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

David Vannier,* Dina Balderes* and David Shore*,†

**Department of Microbiology, College of Physicians and Surgeons of Columbia University, New York, New York 10032 and* †*Département de Biologie Moléculaire, Université de Genève, CH-1211 Genève 4, Switzerland*

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ABSTRACT

In a screen for extragenic suppressors of a silencing defective *rap1^s 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/SDI1/GAM2)* and *RPD3(SDI2)*, 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^s* 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 postion effect are discussed.

ALTHOUGH 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 sub populations 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 (**a** or α) 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 LAURENSON 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

Corresponding author: David Shore, Département de Biologie Moléculaire, Université de Genève, CH-1211 Genève 4, Switzerland.
E-mail: david.shore@molbio.unige.ch

the ORC, Rap1p, 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, Rap1p, and Abf1p have now all been shown to play a role in establishing silencing at either *HMR* or *HML* (KURTZ and SHORE 1991; SUSSEL and SHORE 1991; BELL *et al.* 1993; FOSS *et al.* 1993; KYRION *et al.* 1993; MICKLEM *et al.* 1993; LOO *et al.* 1995). Rap1p, which binds to multiple sites within the poly($C_{1-3}A$) repeats at telomeres, is essential for telomere position effect and telomere length control (CONRAD *et al.* 1990; LUSTIG *et al.* 1990; KYRION *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 (KAYNE *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) Rap1p 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).

RAP1 is an essential gene, whose protein product appears to function as a transcriptional activator in most cases (SHORE 1994). Rap1p 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 Rap1p can be genetically separated from at least one of its silencing functions as evidenced by *rap1^s* mutations that are defective in silencing at *HMR* (when the A site at the *HMR-E* silencer is deleted) yet have no detectable activation or growth defects (SUSSEL and SHORE 1991). In addition, two of the strongest *rap1^s* alleles, *rap1-12* and *rap1-13*, show an increase in telomere length.

In order to identify factors that might be involved in Rap1p-mediated silencing, extragenic suppressors were identified that restore silencing at *HMR* in *rap1^s hmr*ΔA strains (LAMAN *et al.* 1995; SUSSEL *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 *rap1^s* silencing defect, they were able to partially reverse the telomere lengthening phenotype of the stronger *rap1^s* alleles (SUSSEL *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 modulators 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 GABER 1991; VIDAL *et al.* 1991), *SPO13* (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 GABER 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 *RAP1* 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 (SUSSEL and SHORE 1991; SUSSEL *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 (SUSSEL 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, SC-Ura and SC+5-FOA plates. Sporulation assays were performed by growing diploids in 2% potassium acetate with supplementing amino acids and then visually monitoring cultures for the appearance of tetrads at the indicated times (24, 48 and 120 hr). A W303 **a**/α 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 SUSSEL *et al.* 1995). 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 *Eco*RI-*Sal*I 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 2.3-kb fragment was cloned into a *URA3*-marked integrating vector pRS306 (SIKORSKI and HIETER 1989). This plasmid (DV34) was linearized with *Bgl*II, a unique site at nucleotide 1248 of the insert, and targeted to the chromosome via homologous recombination in a *rap1-12 hmrΔA::TRP1 MAT***a** strain (YLS238). 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, YRS38 (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. U62525) were used to search National Institutes of Health, European Molecular Biology Laboratory, and SwissProt sequence databases using the Blastn and Blastp programs (ALTSHUL *et al.* 1990). This search matched Sds3p to a hypothetical 37.6-kD protein in the *SGA1-THS1* intergenic region of chromosome *IX*, accession no. sp[p40505], and the *SDS3* sequence to an unidentified open reading frame (ORF) YIL084C(I9910.12). Comparison of the *SDS3* sequence with known sequences failed to identify any functional motifs or homologies with any other known proteins.

SDS gene disruptions: The *SDS3* gene was mutated by inserting a *Bam*HI fragment containing the *HIS3* gene into a unique *Bgl*II site at position 218 of the predicted 933 bp *SDS3* ORF, creating plasmid DV66. The resulting *sds3::HIS3* allele was removed as a *EcoR*I-*Sal*I fragment from DV66 and used to replace the endogenous *SDS3* locus in a *rap1-12/rap1-13 hmrΔA::TRP1/hmrΔA::TRP1* diploid by one-step gene replacement (ROTHSTEIN 1983). The *SDS3* deletion allele, *sds3Δ::URA3*, was constructed by first cloning the *Cla*I to *Sac*I upstream *SDS3* region into the *Cla*I to *Sac*I sites of LSD24 (a *Hin*dIII *URA3* fragment inserted into pIC19R) resulting in the plasmid DV267. A *Nsi*I to *Sal*I fragment containing the 3′ region of *SDS3* including the last 199 bp of the ORF was cloned into pIC19R to produce DV268. The final *sds3Δ::URA3* construct, DV269, was made by excising the 3′ *SDS3* region from DV268 using *Xho*I and *Bam*HI and inserting it into the *Sal*I and *Bam*HI sites of DV267. The disruption construct deletes *SDS3* from −29 to +781 bp. This *sds3Δ::URA3* fragment was then released from the pIC19R vector using *EcoR*I and used to replace the *SDS3* locus in a *rap1-12 hmrΔA::TRP1* strain. The resulting *sds3Δ::URA3* allele was unable to complement the *sds3-1* sporulation defect as measured in an *sds3-1/sds3Δ::URA3* diploid.

A *rpd3Δ::HIS3* plasmid (provided by R. GABER) and a *sin3-Δ::LEU2* plasmid (provided by D. STILLMAN) were used to disrupt the *RPD3* locus and the *SIN3* locus, respectively, in *rap1-12/rap1-13 hmrΔA::TRP1* diploids in the manner described above. Southern blotting was performed to confirm the mutations. These *sds3::HIS*, *rpd3Δ::HIS3*, or *sin3-Δ::LEU2* alleles were subsequently introduced into all other strains by standard genetic crosses.

Analysis of mRNA levels: Northern analysis was performed as previously described (SUSSEL *et al.* 1995). The *a1* probe was made using the *Xho*I-*Bgl*II fragment from the *HMR Xho*I linker clone no. 238 (ABRAHAM *et al.* 1984). The *α1* probe was labeled from a PCR product of the entire *α1* gene. The *PYK1* probe was made from a 1.9-kb *Eco*RI fragment containing the *PYK1* gene (BURKE *et al.* 1983). The *BCY1* probe was labeled from a 4.2-kb *Bam*HI fragment containing the *BCY1* gene (provided by M. WIGLER). As an internal loading control, we used either *PC4* (gift of A. MITCHELL) or actin (gift of M. A. OSLEY) probes. All probes were labeled using random priming with α-^{32}P dATP. Blots were scanned and quantified using a PhosphorImager (Molecular Dynamics).

***lacZ* reporter assays:** Expression of the *TRK2* gene was monitored by using a 2-*μ* *URA3* plasmid (pAB137) containing a *TRK2-lacZ* fusion (VIDAL *et al.* 1991). Control of the *IME2* gene was assayed by using a *CYC-lacZ* plasmid (pKB1001) in which a large fragment of the *IME2* upstream control region replaces the UAS of the *CYC1-lacZ* fusion (BOWDISH and MITCHELL 1993). Strains with various *SDS* and *RAP1* alleles were then transformed with either pAB137 or pKB1001, colonies were grown in selective SC medium and assayed for *β*-galactosidase activity as previously described (MORETTI *et al.* 1994). The units given are the average from at least five different transformants. The standard deviations for these values were ±25%.

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 *SDS* 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. U62525). A search of the Swiss-Prot database using the Blastp program showed Sds3p to be identical to an unknown ORF, YIL084C (accession no. P40505) (ALTSHUL *et al.* 1990). In order to produce an *SDS3* allele suitable for genetic manipulation, the *HIS3* gene was inserted into a unique *Bgl*II 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

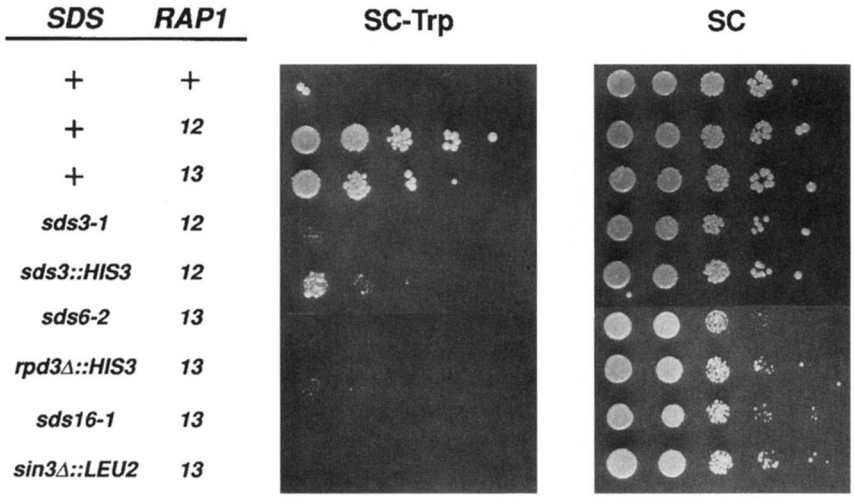

FIGURE 1.—Mutant alleles of *SDS3*, *RPD3(SDS6)* and *SIN3(SDS16)* suppress the silencing defect of a *rap1*^{s} *hmrΔA::TRP1* strain. This assay measures the ability of strains containing an *hmrΔA::TRP1* reporter and the indicated *SDS* and *RAP1* alleles to grow in the presence and absence of tryptophan. Tenfold serial dilutions of overnight cultures were plated onto media lacking tryptophan (SC-Trp) and complete media (SC) and then incubated at 30° for ~2 days.

(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 in a *rap1*^{s} *hmrΔA::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*^{s} *hmrΔA::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, rpd3Δ::HIS3,* showed the same *rap1*^{s} *hmrΔA::TRP1* suppression phenotype as the original *sds6-2* mutation (Figure 1). In subsequent experiments, the *sin3Δ::LEU2* and *rpd3Δ::HIS3* alleles were used in place of the *sds16-1* and *sds6-2* mutations, respectively, to facilitate genetic manipulations. The *rpd3Δ::HIS3* and *sin3Δ::LEU* alleles were found to suppress the *rap1*^{s} *hmrΔA* silencing defect as measured by expression of several different reporters (*ADE2, TRP1* and the endogenous *a1* gene) at *hmrΔA*. Northern blot analysis of steady state *a1* mRNA levels in *rpd3Δ::HIS3 rap1*^{s} *hmrΔA MATα* and *sin3Δ::LEU rap1*^{s} *hmrΔA MATα* strains showed that this suppression was at the level of transcription (SUSSEL *et al.* 1995).

Suppression of *cis*- and *trans*-silencing mutations: Inital experiments with *sds3::HIS3, rpd3Δ::HIS3,* and *sin3Δ::LEU2* showed that they are able to suppress both the *hmrΔA* defect and the *rap1*^{s} silencing defect to restore repression in a *rap1*^{s} *hmrΔA* 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 *hmrΔA* silencer (HARDY *et al.* 1992). Introduction of *sds3::HIS3, rpd3Δ::HIS3,* or *sin3Δ::LEU2* was able to suppress a *RIF1* deletion and restore silencing to a *rif1Δ hmrΔA::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*^{s} rif1Δ double mutant in a *hmrΔA::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 *HM* 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 hmrΔA::TRP1* strain as indicated by a reduced ability of *sds sir1Δ::LEU2 hmrΔA::TRP1* strains to grow on SC-Trp medium (Figure 2B). A 10^2- to 10^3-fold decrease in colony formation on SC-Trp was seen when these strains contained a *rap1*^{s} allele (data not shown). However, in a *sir4Δ::LEU2 hmrΔA::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*^{s} 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 (*hmrΔEΔB::TRP1* and *hmrΔAΔE::ADE2*) (SUSSEL *et al.* 1995). Although the *sds3-1* allele (the first *SDS3* allele examined) did not

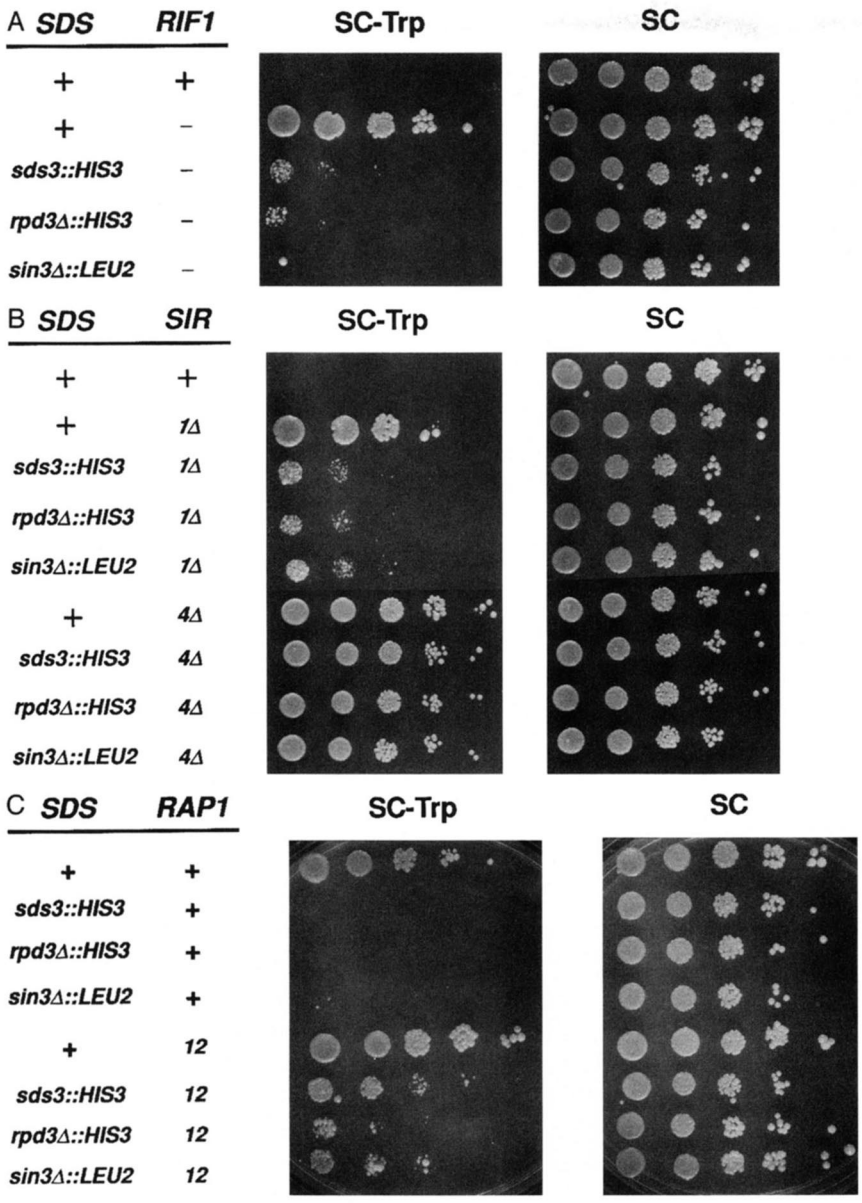

FIGURE 2.—*sds3::HIS3*, *rpd3Δ::HIS3* and *sin3-Δ::LEU2* suppress both *cis*- and *trans*-silencing mutations at *HMR*. (A) Each *sds* mutant is able to restore silencing to a *rif1Δ-::URA3 hmrΔA::RP1* strain as measured by growth on SC-Trp and SC media. This panel compares isogenic strains containing the *hmrΔA::TRP1* reporter and the indicated *RIF1* and *SDS* alleles. (B) *sds* mutants restore silencing at *hmr* in a *sir1Δ::LEU2* background but not in a *sir4Δ::LEU2* background. Isogenic strains containing the *hmrΔA::TRP1* reporter and the indicated *SDS, SIR1* and *SIR4* alleles were examined for their ability to grow in the absence of tryptophan. (C) A silencing defective *hmr* double mutant is suppressed by each of the *sds* mutants. Each strain contains the *hmrΔEΔB::TRP1* reporter in addition to the *SDS* and *RAP1* alleles indicated. Serial dilutions assaying for growth in the absence of tryptophan were performed as described in Figure 1.

have this phenotype, we observed that the *sds3::HIS3* mutation was able to suppress the *hmrΔEΔB::TRP1* reporter (Figure 2C). Furthermore, we found that introduction of *sin3Δ::LEU2*, *rpd3Δ::HIS3* or, to a lesser extent, *sds3::HIS3* partially suppressed a *rap1-12* strain with the *hmrΔEΔB::TRP1* reporter (Figure 2C). However, the *sds* insertion/deletion mutations were unable to affect an *hmrΔAΔE::TRP1* reporter as determined by monitoring growth of 10-fold serial dilutions on SC and SC-Trp plates (data not shown). This discrepancy between the *TRP1* 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 *TRP1* growth assay does not (SUSSEL *et al.* 1993). The presence of the *rap1-12* mutation in these *sds hmrΔAΔE::TRP1* 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 *TRP1* 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

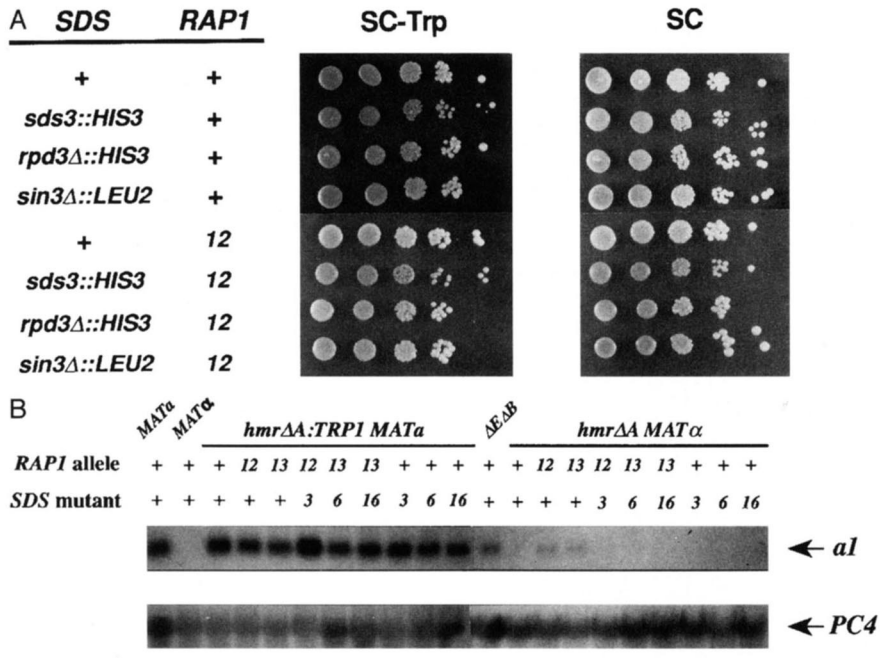

FIGURE 3.—*sds* mutants do not affect expression of *TRP1* and *a1* genes at non-*HMR* loci. (A) Expression of a *his3::TRP1* reporter is unaffected by *sds* or *rap1*^{s} alleles. Strains containing a *his3::TRP1* reporter (the same reporter previously used at *hmr*) and the indicated *sds* and *RAP1* alleles were assayed for the ability to grow on media lacking tryptophan. (B) Northern blot analysis of steady state *a1* mRNA levels shows the *sds* mutations affect expression at *HMR* but not *MAT*. RNA isolated from *MAT***a** *hmrΔA::TRP1* strains or *MATα* *hmrΔA* strains along with the indicated genotypes were transferred to a nylon membrane and hybridized with an α-^{32}P-labeled *a1* fragment. *PC4* mRNA message was used as a loading control.

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 *a1* gene in both *SDS* and *sds* cells, comparing strains in which the only copy of *a1* is either at *HMR* (*hmrΔA MATα*) or at the active *MAT* locus (*MAT***a** *hmrΔA::TRP1*). We observed no significant decreases in the levels of *a1* mRNA from the *MAT* locus (Figure 3B). As observed previously (SUSSEL *et al.* 1995), each of the *sds* mutations resulted in a complete loss of detectable *a1* mRNA from the *HMR* locus in a *rap1*^{s} *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/rpd1* 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-ΔE::TRP1* reporter and combinations of the *sds3::HIS3*, *rpd3Δ::HIS3* and *sin3Δ::LEU2* alleles. In order to get a

TABLE 1

Comparison of *sds3*, *rpd3* and *sin3* phenotypes

SDS allele	Sporulation (%)[a]	*IME2-lacZ* units[b]	*TRK2-lacZ* units[b]
wt	73.9	5.5	26.7
sds3::HIS3	<0.5	79	14.0
rpd3Δ::HIS3	<0.5	91	137.4
sin3Δ::LEU2	<0.5	51	122.9

[a] 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.

[b] These values represent lacZ expression levels from a 2μ-borne *IME2-lacZ* reporter (pKB1001) or a 2μ-borne *TRK2-lacZ* reporter (pAB137) in vegetatively growing haploid strains of the indicated genotype. Details of these plasmids are found in the MATERIALS AND METHODS. Values are the average Miller units of at least four different transformants.

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 *hmrΔAΔE* silencing defect by 30–38% (Table 2). As expected for genes acting in the same pathway, *sin3Δ::LEU2 rpd3Δ::HIS3* double mutant strains 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 *TRP1* 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 *ADH4* 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^s* 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). In this assay for TPE, the *rap1^s* mutation and *sds* mutation had a striking synergistic effect in repressing a telomere proximal *URA3* reporter.

TABLE 2

***sds* double and triple mutant analysis**

SDS alleles	*hmrΔAΔE::TRP1* expression (%)
wt	95 ± 2
sds3	70 ± 2
rpd3	62 ± 5
sin3	70 ± 6
rpd3 sin3	60 ± 10
sds3 rpd3	33 ± 8
sds3 sin3	36 ± 3
sds3 rpd3 sin3	34 ± 6

The values above represent the ability of various combinations of *sds* mutants to suppress the silencing defective *hmrΔAΔE::TRP1* 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.

Effect of *sds* mutations on Rap1p-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

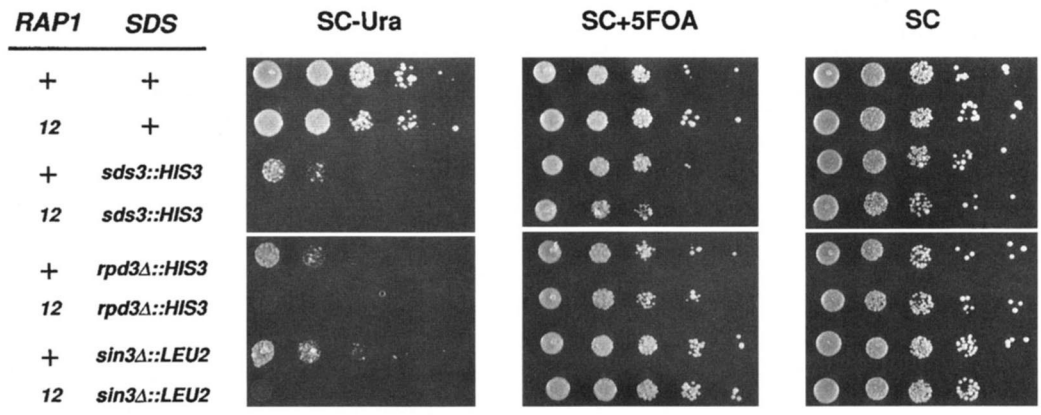

FIGURE 4.—Telomere position effect is increased by each *sds* mutant and is synergystically increased with the addition of a *rap1-12* allele. Each of the above strains contains a telomere proximal *URA3* reporter and the indicated genotype. Expression of the *URA3* reporter is measured by growth of 10-fold serial dilutions on media lacking uracil (SC-Ura). SC media supplemented with 5-fluoroorotic acid (SC+5-FOA) selects for growth of Ura$^-$ colonies, while SC serves as a control for total growth.

quantitating mRNA signals on a PhospohorImager, 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). SUSSEL *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 *rap1^s 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 *rap1^s* silencing de-

TABLE 3

Suppression of 0.5× *SIR4* in *hmrΔA::ADE2* diploids

Genotype	Colony color
wt	pink/white sectored
0.5× *SIR4*	white
0.5× *SIR4 sds3*	white and few white/light pink sectored
0.5× *SIR4 rpd3*	white/pink sectored
0.5× *SIR4 sin3*	white/pink sectored

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 repressed *hmrΔA::ADE2* loci, while white colonies or sectors are populations with derepressed *hmrΔA::ADE2* loci. All strains were plated onto YPD, grown 3 days at 30°, stored for at least 3 days at 4° and then visually examined for colony color.

fect, none of the *sds* mutations described here show specificity for any one of the *rap1*^{s} 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 *hmr*ΔA and *hmr*$\Delta E\Delta B$, and partially at *hmr*$\Delta A\Delta E$. 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.* 1993). 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(SDI1)* and *RPD3(SDI2)* were first identified in a screen for mutants that would allow expression of the *HO* gene in a *swi5* 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 *rpd3* and *sin3* mutants: failure to sporulate as homozygous 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 *CYC1-lacZ*, but does not affect a *CYC1-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 GOTTSCHLING (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 hmr*ΔA silencing defect (SUSSEL *et al.* 1995), while a deletion of *SIN4* partially suppresses the *rap1*^{s} *hmr*ΔA 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*^{s} 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 (*hmr*$\Delta E\Delta B$ for example), while a twofold reduction in Sir4p concentration can result in total derepression of *hmr*ΔA (SUSSEL *et al.* 1993). 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 *rpd3* and *sin3* mutations strongly suppress a 0.5× reduction in *SIR4* dosage, while *sds3* does so to a much lesser extent. This suggests that the *sds* mutants, especially *rpd3* 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*^{s} 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*^{s} *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 (HEBBES *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× *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

ABRAHAM, J., K. A. NASMYTH, J. N. STRATHERN, A. J. S. KLAR and J. B. HICKS, 1984 Regulation of mating-type information in yeast. J. Mol. Biol. **176:** 307–331.

ALTSHUL, S. F., W. GISH, W. MILLER, E. W. MYERS and D. J. LIPMAN, 1990 Basic local alignment search tool. J. Mol. Biol. **215:** 403–410.

APARICIO, O. M., and D. E. GOTTSCHLING, 1994 Overcoming telomeric silencing: a trans-activator competes to establish gene expression in a cell cycle-dependent way. Genes Dev. **8:** 1844–1849.

APARICIO, O. M., B. L. BILLINGTON and D. E. GOTTSCHLING, 1991 Modifiers of position effect are shared between telomeric and silent mating-type loci in S. cerevisiae. Cell **66:** 1279–1287.

BELL, S. P., R. KOBAYASHI and B. STILLMAN, 1993 Yeast origin recognition complex functions in transcription silencing and DNA replication. Science **262:** 1844–1849.

BELL, S. P., and B. STILLMAN, 1992 ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature **357:** 128–134.

BOWDISH, K. S., and A. P. MITCHELL, 1993 Bipartite structure of an early meiotic upstream activation sequence from *Saccharomyces cerevisiae.* Mol. Cell. Biol. **13:** 2172–2181.

BRAND, A. H., L. BREEDEN, J. ABRAHAM, R. STERNGLANZ and K. NASMYTH, 1985 Characterization of a "silencer" in yeast: a DNA sequence with properties opposite to those of a transcriptional enhancer. Cell **41:** 41–48.

BRAND, A. H., G. MICKLEM and K. NASMYTH, 1987 A yeast silencer contains sequences that can promote autonomous plasmid replication and transcriptional activation. Cell **51:** 709–719.

BRAUNSTEIN, M., A. B. ROSE, S. G. HOLMES and J. R. BROACH, 1993 Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. Genes Dev. **7:** 592–604.

BRAUNSTEIN, M., R. E. SOBEL, C. D. ALLIS, B. M. TURNER and J. R. BROACH, 1996 Efficient transcriptional silencing in *Saccharomyces cerevisiae* requires a heterochromatin histone acetylation pattern. Mol. Cell. Biol. **16:** 4349–4356.

BUCHMAN, A. R., W. J. KIMMERLY, J. RINE and R. D. KORNBERG, 1988 Two DNA-binding factors recognize specific sequences at silencers, upstream activating sequences, autonomously replicating sequences, and telomeres in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **8:** 210–225.

BUCK, S. W., and D. SHORE, 1995 Action of a RAP1 C-terminal silencing domain reveals an underlying competition between *HMR* and telomeres in yeast. Genes Dev. **9:** 370–384.

BURKE, R. L., P. TEKAMP-OLSON and R. NAJARIN, 1983 The isolation, characterization and sequence of the pyruvate kinase gene of *Saccharomyces cerevisiae.* J. Biol. Chem. **258:** 2193–2201.

CHEN-CLELAND, T. A., M. M. SMITH, S. LE, R. STERNGLANZ and V. G. ALLFREY, 1993 Nucleosome structural changes during derepression of silent mating-type loci in yeast. J. Biol. Chem. **268:** 1118–1124.

CHIEN, C.-T., S. BUCK, R. STERNGLANZ and D. SHORE, 1993 Targeting of SIR1 protein establishes transcriptional silencing at *HM* loci and telomeres in yeast. Cell **75:** 531–541.

CONRAD, M. N., J. H. WRIGHT, A. J. WOLF and V. A. ZAKIAN, 1990 RAP1 protein interacts with yeast telomeres *in vivo*: overproduction alters telomere structure and decreases chromosome stability. Cell **63:** 739–750.

FOSS, M., F. J. MCNALLY, P. LAURENSON and J. RINE, 1993 Origin recognition complex (ORC) in transcriptional silencing and DNA replication in *S. cerevisiae.* Science **262:** 1838–1844.

GIESMAN, D., L. BEST and K. TATCHELL, 1991 The role of RAP1 in the regulation of the *MAT*alpha locus. Mol. Cell. Biol. **11:** 1069–1079.

GOTTSCHLING, D. E., 1992 Telomere-proximal DNA in *Saccharomyces cerevisiae* is refractory to methyltransferase activity *in vivo.* Proc. Natl. Acad. Sci. USA **89:** 4062–4065.

GOTTSCHLING, D. E., O. M. APARICIO, B. L. BILLINGTON and V. A. ZAKIAN, 1990 Position effect at *S. cerevisiae* telomeres: reversible repression of pol II transcription. Cell **63:** 751–762.

HARDY, C. F. J., L. SUSSEL and D. SHORE, 1992 A RAP1-interacting protein involved in silencing and telomere length regulation. Genes Dev. **6:** 801–814.

HEBBES, T. R., A. W. THORNE, and C. CRANE-ROBINSON, 1988 A direct link between core histone acetylation and transcriptionally active chromatin. EMBO J. **7:** 1395–1402.

HECHT, A., T. LAROCHE, S. STRAHL-BOLSINGER, S. M. GASSER and M. GRUNSTEIN, 1995 Histone H3 and H4 N termini interact with SIR3 and SIR4 proteins: a molecular model for the formation of heterochromatin in yeast. Cell **80:** 583–592.

HENIKOFF, S., 1990 Position-effect variegation after 60 years. Trends Genet. **6:** 422–426.

KAYNE, P. S., U. J. KIM, M. HAN, J. R. MULLEN, F. YOSHIZAKI *et al.*, 1988 Extremely conserved histone H4 N terminus is dispensable for growth but essential for repressing the silent mating loci in yeast. Cell **55:** 27–39.

KIM, Y.-J., S. BJORKLUND, Y. LI, M. H. SAYRE and R. D. KORNBERG, 1994 A multiprotein mediator of transcriptional activation and its interaction with the C-terminal repeat domain of RNA polymerase II. Cell **77:** 599–608.

KLAR, A. J. S., J. N. STRATHERN and J. B. HICKS, 1984 A position-effect control for gene transposition: state of expression of yeast mating-type genes affects their ability to switch. Cell **25:** 517–524.

KURTZ, S., and D. SHORE, 1991 The RAP1 protein activates and silences transcription of mating-type genes in yeast. Genes Dev. **5:** 616–628.

KYRION, G., K. A. BOAKYE and A. J. LUSTIG, 1992 C-terminal truncation of RAP1 results in the deregulation of telomere size, stability, and function in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **12:** 5159–5173.

KYRION, G., K. LIU, C. LIU and A. J. LUSTIG, 1993 RAP1 and telomere structure regulate telomere position effects in *Saccharomyces cerevisiae.* Genes Dev. **7:** 1146–1159.

LAMAN, H., D. BALDERES and D. SHORE, 1995 Disturbance of normal cell cycle progression enhances the establishment of transcriptional silencing in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **15:** 3608–3617.

LAURENSON, P., and J. RINE, 1992 Silencers, silencing, and heritable transcriptional states. Microbiol. Rev. **56:** 543–560.

LI, Y., S. BJORKLUND, Y. W. JIANG, Y.-J. KIM, W. S. LANE *et al.*, 1995 Yeast global transcriptional regulators Sin4 and Rgr1 are components of mediator complex/RNA polymerase II holoenzyme. Proc. Natl. Acad. Sci. USA **92:** 10864–10868.

LOO, S., P. LAURENSON, M. FOSS, A. DILLIN and J. RINE, 1995 Roles of *ABF1, NPL3,* and *YCL54* in silencing in *Saccharomyces cerevisiae.* Genetics **141:** 889–902.

LOO, S., and J. RINE, 1994 Silencers and domains of generalized repression. Science **264:** 1768–1771.

LUSTIG, A. J., S. KURTZ, and D. SHORE, 1990 Involvement of the silencer and UAS binding protein RAP1 in regulation of telomere length. Science **250:** 549–553.

MAHONEY, D. J., and J. R. BROACH, 1989 The *HML* mating-type cassette of *Saccharomyces cerevisiae* is regulated by two separate but functionally equivalent silencers. Mol. Cell. Biol. **9:** 4621–4630.

MICKLEM, G., A. ROWLEY, J. HARWOOD, K. NASMYTH and J. F. X. DIFFLEY, 1993 Yeast origin recognition complex is involved in DNA replication and transcriptional silencing. Nature **366:** 87–89.

MORETTI, P., K. FREEMAN, L. COODLEY and D. SHORE, 1994 Evidence that a complex of SIR proteins interacts with the silencer and telomere-binding protein RAP1. Genes Dev. **8:** 2257–2269.

NASMYTH, K. A., 1982 The regulation of yeast mating-type chromosome structure by *SIR*: an action at a distance affecting both transcription and transposition. Cell **30:** 567–578.

NASMYTH, K., D. STILLMAN and D. KIPLING, 1987 Both positive and negative regulators of HO transcription are required for mother-cell-specific mating-type switching in yeast. Cell **48:** 579–587.

NISHIZAWA, M., R. ARAKI and Y. TERANISHI, 1989 Identification of an upstream activating sequence and an upstream repressible sequence of the pyruvate kinase gene of the yeast *Saccharomyces cerevisiae.* Mol. Cell. Biol. **9:** 442–451.

ORLANDO, V., and R. PARO, 1995 Chromatin multiprotein complexes involved in the maintenance of transcription patterns. Curr. Opin. Genet. Dev. **5:** 174–179.

PILLUS, L., and J. RINE, 1989 Epigenetic inheritance of transcriptional states in *S. cerevisiae.* Cell **59:** 637–647.

RIGGS, A. D., and G. D. PFEIFFER, 1992 *X*-chromosome inactivation and cell memory. Trends Genet. **8:** 169–174.

RINE, J., and I. HERSKOWITZ, 1987 Four genes responsible for a position effect on expression from *HML* and *HMR* in *Saccharomyces cerevisiae.* Genetics **116:** 9–22.

ROSE, M. D., P. NOVICK, J. H. THOMAS, D. BOTSTEIN and G. R. FINK, 1987 A *Saccharomyces cerevisiae* genomic plasmid bank based on a centromere-containing shuttle vector. Gene **60:** 237–243.

ROSE, M. D., F. WINSTON, F. and P. HIETER, 1990 *Methods in Yeast Genetics: A Laboratory Course Manual.* Cold Spring Harbor Press, Cold Spring Harbor, NY.

ROTHSTEIN, R., 1983 One-step gene disruption in yeast. Methods Enzymol. **101:** 202–211.

SHORE, D., 1994 RAP1: a protean regulator in yeast. Trends Genet. **10:** 408–412.

SHORE, D., and K. NASMYTH, 1987 Purification and cloning of a DNA binding protein from yeast that binds to both silencer and activator elements. Cell **51:** 721–732.

SHORE, D., D. J. STILLMAN, A. H. BRAND and K. A. NASMYTH, 1987 Identification of silencer binding proteins from yeast: possible roles in SIR control and DNA replication. EMBO J. **6:** 461–467.

SIKORSKI, R., and P. HIETER, 1989 A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae.* Genetics **122:** 19–27.

SINGH, J., and A. J. S. KLAR, 1992 Active genes in budding yeast display enhanced *in vivo* accessibility to foreign DNA methylases: a novel *in vivo* probe for chromatin structure of yeast. Genes Dev. **6:** 186–196.

STERNBERG, P. W., M. J. STERN, I. CLARK and I. HERSKOWITZ, 1987 Activation of the yeast *HO* gene by release from multiple negative controls. Cell **48:** 567–577.

STILLMAN, D. J., S. DORLAND and Y. YU, 1994 Epistasis analysis of suppressor mutations that allow *HO* expression in the absence of the yeast *SWI5* transcriptional activator. Genetics **136:** 781–788.

STRICH, R., M. R. SLATER and R. E. ESPOSITO, 1989 Identification of negative regulatory genes that govern the expression of early meiotic genes in yeast. Proc. Natl. Acad. Sci. USA **86:** 10018–10022.

SURANI, M. A., 1994 Genomic imprinting: control of gene expression by epigenetic inheritance. Curr. Opin. Cell Biol. **6:** 390–395.

SUSSEL, L., and D. SHORE, 1991 Separation of transcriptional activation and silencing functions of the *RAP1*-encoded repressor/activator protein 1: Isolation of viable mutants affecting both silencing and telomere length. Proc. Natl. Acad. Sci. USA **88:** 7749–7753.

SUSSEL, L., D. VANNIER and D. SHORE, 1993 Epigenetic switching of transcriptional states: *cis*- and *trans*-acting factors affecting establishment of silencing at the *HMR* locus in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **13:** 3919–3928.

SUSSEL, L., D. VANNIER and D. SHORE, 1995 Suppresso.: of defective silencing in yeast: effects on transcriptional repression at the HMR locus, cell growth, and telomere structure. Genetics **141:** 873–888.

TAUNTON, J., C. A. HASSIG and S. L. SCHREIBER, 1996 A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. Science **272:** 408–411.

THOMAS, B. J., and R. ROTHSTEIN, 1989 Elevated recombination rates in transcriptionally active DNA. Cell **56:** 619–630.

THOMPSON, J. S., X. LING and M. GRUNSTEIN, 1994 Histone H3 amino terminus is required for telomeric and silent mating locus repression in yeast. Nature **369:** 245–247.

VIDAL, M., and R. F. GABER, 1991 *RPD3* encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **11:** 6317–6327.

VIDAL, M., R. STRICH, R. E. ESPOSITO and R. F. GABER, 1991 *RPD1(SIN3/UME4)* is required for maximal activation and repression of diverse yeast genes. Mol. Cell. Biol. **11:** 6306–6317.

WANG, H., I. CLARK, P. R. NICHOLSON, I. HERSKOWITZ and D. J. STILLMAN, 1990 The *Saccharomyces cerevisiae SIN3* gene, a negative regulator of *HO,* contains four paired amphipathic helix motifs. Mol. Cell. Biol. **10:** 5927–5936.

WANG, H., and D. J. STILLMAN, 1993 Transcriptional repression in *Saccharomyces cerevisiae* by a SIN3-LexA fusion protein. Mol. Cell. Biol. **13:** 1805–1814.

YOSHIMOTO, H., M. OHMAE and I. YAMASHITA, 1992 The *Saccharomyces cerevisiae GAM2/SIN3* protein plays a role in both activation and repression of transcription. Mol. Gen. Genet. **233:** 327–330.

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