Evidence That the Transcriptional Regulators SIN3 and RPD3, and a Novel Gene (SDS3) with Similar Functions, Are Involved in Transcriptional Silencing in S. cerevisiae

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ABSTRACT

In a screen for extragenic suppressors of a silencing defective rap1 HmrΔA strain, recessive mutations in 21 different genes were found that restored repression to HMR. We describe the characterization of three of these: SDS (suppressors of defective silencing) genes. SDS16 and SDS6 are known transcriptional modifiers, SIN3/RPD1/UME4/SD11/GAM2 and RPD3/SD12, respectively, while the third is a novel gene, SDS3. SDS3 shares the meiotic functions of SIN3 and RPD3 in that it represses IME2 in haploid cells and is necessary for sporulation in diploid cells. However, SDS3 mutations differ from sin3 and rpd3 mutations in that they do not derepress TRK2. These Sds mutations suppress a variety of Cis- and Trans-defects, which impair the establishment of silencing at HMR. Any one of the SDS mutations slightly increases telomere position effect while a striking synergistic increase in repression is observed in a rap1' background. Epistasis studies suggest that SDS3 works in a different pathway from RPD3 and SIN3 to affect silencing at HMR. Together these results show that defects in certain general transcriptional modifiers can have a pronounced influence on position-effect gene silencing in yeast. Mechanisms for this increase in position effect are discussed.

A LTHOUGH most studies of gene expression in eukaryotes have focused on the activation of tissue-specific genes, it is clear that both general and specific mechanisms of stable gene repression play a critical role in the development and maintenance of differentiated cell types. Heterochromatin, the densely staining regions of eukaryotic chromosomes that replicate late in S phase and contain few active genes, may provide a model to understand many specific forms of gene regulation. Heterochromatin can influence the expression of nearby genes resulting in their repression in subpopulations of cells in an organism, a phenomenon called position-effect variegation (PEV) (reviewed in Henikoff 1990). Recent studies indicate that PEV may be related to the inheritance of stable patterns of homeotic gene expression in Drosophila (Orlando and Paro 1995). These studies in Drosophila have raised intriguing parallels to other "epigenetic" regulatory phenomena observed in mammals, such as gene imprinting (Surani 1994) and X-chromosome inactivation (Riggs and Pfeiffer 1992). Such examples of heritable gene regulation are well documented but their underlying mechanisms are still obscure.

In the yeast Saccharomyces cerevisiae, position effects on gene expression occur at telomeres and the silent mating-type loci (HM) and may be analogous to the formation of heterochromatin in more complex eukaryotes. Repression of the silent mating-type loci is vital for the proper regulation of the yeast life cycle since it permits haploid cells to exhibit a unique mating phenotype (α or a) by expression from the MAT locus. Identical mating-type genes are present at the HM loci (usually a information at HMR and α information at HML), but they are transcriptionally silent and thus do not influence the cell type. The silent loci serve a positive function by acting as donors of information in a mating-type switching event that allows haploid cells to rapidly reach the diploid state (reviewed in Laurensen and Rine 1992). Chromatin at the HM loci and telomeres appears to be in an altered conformation, which results in transcriptional silencing, reduced DNA repair, and protection of the DNA from nucleases or other enzymes (Nasmyth 1982; Klar et al. 1984; Gottschling 1992; Singh and Klar 1992; Chen-Cleland et al. 1993; Loo and Rine 1994).

Cis elements on either side of HML and HMR, called silencers, are necessary for the transcriptional inactivation of these loci (Brand et al. 1985; Mahoney and Broach 1989). Silencers consist of an autonomously replicating sequence (ARS) consensus site (ACS), which is a binding site for the origin recognition complex (ORC) (Bell and Stillman 1992), and binding sites for either or both of two multifunctional regulatory proteins, Rap1p and Abf1p (Shore and Nasmyth 1987; Shore et al. 1987; Buchman et al. 1988). The telomere distal HMR-E silencer is comprised of binding sites for
the ORC, Raplp, and Abf1p, which are often referred to as the A, E, and B sites, respectively (Brand et al. 1987). Telomere-proximal to the a1 and a2 genes of HMR lies the HMR-I silencer, which is composed of an A site and a B site (Buchman et al. 1988). In the presence of this HMR-I silencer, the three sites of HMR-E are redundant as any two are sufficient for the establishment of silencing. The ORC, Raplp, and Abf1p have now all been shown to play a role in establishing silencing at either HMR or HML (Kurtz and Shore 1991; Susse and Shore 1991; Bell et al. 1993; Foss et al. 1993; Kyron et al. 1993; Micklem et al. 1993; Loo et al. 1995). Raplp1, which binds to multiple sites within the poly(C14-A) repeats at telomeres, is essential for telomere position effect and telomere length control (Conrad et al. 1990; Lustig et al. 1990; Kyron et al. 1992, 1993; Moretti et al. 1994). In addition to the silencer- and telomere-binding proteins, Sir2p, Sir3p, Sir4p (Rine and Herskowitz 1987) and the N termini of histones H3 and H4 (Kaye et al. 1988; Thompson et al. 1994) play an important or essential role in repression. Recent data suggests that in the proper context (e.g., at HMR or at telomeres) Raplp1 targets Sir3p and Sir4p to the chromosome through direct protein-protein interactions (Moretti et al. 1994). Sir3p and Sir4p are in turn capable of interacting with the N-terminal tails of histones H3 and H4, and this is believed to underlie the formation of repressed chromatin (heterochromatin) in yeast (Hecht et al. 1995).

Raplp1 is an essential gene, whose protein product appears to function as a transcriptional activator in most cases (Shore 1994). Raplp binding sites are found upstream of a number of glycolytic and ribosomal protein genes, where the protein is believed to play an important role in the activation of these genes. The essential function of Raplp1 can be genetically separated from at least one of its silencing functions as evidenced by raplp1 alleles, rapl-12 and rapl-13, show an increase in telomere length.

In order to identify factors that might be involved in Raplp-mediated silencing, extragenic suppressors were identified that restore silencing at HMR in raplp1 hmr2A strains (Laman et al. 1995; Susse et al. 1993, 1995). Fifty-seven extragenic mutations were identified that defined 21 different suppressor of defective silencing (SDS) complementation groups. Here we describe the cloning and further characterization of three of these SDS genes: SDS3, SDS6, and SDS16. Initial characterization of sds3, sds6 and sds16 mutants showed that, in addition to suppressing the raplp1 silencing defect, they were able to partially reverse the telomere lengthening phenotype of the stronger raplp1 alleles (Susse et al. 1995). We now show that null alleles of all three genes share the property of being able to bypass the normal requirements for HMR-E silencer function. In addition, we found that mutations in each of these three genes result in an increase in telomere position effect. SDS6 and SDS16 are identical to RPD3(SDI2) and SIN3(RPD1/UME4/SDI1/GAM2), respectively. Both of these genes encode global transcriptional modifiers that have been shown to affect the expression of a variety of genes such as HO (Nasmyth et al. 1987; Sternberg et al. 1987), TRK2 (Vidal and Gabel 1991; Vidal et al. 1991), and SP013 (Strich et al. 1989), ADR6/GAM3 (Yoshimoto et al. 1992) and IME2 (Bowdish and Mitchell 1993). Targeting experiments with a LexA-Sin3p fusion protein suggest that Sin3p functions as a transcriptional repressor (Wang and Stillman 1993), and a recent study has found Rpd3p to be 60% identical to a mammalian histone deactylase (Taunton et al. 1996). Epistasis analysis suggests that SIN3 and RPD3 work in the same pathway to affect the transcription of their target genes (Vidal and Gabel 1991; Stillman et al. 1994). SDS3 is a novel gene that shares many of the same properties as RPD3 and SIN3. However, sds3 does not appear to act in the same pathway as rpd3 and sin3 to restore silencing at HMR. Possible mechanisms for the action of suppression by these genes are discussed.

**MATERIALS AND METHODS**

**Yeast strains and methods**: Yeast strains used in this study are all isogenic to W303-1A or -1B (Thomas and Rothstein 1989). SDS and Raplp1 mutations were introduced either by the one-step gene replacement method (Rothstein 1983) or by genetic crosses using standard procedures (Rose et al. 1990). The HMR::TRP1, his3::TRP1 and HMR::ADE2 reporter genes have been described previously (Susse and Shore 1991; Susse et al. 1993). Yeast were grown in rich medium (YPD) or synthetic minimal media (SC) as described (Rose et al. 1990). Expression of the TRP1 gene fragment at the HMR and HIS3 loci was assayed by examining 10-fold serial dilutions of overnight cultures on SC and SC-Trp media as previously described (Susse and Shore 1991). Telomeric silencing was measured in strains in which the URA3 gene was targeted to the telomere-proximal ADH4 locus on chromosome VII-L using plasmid pVII-L URA3-TEL (provided by D. Gottschling) as previously described (Gottschling et al. 1990). Expression of this telomeric URA3 reporter was monitored by plating serial dilutions of overnight cultures grown in rich medium onto SC-Ura and SC+5-FOA plates. Sporulation assays were performed by growing diploids in 2% potassium acetate with supplementing amino acids and then visualizing cultures for the appearance of tetrads at the indicated times (24, 48 and 120 hr). A W303 a/a diploid was used as a positive control for this assay.

**Cloning of SDS genes**: The SDS genes were cloned by screening a centromere-based (YCp50) yeast genomic library (Rose et al. 1987) for plasmids that were able to complement the recessive sds3-1, sds6-2 or sds16-1 alleles (as described in Susse et al. 1993). Six clones (representing four overlapping genomic fragments) were isolated, which complemented the sds3-1 allele. Further subcloning localized the sds3-1 complementing region to a 2.3-kb EcoRI-SalI genomic fragment. Two identical sds6-2 complementing plasmids were isolated. This plasmid was also able to complement the sds6-1, -6-3 and -6-
4 alleles (data not shown). Subcloning and sequence analysis revealed this genomic fragment to contain the RPD3 gene. Twelve plasmids containing overlapping regions were isolated by their ability to complement the sds16-1 allele. Sequence analysis of one of these clones showed that the common region contained the SIN3 gene.

**SDS linkage analysis:** To confirm that the 2.3-kb genomic fragment that complemented the sds3-1 mutation contained the SDS3 gene, we performed a genetic linkage analysis. The 2.5-kb fragment was cloned into a URA3-marked integrating vector pRS306 (Sikorski and Hieter 1989). This plasmid (DV34) was linearized with BglII, a unique site at nucleotide 1248 of the insert, and targeted to the chromosome via homologous recombination in a rap1-12 hmrΔA::TRP1 MATa strain (YLS288). The proper integration was confirmed by Southern blotting. The resulting strain, YDV21 (Ura+, Trp+), was crossed to a sds3-1 rap1-12 hmrΔA::TRP1 strain, YRS58 (Ura+, Trp+), and the diploid was sporulated. All 19 tetrads examined were of the parental ditype, i.e., there were no Ura+ segregants in which the hmrΔA::TRP1 reporter was silenced (Trp+ phenotype). This result shows that the cloned DNA is tightly linked to the SDS3 locus. A similar approach was used to show that DNA that complemented the sds6-2 mutation is tightly linked to the SDS6 (RPD3) locus. In this case, the cloned DNA was marked with the HIS3 gene and targeted to the chromosome by homologous recombination. No recombination events were observed between HIS3 and sds6-2 in the 28 tetrads examined. Similarly, the HIS3 gene was used to tag the cloned DNA that complemented the sds16-1 mutation. In the manner described above, subsequent tetrad analysis showed this DNA to be tightly linked to the SDS16 (SIN3) locus (no recombinants in over 20 tetrads analyzed).

**SDS3 sequence analysis:** The complete SDS3 nucleotide and Sds3p peptide sequences (GenBank accession no. U62255) were used to search National Institutes of Health, European Molecular Biology Laboratory, and SwissProt sequence databases using the Blastn and Blastp programs (Altschul et al. 1990). This search matched Sds3p to a hypothetical 37.6-kD protein in the SCA1-THS1 intergenic region of chromosome IX, accession no. sp[40505], and the SDS3 sequence to an unidentified open reading frame (ORF) YII.084C (19910.12). Comparison of the SDS3 sequence with known sequences failed to identify any functional motifs or homologies with any other known proteins.

**Sds3 gene disruptions:** The SDS3 gene was mutated by inserting a BamHI fragment containing the HIS3 gene into a unique BglII site at position 218 of the predicted 933 bp SDS3 ORF, creating plasmid DV66. The resulting sds3::HIS3 allele was removed as a EcoRI-SalI fragment from DV66 and used to replace the endogenous SDS3 locus in a rap1-12::rap1-13 hmrΔA::TRP1/MATα strain (YLS288). The proper integration was confirmed by Southern blotting. The resulting strain, YDV21 (Ura+, Trp+), was crossed to a sds3-1 rap1-12 hmrΔA::TRP1 strain, YRS58 (Ura+, Trp+), and the diploid was sporulated. All 19 tetrads examined were of the parental ditype, i.e., there were no Ura+ segregants in which the hmrΔA::TRP1 reporter was silenced (Trp+ phenotype). This result shows that the cloned DNA is tightly linked to the SDS3 locus. A similar approach was used to show that DNA that complemented the sds6-2 mutation is tightly linked to the SDS6 (RPD3) locus. In this case, the cloned DNA was marked with the HIS3 gene and targeted to the chromosome by homologous recombination. No recombination events were observed between HIS3 and sds6-2 in the 28 tetrads examined. Similarly, the HIS3 gene was used to tag the cloned DNA that complemented the sds16-1 mutation. In the manner described above, subsequent tetrad analysis showed this DNA to be tightly linked to the SDS16 (SIN3) locus (no recombinants in over 20 tetrads analyzed).

**RESULTS**

**SDS3 is a novel gene:** The SDS3 gene was cloned by complementation of the recessive sds3-1 allele. We isolated a 2.3-kb genomic fragment that complemented the sds3-1 mutation and was tightly linked to the SDS3 locus (see MATERIALS AND METHODS). Sequence analysis of the 2.3-kb genomic fragment identified a 933-bp open reading frame (ORF) encoding a protein with a predicted molecular weight of 37.6 kD (GenBank accession no. U62255). A search of the Swiss-Prot database using the Blastp program showed Sds3p to be identical to an unknown ORF, YII.084C (accession no. P40505) (Altschul et al. 1990). In order to produce an SDS3 allele suitable for genetic manipulation, the HIS3 gene was inserted into a unique BglII site at nucleotide +218 of the predicted ORF. This sds3::HIS3 plasmid (DV66) was unable to complement the sds3-1 mutation as determined in a rap1-12 hmrΔA::TRP1 background, providing further evidence that the cloned gene is SDS3 (data not shown).

The sds3::HIS3 allele was used to replace the endogenous SDS3 locus and the resulting sds3::HIS3 rap1-12 hmrΔA::TRP1 strain was viable and unable to grow on medium lacking tryptophan (Figure 1), indicating that the sds3::HIS3 mutation suppresses the silencing defect in this strain. Northern analysis of an isogenic strain containing the endogenous a1 gene at HMR confirmed that this suppression occurs at the transcriptional level.
(SUSSEL et al. 1995). In subsequent experiments, the sds3::HIS3 disruption was used instead of the sds3-1 mutation. Additionally, a sds3 deletion allele was made by replacing sequences from −29 to +781 with the URA3 gene, deleting all but the last 199 bp of the SDS3 ORF. The sds3Δ::URA3 allele completely suppressed the silencing defect to a rap1′ hmrAΔ::TRP1 strain, as do the sds3::HIS3 and sds3-1 alleles (data not shown).

SDS6 and SDS16 are known transcriptional modifiers, RPD3 and SIN3, respectively: The genes encoding SDS6 and SDS16 were isolated from a yeast genomic library by their ability to complement the sds6-2 and sds16-1 mutations, respectively. Partial sequence analysis of the sds16-1 complementing clone showed that it contains the SIN3 gene, which encodes a transcriptional regulator (WANG et al. 1990). Consistent with the notion that sds16-1 is an allele of SIN3, segregation analysis confirmed that the two are tightly linked (see MATERIALS AND METHODS). Furthermore, a sin3Δ::LEU2 disruption also has the ability to suppress the rap1′ hmrAΔ::TRP1 mutation (Figure 1). Restriction mapping and DNA sequence analysis showed the sds6-2 complementing clone to contain RPD3, a transcriptional modifier that is reported to function together with SIN3 (RPD1) (VIDAL and GABER 1991; STILLMAN et al. 1994). The RPD3 locus and sds6-2 are tightly linked (see MATERIALS AND METHODS), and a genomic deletion of RPD3, rap3Δ::HIS3, showed the same rap1′ hmrAΔ::TRP1 suppression phenotype as the original sds6-2 mutation (Figure 1). In subsequent experiments, the sin3Δ::LEU2 and rap3Δ::HIS3 alleles were used in place of the sds6-1 and sds6-2 mutations, respectively, to facilitate genetic manipulations. The rap3Δ::HIS3 and sin3Δ::LEU alleles were found to suppress the rap1′ hmrA silencing defect as measured by expression of several different reporters (ADE2, TRP1 and the endogenous a1 gene) at hmrA. Northern blot analysis of steady state a1 mRNA levels in rap3Δ::HIS3 rap1′ hmrAΔ MATαu and sin3Δ::LEU rap1′ hmrAΔ MATαu strains showed that this suppression was at the level of transcription (SUSSEL et al. 1995).

**Suppression of cis- and trans-silencing mutations:** Initial experiments with sds3::HIS3, rap3Δ::HIS3, and sin3-Δ::LEU2 showed that they are able to suppress both the hmrA defect and the rap1′ silencing defect to restore repression in a rap1′ hmrAΔ strain (data not shown). As this suggests a general role for these sds mutants in modulating silencing, we examined their ability to suppress a variety of other silencing defects. Disruption of the RIF1 gene, encoding a Rap1p-interacting factor, leads to derepression of an hmrA silencer (HARDY et al. 1992). Introduction of sds3::HIS3, rap3Δ::HIS3, or sin3Δ::LEU2 was able to suppress a RIF1 deletion and restore silencing to a rif1Δ hmrAΔ::TRP1 strain as assayed by growth on SC-Trp plates (Figure 2A). In addition, each of the sds mutants was able to suppress the silencing defect of a rap1′ rif1Δ double mutant in a hmrAΔ::TRP1 strain (data not shown).

We also tested the ability of each of the sds mutations to restore silencing in sir1 and sir4 strains. Previous studies have implicated Sir1p in the establishment of silencing at the HML loci and Sir2p, Sir3p and Sir4p with the maintenance of the repressed state (PILLUS and RINE 1989). Any one of the sds mutations partially restored silencing to a sir1Δ::LEU2 hmrAΔ::TRP1 strain as indicated by a reduced ability of sds sir1Δ::LEU2 hmrAΔ::TRP1 strains to grow on SC-Trp medium (Figure 2B). A 102- to 105-fold decrease in colony formation on SC-Trp was seen when these strains contained a rap1′ allele (data not shown). However, in a sir4Δ::LEU2 hmrAΔ::TRP1 strain the sds mutations did not affect growth on SC-Trp medium, indicating that they were unable to suppress the silencing defect of a SIR4 deletion (Figure 2B). Addition of the rap1′ allele had no effect on the tryptophan prototrophy of these strains (data not shown).

We previously found that two of the three sds mutations, sds6-2 and sds16-1, were able to suppress HMR-E silencer double-mutations (hmrAΔΔ::TRP1 and hmrAΔΔE::ADE2) (SUSSEL et al. 1995). Although the sds3-1 allele (the first SDS3 allele examined) did not...
have this phenotype, we observed that the sds3::HIS3 mutation was able to suppress the hmrΔEΔB::TRPI reporter (Figure 2C). Furthermore, we found that introduction of sin3A::LEU2, rpd3A::HIS3 or, to a lesser extent, sds3::HIS3 partially suppressed a rapI-12 strain with the hmrΔEΔB::TRPI reporter (Figure 2C). However, the sds insertion/deletion mutations were unable to affect an hmrΔEΔA::TRPI reporter as determined by monitoring growth of 10-fold serial dilutions on SC and SC-Trp plates (data not shown). This discrepancy between the TRPI and ADE2 reporters is probably due to the fact that the ADE2 colony color assay readily detects a small fraction of cells in the repressed state whereas the TRPI growth assay does not (SusSs et al. 1993). The presence of the rapI-12 mutation in these sds hmrΔEΔA::TRPI strains had no effect on growth on SC-Trp plates. These results suggest that the sds mutations are able to suppress defects in the establishment of silencing but not defects in factors required for maintenance of the repressed state.

Mutations in SDS3, RPD3, and SIN3 restore a position effect: One possible explanation for the ability of the sds3, rpd3, and sin3 mutants to suppress a variety of silencing defects is that they merely reduce the transcriptional activation of the reporter genes we have examined, rather than restoring silencing at HMR. Although none of the mutations restore silencing at hmr in a sir4 strain (see above), we tested this model more directly by asking whether suppression is position-dependent. We moved the TRPI reporter from hmr to a new site not associated with transcriptional silencing, the HIS3 locus, and tested the ability of the sds mutations to affect its expression at this locus. None of the three sds mutations had any effect on the ability of these strains to grow on medium lacking tryptophan (Figure 3A). Thus it appears that the ability of the sds mutations
to decrease transcription is dependent on the context of the reporter, and in this case, to residual silencer function at HMR in the suppressible mutant strains.

As a second control, we examined steady-state mRNA levels of the endogenous al gene in both SDS and sds cells, comparing strains in which the only copy of al is either at HMR (hmrΔA MATα) or at the active MAT locus (MATα hmrΔA::TRPl). We observed no significant decreases in the levels of al mRNA from the MAT locus (Figure 3B). As observed previously (Susse et al. 1995), each of the sds mutations resulted in a complete loss of detectable al mRNA from the HMR locus in a rap1Δ hmrΔA background. Again it appears that the ability of the sds mutations to suppress reporter expression is context dependent, since only genes at HMR become transcriptionally silent.

Comparison of sds3 and rpd3/sin3 phenotypes: In the assays described above, the sds3, rpd3, and sin3 mutants have very similar phenotypes. Because SDS3 is a novel gene, we examined other phenotypes characteristic of rpd3 and sin3 mutations to determine whether SDS3 shares other properties of these two global regulatory genes. Diploid cells with homozygous null mutations of Sin3 or Rpd3 are unable to undergo sporulation (Vidal and Gaber 1991; Vidal et al. 1991). We constructed diploid strains homozygous for the sds3::HIS3 disruption and assayed them for their ability to sporulate. After 5 days under starvation conditions, <0.5% of the homozygous sds3::HIS3 diploids examined formed tetrads compared with 73.9% for wild-type W303 diploids (Table 1).

SIN3 and RPD3 act as repressors of certain meiosis-specific genes, such as IME2 (Bowdish and Mitchell 1993). To test the effect of SDS3 on IME2 expression, we used a CYC1-lacZ reporter gene containing the IME2 upstream regulatory region in place of the CYC1 UAS element (pKB1001). When this reporter is transformed into haploid cells, little or no transcriptional activity is seen in vegetatively growing cells. The addition of a sin3 or an rpd3 mutation to this background alleviates the repression of the IME2-upstream region and lacZ expression is seen (Table 1), as expected (Bowdish and Mitchell 1993). We found that addition of the sds3::HIS3 disruption also alleviates repression of the IME2 UAS and yields β-galactosidase values comparable to those seen with either the sin3 or rpd3 mutations.

The rpd3 and sin3/rpd3 mutations also lead to the upregulation (i.e., derepression) of a gene encoding a low-affinity K+ pump, TRK2, thereby allowing strains lacking the high-affinity K+ pump Trk1p to grow on media with low levels of potassium (Vidal and Gaber 1991; Vidal et al. 1991). To determine if sds3 might share this same phenotype, we used a TRK2-lacZ fusion to assay for levels of TRK2 expression. Both the rpd3 and sin3 mutations cause an increase in expression of the TRK2-lacZ reporter, as expected (Table 1). However, sds3 mutation is unable to derepress the TRK2-lacZ reporter as the level of lacZ expression is similar to that seen in wild-type cells. Thus sds3 appears to share some, but not all, of the phenotypes seen with rpd3 and sin3 mutations.

SDS3 acts in a different pathway from SIN3/RPD3 to affect silencing: Earlier studies have indicated that RPD3 and SIN3 work together, either in the same pathway or as a complex (Vidal and Gaber 1991; Stillman et al. 1994). To determine if SDS3 might also be in this SIN3/RPD3 group, we constructed strains with an hmrΔA::TRPl reporter and combinations of the sds3::HIS3, rpd3Δ::HIS3 and sin3Δ::LEU2 alleles. In order to get a...
more accurate measure of repression, we counted colonies on SC and SC-Trp plates. Due to the sensitive nature of this assay, we were able to see that any one of the sds mutations suppressed the hmrΔAAΔE silencing defect by 30–38% (Table 2). As expected for genes acting in the same pathway, sin3Δ::LEU2 rpd3Δ::HIS3 and sds3::HIS3 sin3Δ::LEU2 double mutants showed a comparable extent of repression as strains with either single mutation (a 40% decrease in Trp+ colonies). However, both sds3::HIS3 rpd3Δ::HIS3 and sds3::HIS3 sin3Δ::LEU2 double mutants showed a reduction of >60% in the number of Trp+ colonies. A strain containing all three mutations showed approximately the same level of TRPI repression, 66%, as the sds3::HIS3 rpd3Δ::HIS3 and sds3::HIS3 sin3Δ::LEU2 double mutant strains (67 and 64%, respectively). This additive increase in suppression implies that SDS3 works in a different pathway than SIN3 and RPD3 to affect silencing at the HMR locus.

**Effects on telomeric silencing:** Transcriptional silencing similar to that seen at the HM loci is also observed for genes placed near telomeres in yeast (Aparicio et al. 1991; Gottschling et al. 1990). We therefore examined the effect of sds mutations on telomeric silencing (telomere position effect, or TPE) using a strain in which the URA3 gene is placed immediately adjacent to a telomere created at the ADH1 locus (Gottschling et al. 1990). The expression of this telomeric URA3 reporter is influenced by TPE and results in two distinct populations of cells: Ura+, in which the URA3 gene is expressed and Ura−, in which the URA3 gene is repressed. Ura+ cells are able to grow on SC-Ura plates and Ura− cells are able to grow on SC plates containing 5-fluoroorotic acid (5-FOA). Strains that have this telomere reporter and any one of the three sds mutations showed a slight decrease in Ura− colonies compared with the parental wild-type strain (Figure 4). Growth was seen on all three types of plates (SC, SC-Ura, and SC+5-FOA), indicating that there were separate populations of repressed and derepressed cells within these cultures. Previous studies which quantified viability on SC vs. SC+5-FOA media have shown that the rap1 allele rap1-12 also slightly increases telomeric silencing of URA3 (Buck and Shore 1995), though this effect is too small to be seen with 10-fold serial dilutions on either SC+5-FOA media or SC-Ura plates. However, when any one of the sds mutations was included in this rap1-12 background, the state of repression of URA3 at the telomere was greatly increased as evidenced by a lack of Ura− colonies (Figure 4).

**TABLE 1**

<table>
<thead>
<tr>
<th>SDS allele</th>
<th>Sporulation (%)</th>
<th>IME2-lacZ units</th>
<th>TRK2-lacZ units</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>73.9</td>
<td>5.5</td>
<td>26.7</td>
</tr>
<tr>
<td>sds3::HIS3</td>
<td>&lt;0.5</td>
<td>79</td>
<td>14.0</td>
</tr>
<tr>
<td>rpd3Δ::HIS3</td>
<td>&lt;0.5</td>
<td>91</td>
<td>137.4</td>
</tr>
<tr>
<td>sin3Δ::LEU2</td>
<td>&lt;0.5</td>
<td>51</td>
<td>122.9</td>
</tr>
</tbody>
</table>

*The ability to sporulate was visually measured in homozygous diploid strains after 5 days in starvation media. The numbers represent the percent of tetrads found upon examination of >2000 diploids for each strain.

<table>
<thead>
<tr>
<th>SDS allele</th>
<th>hmrΔAAΔE::TRPI1 expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>sds3</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>rpd3</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>sin3</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>rpd3 sin3</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>sds3 rpd3</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>sds3 sin3</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>sds3 rpd3 sin3</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

The values above represent the ability of various combinations of sds mutants to suppress the silencing defective hmrΔAAΔE::TRPI1 reporter. Percentages were determined by plating dilutions of identical cultures and then dividing the number of colonies on SC-Trp plates by the number seen on SC plates. sds3, rpd3 and sin3 represent the sds3::HIS3, rpd3Δ::HIS3, and sin3Δ::LEU2 alleles, respectively.

**TABLE 2**

<table>
<thead>
<tr>
<th>SDS8 alleles</th>
<th>hmrΔAAΔE::TRPI1 expression (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>wt</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>sds3</td>
<td>70 ± 2</td>
</tr>
<tr>
<td>rpd3</td>
<td>62 ± 5</td>
</tr>
<tr>
<td>sin3</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>rpd3 sin3</td>
<td>60 ± 10</td>
</tr>
<tr>
<td>sds3 rpd3</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>sds3 sin3</td>
<td>36 ± 3</td>
</tr>
<tr>
<td>sds3 rpd3 sin3</td>
<td>34 ± 6</td>
</tr>
</tbody>
</table>

**Effect of sds mutations on Raplp-mediated activation:** One possible mechanism for the suppression of the rap1s silencing defect by the sds mutations is that they impair the ability of Rap1p to activate transcription such that its silencing function is favored. To examine this possibility, we examined the effects of the sds mutations on the expression of three native Rap1p-driven genes (MATα1, PYK1, and BCY1) at their endogenous loci (Nishizawa et al. 1989; Giesman et al. 1991; Kurtz and Shore 1991). Using Northern blot analysis and
quantitating mRNA signals on a PhosphorImager, we failed to detect any significant changes in the level of MATα1, PYK1, or BCY1 mRNA in the presence of any of the sds mutations (data not shown). Addition of the rap1-12 allele had no effect on the expression of the above genes in any of these strains. These experiments suggest that the sds mutations do not specifically impair the ability of Rap1p to activate transcription of its normal target genes.

**Silencing factor dosage and sds mutations:** Another possible mechanism for the suppression seen with these sds mutations is that they are upregulating the expression or activity of silencing factors and thus enhancing repression (see Laman et al. 1995; Susse1 et al. 1993) showed that reduction of SIR4 dosage by one half was able to derepress a hmrΔA::ADE2 reporter. An hmrΔA::ADE2 strain has pink/white sectored colonies. In diploid cells with only a single wild-type copy of SIR4 (SIR4/sir4Δ:: LEU2), this reporter is completely derepressed and the cells are uniformly white. The addition of homozygous rpd3Δ::HIS3 or sin3Δ::LEU2 mutations to this diploid strain results in cells which are white with pink sectors (Table 3). However, cells with an sds3::HIS3/sds3HIS3 SIR4/sir4Δ::LEU2 hmrΔA::ADE2/hmrΔA::ADE2 genotype are mostly white with relatively few white/pink sectored cells. This indicates that the rpd3 and sin3 mutations are more efficient at suppressing a 0.5× reduction in SIR4 dosage than the sds3::HIS3 allele. These experiments suggest that the sds alleles, especially sin3 and rpd3, could be upregulating the expression or activity of silencing factor(s) (at least SIR4), which might account for their ability to suppress a number of defects in the establishment of transcriptional silencing. These results also agree with our epistasis experiments in suggesting that Sds3p is working in a different manner to influence transcriptional silencing than Rpd3p and Sin3p.

**DISCUSSION**

We have described the cloning and characterization of three genes identified in a screen for mutations which restore silencing to a rap1Δ hmrΔA strain. Two of the genes identified, RPD3 and SIN3, have been previously described as modulators of transcription that appear to work together to allow maximal levels of transcriptional activation or repression (Vidal and Gaber 1991; Stillman et al. 1994). The third gene, SDS3, encodes a novel 327 amino acid protein, mutations in which cause some of the same phenotypes as sin3 and rpd3 mutants. Results in this paper suggest that SDS3 also acts to modulate levels of transcription. However, SDS3 differs from RPD3 and SIN3 in that it does not appear to be involved in repression of TRK2. Furthermore, epistasis studies suggest that RPD3 and SIN3 work in the same pathway to affect silencing while SDS3 does not. Disruptions of any one of these global transcriptional modulators are able to increase position effect at the silent mating locus HMR and at a telomere.

**Mutations in SDS3, RPD3, and SIN3 improve the establishment of silencing:** Although originally isolated in a screen to find suppressors of a rap1Δ silencing de-

**TABLE 3**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Colony color</th>
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</thead>
<tbody>
<tr>
<td>wt</td>
<td>pink/white sectored</td>
</tr>
<tr>
<td>0.5× SIR4</td>
<td>white</td>
</tr>
<tr>
<td>0.5× SIR4 sds3</td>
<td>white and few white/light pink sectored</td>
</tr>
<tr>
<td>0.5× SIR4 rpd3</td>
<td>white/pink sectored</td>
</tr>
<tr>
<td>0.5× SIR4 sin3</td>
<td>white/pink sectored</td>
</tr>
</tbody>
</table>

The sds mutations suppress a 0.5× reduction in SIR4 dosage. All strains are diploid hmrΔA::ADE2 cells with the indicated SIR4 and SDS alleles. 0.5× SIR4 denotes heterozygous alleles of SIR4 (SIR4/sir4Δ::LEU2), while sds3, rpd3 and sin3 represent homozygous alleles of sds3::HIS3, rpd3Δ::HIS3, or sin3Δ::LEU2, respectively. Pink sectors represent populations with derepressed hmrΔA::ADE2 loci, while white colonies or sectors are populations with repressed hmrΔA::ADE2 loci. All strains were plated onto YPD, grown 3 days at 30°C, stored for at least 3 days at 4°C and then visually examined for colony color.
fect, none of the sds mutations described here show specificity for any one of the rap1' alleles. Instead these suppressor mutations appear to act in a general manner to improve the establishment of silencing. To begin with, these mutations can restore silencing in strains with various cis-acting mutations at HMR-E, such as hmrΔA and hmrΔEΔB, and partially at hmrΔAΔE. The sds mutations are also able to suppress a deletion of the HMR-I silencer but are unable to reverse the effect of mutations in both the E and I silencers, such as a deletion of the Rap1p binding site at HMR-E combined with a deletion of the HMR-I silencer (data not shown). This suggests that some minimal cis elements are needed in order for these three sds mutations to enhance the establishment of silencing. Consistent with this idea, the ability of the sds mutations to reduce transcription appears to be position dependent, since they reduce the expression of the a1 and TRP1 genes only when they are located at HMR and not at other chromosomal loci where silencing does not normally occur. Furthermore, these sds mutations suppress defects in trans-acting factors thought to be specifically involved in establishment of repression at HMR (Sir1p and Rap1p) (PILLUS and RINE 1989; CHIEN et al. 1993; SUSSEL et al. 1995). However, they fail to suppress a deletion of SIR4, which is believed to be a structural component required to maintain repression at HM loci and telomeres (HECHT et al. 1995). These results suggest that the sds mutations improve the establishment of silencing at HMR and telomeres (and hence the stability of the repressed state) but do not bypass the requirement for silencer elements or proteins (such as Sir4p) that are needed for maintenance of the repressed state.

A general role for SDS3 in transcriptional regulation: Previous studies suggest that RPD3 and SIN3 are required for the proper range of transcriptional regulation because mutations in these genes either reduce activated levels of expression or increase (i.e., derepress) basal expression of a number of different genes. Using the well-characterized RPD3 and SIN3 genes as a paradigm, we have shown that SDS3 shares many, but not all of their regulatory functions. SIN3(SDIl) and RPD3(SDl2) were first identified in a screen for mutants that would allow expression of the HO gene in a sin5 background (NASMYTH et al. 1987; STERNBERG et al. 1987), a phenotype shared with the sds3::HIS3 mutation (D. STILLMAN, personal communication). In addition, sds3 mutants exhibit two meiosis-related phenotypes of rap3 and sin3 mutants: failure to sporulate as homzygous diploids and derepression of the meiosis-specific regulator IME2 in vegetatively growing haploid cells. However, SDS3 does not share all of the RPD3/SIN3 phenotypes since an sds3 mutation is unable to relieve the repression of a TRK2-lacZ reporter in TRK1' cells. We also found that the sds3::HIS3 allele decreases the expression of activated lacZ reporter genes, TEF2-lacZ (a Rap1p-driven reporter) and CYCI-lacZ, but does not affect a CYCl-lacZ reporter lacking its UAS element (D. VANNIER and D. SHORE, unpublished data). Taken together, these results suggest that SDS3 encodes a factor necessary for either positive or negative expression of certain genes, and that its function is similar, but not identical to that of RPD3 and SIN3. Further studies are currently underway to elucidate the mode of action of Sds3p.

Possible mechanisms for suppression by SDS3, RPD3, and SIN3 mutants: Several studies have demonstrated an inverse correlation between promoter strength and susceptibility to silencing. APARICIO and GOTTSCALING (1994) have shown that mutation of the URA3 activator Ppr1p greatly enhances silencing of a telomere proximal URA3 reporter, whereas overexpression of PPR1 weakens silencing. Gal11p and Sin4p have been identified as components of the RNA polymerase II holoenzyme complex, which is required for activated transcription in vitro (Kim et al. 1994; Li et al. 1995). A deletion of GAL11 suppresses the rap1s hmrΔA silencing defect (SUSSEL et al. 1995), while a deletion of SIN4 partially suppresses the rap1' hmrΔA defect (data not shown). One possibility is that the sds mutations described here are decreasing the efficiency of activated transcription and thus favoring the silenced state at HMR and telomeres. Given that Sin3p are Rpd3p do not appear to be associated with the transcriptional activation machinery, their effect is likely to be indirect. Studies are currently underway to assess Sds3p's role in transcriptional activation. It should be emphasized that the effects of the sds mutations on activated transcription may be very subtle and thus only detectable at loci that are subjected to silencing. This may also explain why others had not previously identified SDS3 in any of the genetic screens which resulted in alleles of SIN3 and RPD3.

Another mechanism for the sds suppression relates to the observation that rap1' mutations are suppressed by increased gene dosage of either SIR1 or SIR4 (SUSSEL and SHORE 1991). Under certain circumstances, the HMR locus has been found to be very sensitive to the concentration of Sir4p. A twofold increase in SIR4 dosage can restore silencing in defective hmr strains (hmrΔEΔB for example), while a twofold reduction in Sir4p concentration can result in total derepression of hmrΔA (SUSSEL et al. 1995). Given the fact that mutations in SDS3, RPD3, or SIN3 can increase the expression of some genes, it is possible that they improve silencing by increasing the expression or activity of SIR genes or other genes that influence silencing. As these mutants suppress a SIR1 deletion, it is unlikely that they act (only) by increasing SIR1 expression. SUSSEL et al. (1995) failed to see any increases in SIR4 mRNA levels due to the sds mutations. However, experiments presented here show that rap3 and sin3 mutations strongly suppress a 0.5x reduction in SIR4 dosage, while sds3 does so to a much lesser extent. This suggests that the sds mutants, especially rap3 and sin3, could in principle work by increasing the expression or activity of SIR4 or, perhaps, other factors involved in the
establishment of silencing. Since Sin3p has been shown to be a transcriptional repressor (Wang and Stillman 1993), this raises the possibility that a SIN3 deletion increases the expression of genes which promote silencing. Finally, it should be noted that all three of the sds mutants described here suppress the telomere lengthening phenotype of rap1+ mutants, which could itself lead to an effective increase of Sir protein concentrations at HMR (Buck and Shore 1995). However, this mechanism would not explain the effects of sds mutants in RAP1+ strains, where no telomere length changes are seen, nor would it explain the dramatic increase in TPE in rap1+ sds double mutants.

A recent study has assigned a putative function to Rpd3p. Taunton et al. (1996) identified a mammalian histone deacetylase (HD1) that has 60% sequence identity to Rpd3p. This strongly suggests that Rpd3p is also a deacetylase, although this has yet to be shown directly. Transcriptionally active regions are associated with hyperacetylated nucleosomes while transcriptionally silent loci are associated with histone hypoacetylation (Hessels et al. 1988; Braunstein et al. 1993). Histone H4 isolated from regions of silenced chromatin was found to be preferentially acetylated at the position 12 lysine and unacetylated at other lysine residues (Braunstein et al. 1996). The authors in this study went on to suggest that a specific pattern of histone acetylation is required for efficient transcriptional silencing. This raises the possibility that Rpd3p is directly involved in the deacetylation of histone H4 at position 12 and that by deleting RPD3 we are increasing the availability of "silencing-competent" histone H4.

In conclusion, we have shown that mutations in two previously characterized transcriptional modifiers, SIN3 and RPD3, are able to increase position-effect repression at HMR and at a telomere. In addition, we have identified a novel gene, SDS3, with similar functions. Mutations in any one of these three genes have rather subtle effects, either slightly decreasing levels of activated transcription or relieving repression of other genes. Given the epistasis analysis results and the 0.5 x SIR4 suppression data it is likely that Sds3p acts in a different manner than Rpd3p and Sin3p to influence transcriptional silencing. Various mechanisms have been suggested above. Nonetheless, our results show that these mutations can have relatively dramatic effects on the expression of genes that are subjected to transcriptional silencing. These results highlight the delicate balance between transcriptional activation and silencing which exists at the silent HM mating-type loci and telomeres in yeast.

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LITERATURE CITED


Position-Effect Repression in Yeast


