Trk2, for potassium uptake. Strains with a *trk1* mutation require >5 mm potassium in the medium, and limiting the potassium concentration to 0.2 mm results in no growth. The *TRK2* gene, encoding the low-affinity transporter, is normally expressed at very low levels, and mutations such as *sin3* and *rpd3* that increase *TRK2* expression restore growth to a *trk1* mutant.

We constructed isogenic *trk1*, *trk1 sds3*, *trk1 sin3*, and *trk1 sds3 sin3* strains and determined the ability of these strains to grow on low-potassium medium (Figure 4). *trk1* strains grow very poorly, with a doubling time of

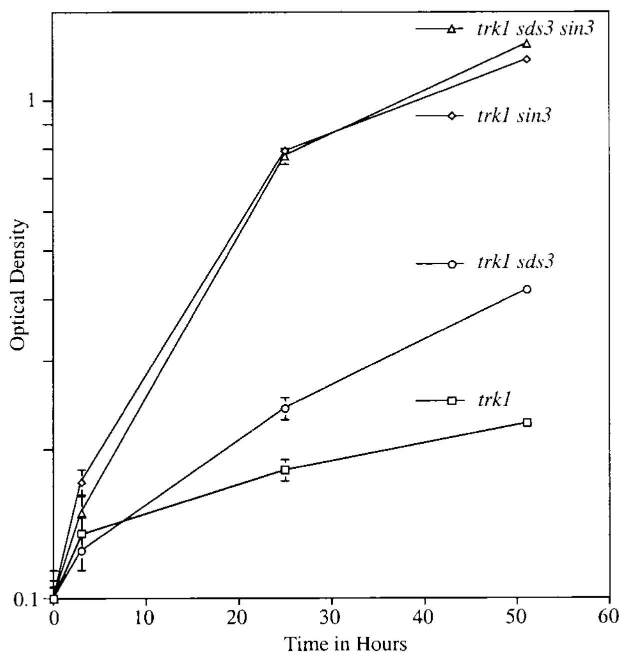

Figure 4.—*SDS3* regulates *TRK2* expression and is not additive with *sin3.* Cells lacking the high-affinity potassium transporter *TRK1* must have increased expression of the *TRK2* low-affinity potassium transporter in order to survive on low potassium medium. Strains were pregrown in rich medium (replete potassium) and then diluted to a low density in synthetic complete medium supplemented with 0.2 mm potassium (limiting potassium) and grown at 30°. Cell growth was monitored over time by optical density at 660 nm for three independent cultures. Growth rates are plotted with standard deviations shown as error bars for each time point. The strains used were SY562 (*trk1*), SY564 (*trk1 sds3*), SY566 (*trk1 sin3*), and SY568 (*trk1 sds3 sin3*).

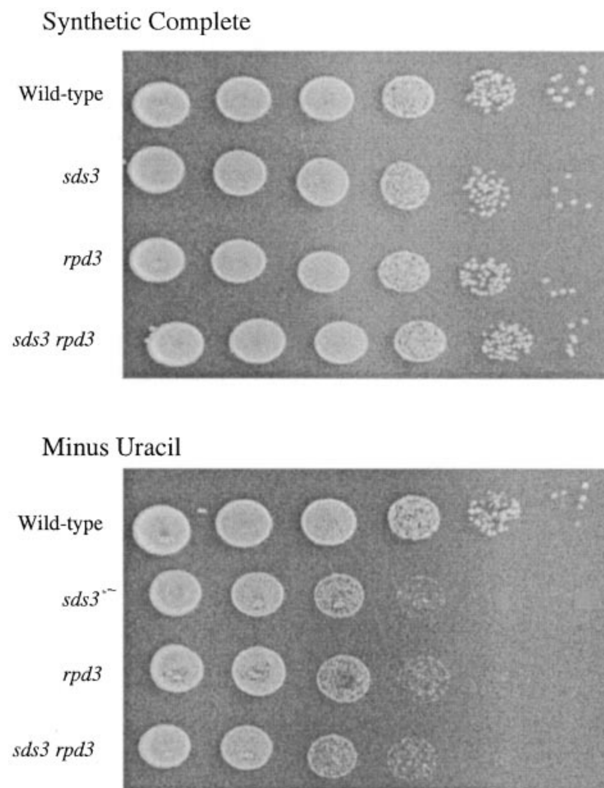

Figure 5.—*sds3* is not additive with *rpd3* in telomeric silencing. A *URA3* gene located 15 kb from the telomere on chromosome VII weakly silenced by telomeric heterochromatin, but mutations that increase telomeric silencing will result in decrease plating efficiency on media lacking uracil. Mutations in *SDS3* and *RPD3* both increase telomeric silencing, but the effect was not additive in *sds3 rpd3* double mutants. Serial dilutions (10-fold) of each culture were spotted to medium lacking uracil or to synthetic complete medium, as a control. The strains used were DY5888 (wild type), DY5892 (*sds3*), DY5894 (*rpd3*), and DY5900 (*sds3 rpd3*).

~53 hr. As expected, disruption of the *SIN3 (rpd1)* or *RPD3* gene resulted in a significant increase in growth rate under limiting potassium, to ~11 hr (Figure 4 and data not shown). The *trk1 sds3* mutant grows much better than the *trk1* single mutant, with a doubling time of 34 hr. Significantly, the *trk1 sds3* strain does not grow as well as the *trk1 sin3* mutant, suggesting that the *sds3* mutation has less of an effect on *TRK2* expression than *sin3.* Finally, the *trk1 sin3* and *trk1 sds3 sin3* strains grow at equivalent rates on low-potassium medium (Figure 4). Thus, the *sds3*, *sin3*, and *rpd3* mutants all suppress the *trk1* defect, and the mutations are not additive. These results suggest that *SDS3* does regulate *TRK2* and functions in the same genetic pathway as *RPD3* and *SIN3.*

Vannier *et al.* (1996) suggested that *SDS3* is functionally different from *RPD3* and *SIN3*, based on an additive effect in silencing with the *hmr*ΔAΔ*E::TRP1* reporter. We have compared the *sds3* single mutant to *sds3 sin3* and *sds3 rpd3* double mutants with a variety of transcriptional reporters, and we have not seen any additive effects. As a silencing assay to examine whether *sds3* is additive with *sin3* or *rpd3*, we constructed isogenic strains with a *URA3* reporter integrated near the telomere of chromosome VII. This telomeric reporter does not require any specific mutations at the *HMR-E* or *RAP1* loci and gives a significantly stronger signal than the *hmr*ΔAΔ*E::TRP1* reporter. Mechanistically, the *URA3* telomere-silencing assay is thought to be similar to the *HMR*-silencing assay, as both are dependent upon the *SIR2*, *SIR3*, *SIR4*, and *RAP1* genes (Aparicio *et al.* 1991).

The results of the telomere-silencing assay are shown in Figure 5. In this assay, one measures the fraction of cells with a transcriptionally inactive telomere-linked *URA3* gene by determining the fraction of cells incapable of growth on medium lacking uracil. It has been shown that DNA near telomeres is assembled into a heterochromatic state that represses transcription (Grunstein 1998), with the efficiency of this silencing decreasing with distance from the telomere (Renauld *et al.* 1993). With the *URA3* reporter placed 15 kb from the telomere, silencing is quite inefficient in wild-type strains, with nearly 100% of cells growing without added

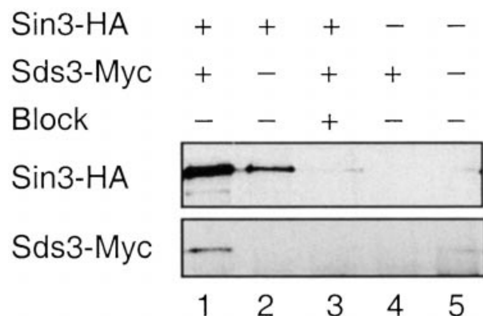

Figure 6.—Sds3 and Sin3 coimmunoprecipitate. Extracts were prepared from strains expressing Sin3-HA and/or Sds3-Myc, as indicated, precipitated with antibody to HA, and the immunoprecipitates were probed in Western blots for Sin3-HA and Sds3-Myc. An excess of blocking peptide was added to the sample in lane 3. Strains DY5699 (wild type) and DY5870 (Sds3-Myc) and plasmid M3561 (Sin3-HA) were used.

uracil. For the *sds3* and *rpd3* mutant strains, only ∼10% of cells grow on the plate lacking uracil, showing that the two mutations cause a quantitatively similar increase in silencing. Importantly, telomeric silencing in the *sds3 rpd3* double mutant was the same as in the *sds3* and *rpd3* single mutants. Thus, there is no additive effect between *sds3* and *rpd3*, and we believe that *SDS3* functions in the same genetic pathway as *RPD3* and *SIN3.*

Sds3 is present in the Sin3 complex: Sin3 and Rpd3 are physically associated (Kadosh and Struhl 1997; Kasten *et al.* 1997). We used immunoprecipitation methods to determine whether Sds3 is present in the Sin3 complex. Strains were constructed that expressed epitope-tagged versions of Sin3 and Sds3. Sin3 contained a 3 × HA epitope tag, and Sds3 contains a 13 × Myc tag, both as C-terminal fusions. When extracts were prepared from strains and immunoprecipitated with anti-HA antibody, the Sin3-HA fusion protein was detected in Western blots (Figure 6, lanes 1 and 2). The Western blot signal was absent from strains not expressing the Sin3-HA fusion (Figure 6, lanes 4 and 5), and the signal was also abolished by addition of blocking peptide (Figure 6, lane 3). Sds3-Myc coprecipitates with Sin3-HA (Figure 6, lane 1), and this Western blot signal was absent from strains lacking the Myc-tagged Sds3 (Figure 6, lane 2). These experiments show clearly that Sds3 is present in the Sin3 complex.

DISCUSSION

Sin3 is a transcriptional repressor that is targeted to specific promoters by interacting with DNA-binding proteins. Sin3 is present in a large multiprotein complex that includes the Rpd3 histone deacetylase, and thus Sin3 functions as a repressor, at least in part, by altering the acetylation state of chromatin. We set up a genetic screen to identify other genes that play a role in Sin3-mediated repression. The screen used a LexA-Sin3 fusion protein that represses transcription of promoters with LexA binding sites, and a number of mutations that reduced this repression were isolated. In this article we describe four genes, *RPD3*, *SDS3*, *CBK1*, and *HYM1*, that play a role in Sin3 function.

The fact that we obtained *RPD3* in the screen validates our selection strategy. In addition to the fact that Rpd3 is physically associated with Sin3, *rpd3* mutations have similar effects on transcriptional regulation as *sin3* mutants. Additionally, the phenotype of a *sin3 rpd3* double mutant is similar to the single mutants, suggesting that Sin3 and Rpd3 function together (Stillman *et al.* 1994).

A variety of experimental observations suggest that *SDS3* functions in the same genetic pathway as *SIN3* and *RPD3.* We have shown that *sds3* mutations have a similar effect on transcriptional regulation as *sin3* and *rpd3* mutations. Eight different *SIN3*-responsive transcription units (*CYC1-LexA-HIS3*, *CYC1-LexA-LacZ*, *STE6-LacZ*, *PHO5*, *IME2-LacZ*, *INO1-LacZ*, *TRK2*, and telomeric silencing) were used to determine the effect of an *sds3* mutation on gene expression. In every case, *sds3* has the same effect as *sin3* and *rpd3.* Moreover, in quantitative assays, *sds3* was similar to *sin3* and *rpd3*, except in the *TRK2* assay for suppression of the poor growth in low-potassium medium due to a *trk1* gene deletion. The Swi/Snf complex provides an example where mutations in different components all have related phenotypes, but there can be differences in the phenotypic severity (Cairns *et al.* 1996). We conclude that an *sds3* mutation causes the same phenotypes as *sin3* and *rpd3.*

Analysis of double mutant strains also suggests that *SDS3* functions with *SIN3* and *RPD3.* We have examined expression from all of the *SIN3*-dependent reporters in *sds3 rpd3* or *sds3 sin3* double mutants. In all cases, we fail to see any additive effects in the double mutants, compared to the *rpd3* or *sin3* single mutants. Importantly, this is also true in the *TRK2* bioassay, where the *sds3* mutant is a less effective suppressor, showing that they are not functioning in different pathways. Of course, an *sds3* mutation could reduce repression by reducing expression of either *SIN3* or *RPD3.* However, an *sds3* mutation affects repression by both endogenous Sin3 and the LexA-Sin3 fusion protein that is expressed by a different promoter, and Western immunoblots showed that Rpd3 and LexA-Sin3 levels are unaffected.

SDS3 was identified in a screen for mutations that cause increased silencing of a crippled *HMR* silencer (Vannier *et al.* 1996). This screen also identified *SIN3* and *RPD3*, suggesting a connection between *SDS3* and *SIN3/RPD3.* These authors concluded that *SDS3* was in a different genetic pathway based upon a lack of derepression of a *TRK2-lacZ* plasmid reporter and additive effects in double mutants observed with a *TRP1* gene present at a crippled silencer. However, we found that an *sds3* mutation does affect *TRK2* expression, based on a bioassay involving growth on low potassium, and we do not see any additive effects on silencing in

sds3 rpd3 and *sds3 rpd3* double mutant strains. We also conducted double mutant analysis for all of our transcription assays, and we observed no additive effects with *sds3.* Finally, coimmunoprecipitation experiments show that Sds3 is physically present in the Sin3 complex. We conclude that *SDS3* functions in the same genetic pathway as *RPD3* and *SIN3.*

The screen that originally identified *sds3* used strains sensitized for silencing, with either the *hmr*Δ*A::ADE2* or *hmr*Δ*A::TRP1* reporter in a *rap1[s]* strain (Sussel *et al.* 1995; Vannier *et al.* 1996). There are two mutations that increase the sensitivity for changes in silencing, one the Δ*A* mutation in the ORC binding site in the *HMR-E* silencer, and the other the *rap1[s]* mutation in the gene encoding the Rap1 protein that binds to silencers. Quantitative analysis showed that the *sds3* mutation caused a 1000-fold increase in silencing (Sussel *et al.* 1995; Vannier *et al.* 1996). For the experiments showing an additive effect with *sds3*, the authors used a *hmr*Δ*A*Δ*E::TRP1* reporter, which has mutations in both the ORC and Rap1 binding sites in the *HMR-E* silencer (Vannier *et al.* 1996). With this reporter system, 95% of wild-type cells are Trp^+, 70% of *sds3* and *sin3* mutants are Trp^+, and 36% of *sds3 sin3* double mutants are Trp^+. We feel that the dynamic range of this *hmr*Δ*A*Δ*E::TRP1* assay is quite different from the 1000-fold seen with the *hmr*Δ*A::TRP1 rap1[s]* assay system, and thus an apparent additive effect was observed with this assay system for the *sds3 sin3* double mutant.

CBK1 and *HYM1* appear to function in the same genetic pathway. In addition to reducing repression by LexA-Sin3, *cbk1* and *hym1* mutations both cause defects in cell separation and altered colony morphology. Similar effects on expression of *SIN3*-dependent genes are seen in both *cbk1* and *hym1* mutants. The effects of *cbk1* and *hym1* are specific to *SIN3*, as these mutations have no effect on repression by a LexA-Ssn6 fusion protein. Of the seven transcriptional effects analyzed, three (*INO1-LacZ*, *TRK2*, and telomeric silencing) were unaffected by these mutations, while *cbk1* and *hym1* mutations have weak effects on four (*CYC1-LexA-LacZ*, *STE6-LacZ*, *IME2-LacZ*, and *PHO5*) *SIN3*-dependent genes. Finally, *cbk1 hym1* double mutants show no additive effects, compared to single mutants, in any assay, suggesting that *CBK1* and *HYM1* function together.

The transcriptional effects of *cbk1* and *hym1* mutations can be separated genetically from *SIN3* and *RPD3.* These mutations cause extremely weak derepression at *IME2-LacZ* and *PHO5*, and the derepression at *PHO5* is affected by growth on solid *vs.* liquid medium. Importantly, the effect is additive when *cbk1* or *hym1* mutations are combined with *sin3* or *rpd3.* This result suggests that *CBK1* and *HYM1* are in a separate genetic pathway from *SIN3* and *RPD3*, in terms of regulation of *IME2-LacZ* and *PHO5.* The increased derepression observed at *PHO5*, which is not dependent upon growth conditions, is greater than the sum of the effects of the two mutations and may represent a synergistic effect. Thus, at *IME2:LacZ* and *PHO5*, *CBK1* and *HYM1* function through a mechanism that is independent of Sin3 and Rpd3, suggesting that *CBK1/HYM1* are in a different pathway from *SIN3/RPD3.*

However, double mutant analysis using *CYC1-LexA-LacZ* and *STE6-LacZ* indicates that at these two promoters *cbk1* and *hym1* have modest effects that are genetically inseparable from the effect of the Sin3/Rpd3 complex. At these two promoters, the function of *CBK1* and *HYM1* appears to be through the Sin3/Rpd3 complex, since the transcriptional effect in strains lacking both *CBK1* and *RPD3* is no more severe than in the *rpd3* single mutant. Thus, the function of *CBK1* and *HYM1* at these two promoters differs from that seen at *IME2:LacZ* and *PHO5.*

We conclude from our analysis that *cbk1* and *hym1* mutations have different effects at distinct promoters regulated by *SIN3.* Some promoters, such as *CYC1-LexA-LacZ* and *STE6-LacZ*, show modest effects in *cbk1* and *hym1* mutants and do not show additive effects when combined with *rpd3* mutations. At *IME2-LacZ* and *PHO5*, there are weak or conditional effects that are additive with *rpd3* or *sin3.* Given that Cbk1 shows strong homology to serine/threonine kinases, it seems likely that its function must be the phosphorylation of some substrate. Although *HYM1* has high homology (e^{-40} to e^{-72}) to genes in mouse, *Drosophila melanogaster*, *Caenorhabditis elegans*, *Arabidopsis thaliana*, and *A. nidulans*, the predicted amino acid sequence provides no clues as to function. Perhaps Hym1 is a subunit of the Cbk1 kinase complex, providing a substrate recognition function. The additive effects between mutations in *CBK1/HYM1* and *SIN3/RPD3*, at least at some promoters, suggest that Sin3 and Rpd3 are not the relevant substrates of the Cbk1 kinase. We propose that the Cbk1 kinase may phosphorylate a chromatin protein. Phosphorylation of such a protein could have different consequences at different promoters, consistent with the different effects of *cbk1* and *hym1* mutations at different genes.

We thank members of the Stillman Lab for many helpful suggestions, Janet Shaw for advice on UV mutagenesis, Brad Cairns for advice on immunoprecipitation, Jarmilla Janatova for providing antibodies, and Karen Freedman for making the *trk1::ADE2* strain. We thank Jef Boeke, Fred Cross, Rick Gaber, Ira Herskowitz, Yi Wei Jiang, Mark Longtine, John Lopes, David Shore, and Kevin Struhl for providing plasmids and strains. This work was supported by grants from the National Institutes of Health awarded to D.J.S.

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Communicating editor: M. Hampsey