MOLECULAR AND CELLULAR BIOLOGY, July 1993, p. 3919–3928
0270-7306/93/073919-10$02.00/0

Vol. 13, No. 7

Epigenetic Switching of Transcriptional States: *cis*- and *trans*-Acting Factors Affecting Establishment of Silencing at the *HMR* Locus in *Saccharomyces cerevisiae*

LORI SUSSEL, DAVID VANNIER, AND DAVID SHORE*

Department of Microbiology, College of Physicians & Surgeons of Columbia University, 701 W. 168th Street, New York, New York 10032

Received 2 December 1992/Returned for modification 1 March 1993/Accepted 30 March 1993

In this study, we used the *ADE2* gene in a colony color assay to monitor transcription from the normally silent *HMR* mating-type locus in *Saccharomyces cerevisiae*. This sensitive assay reveals that some previously identified *cis*- and *trans*-acting mutations destabilize silencing, causing genetically identical cells to switch between repressed and derepressed transcriptional states. Deletion of the autonomously replicating sequence (ARS) consensus element at the *HMR-E* silencer or mutation of the silencer binding protein RAP1 (*rap1^s*) results in the presence of large sectors within individual colonies of both repressed (Ade$^-$, pink) and derepressed (Ade$^+$, white) cells. These results suggest that both the ARS consensus element and the RAP1 protein play a role in the establishment of repression at *HMR*. In diploid cells, the two copies of *HMR* appear to behave identically, suggesting that the switching event, though apparently stochastic, reflects some property of the cell rather than a specific event at each *HMR* locus. In the *ADE2* assay system, silencing depends completely upon the function of the *SIR* genes, known *trans*-acting regulators of the silent loci, and is sensitive to the gene dosage of two *SIR* genes, *SIR1* and *SIR4*. Using the *ADE2* colony color assay in a genetic screen for suppressors of *rap1^s*, silencer ARS element deletion double mutants, we have identified a large number of genes that may affect the establishment of repression at the *HMR* silent mating-type locus.

Position-effect control of transcription plays an essential role in determining the mating type of haploid cells (**a** or α) in the yeast *Saccharomyces cerevisiae* (reviewed in reference 26). The yeast genome contains three copies of mating-type genes, all of which are located on chromosome III. Only those genes present at the *MAT* locus (either **a** or α) are actually expressed. Two additional silent loci, called *HMR* and *HML*, also contain mating-type genes (**a** and α, respectively), but transcription of these genes is prevented by the presence of flanking *cis*-acting regulatory elements called silencers. The silent loci act as donors of information in a mating-type switching event that replaces sequences at *MAT* with those of the opposite mating type.

Repression of the two silent mating-type loci requires the function of a number of known *trans*-acting regulators, including the four *SIR* genes (37), histone H4 (22, 32, 35), the *NAT1/ARD1* N-terminal acetyltransferase (34, 45), and either one of two silencer-binding proteins, repressor/activator protein 1 (RAP1) and autonomously replicating sequence (ARS) binding factor 1 (ABF1) (7, 8, 23, 41, 42). RAP1 and ABF1 proteins bind to silencer regulatory sites called E and B, respectively. The silencers at both *HML* and *HMR* function as ARSs and contain ARS consensus elements (called A elements) that are important for silencer function (1, 7, 13, 24). Furthermore, the *HMR-E* silencer (found to the left of the repressed locus) functions as a chromosomal origin of DNA replication, and its ability to do so is closely correlated with silencer function (38). Using a temperature-sensitive *sir3* mutant, Miller and Nasmyth (33) showed that the reestablishment of silencing requires progression through S phase. Taken together, these results suggest that silencing results from the assembly of a repressed state of chromatin that is initiated at the silencer element at the time of DNA replication.

Pillus and Rine (36) have shown that in cells carrying null mutations in the *SIR1* gene, silencing at *HML* is not abolished but is destabilized. In *sir1* mutant cells, the *HML* silent locus switches back and forth between repressed and derepressed states. This epigenetic effect on silencing results in a mixed population, with respect to expression of the *HML* locus, in a culture of genetically identical *sir1* cells. On average, 20% of cells in a population are repressed and 80% derepressed. Remarkably, switching between the repressed and derepressed states occurs at a low frequency. Thus, cells in either state have a >99% probability of giving rise to progeny in the same state. Pillus and Rine explained their results by proposing that silencing can be considered to consist of two separable processes, establishment and maintenance, and that *SIR1* is required only for the establishment function. Previous studies by Miller and Nasmyth (33) with a temperature-sensitive mutation in *SIR3* had first demonstrated an efficient establishment function that operates during S phase of the cell cycle. The phenotype of *sir1* mutant cells suggests further that an additional mechanism that promotes the inheritance of repression in the absence of an establishment function exists. More recently, *cis*-acting elements within the *HML-E* silencer that are required for efficient establishment of repression at *HML* in *SIR1* wild-type cells missing the *HML-I* silencer have been identified (29). An intact silencer element and a functional *SIR1* gene thus appear to be important for the establishment of repression at *HML*.

The molecular nature of the establishment function at the *HMR* silent locus has not been determined. Though similar in overall organization, the *HMR* silencers show distinct structural and functional differences in comparison to those at *HML*. For example, at *HML* the two silencers, called E

* Corresponding author.

and I (located to the left and right of the locus, respectively), appear to be functionally equivalent. Neither E nor I is necessary for silencing, since deletions of either element alone have no effect on repression (13, 28). However, at *HMR* the E silencer is essential for repression (1, 6), whereas chromosomal deletions of the I element have no effect on silencing (6). In addition, the two *HM* loci show differing sensitivity to the loss of various *trans*-acting regulators. For example, *HML* is severely derepressed in cells containing either *NAT1* or *ARD1* mutations (34, 45), whereas *HMR* is not affected. Deletions of the N-terminal tail of histone H4 also strongly derepress *HML* but have little or no affect on *HMR* silencing (22). Finally, the *HMR-E* silencer functions as a chromosomal origin of replication (38), whereas the *HML* silencers apparently do not (12).

The recent discovery that telomeres in *S. cerevisiae* are also regions of silenced chromatin (16) indicates that several components of mating-type gene silencing system may have a more widespread affect than previously thought. Repression of genes placed near artificial telomeres, like mating-type gene silencing, requires the function of *SIR2-4*, histone H4, and the *NAT1/ARD1* N-terminal acetyltransferase (2). Telomeric silencing, however, appears to be inherently unstable, since genes placed near telomeres switch between on and off states even in wild-type cells. In this regard it is interesting that telomeric silencing is unaffected by mutations in *SIR1*. The nature of the telomeric silencer element remains unclear. However, telomeres contain many high-affinity binding sites for the silencer binding protein RAP1 within poly($C_{1-3}A$) sequences (9, 27), suggesting that RAP1 may also play a role in telomeric silencing.

We are interested in the role of the RAP1 protein in mating-type gene silencing. Binding sites for RAP1, a relatively abundant sequence-specific DNA binding protein, are found at both the *HMR-E* and *HML-E* silencers, where deletion analyses indicate that they are important for silencer function (7, 8, 23, 42). RAP1, which is encoded by an essential gene, also binds to the upstream activation sites (UASs) of genes, including a number of ribosomal protein and glycolytic enzyme genes, where it appears to be an activator of transcription (9, 10, 20, 41). Experiments in which silencer and UAS binding sites for RAP1 are exchanged suggest that the context of the RAP1 binding site determines its regulatory activity, presumably through interactions with other proteins at these loci (9, 41). Genetic studies using temperature-sensitive (ts) lethal mutations in *RAP1* demonstrate a direct role for the protein in the activation of at least one gene containing a RAP1 UAS (*MAT*α) (15, 25). One ts mutant is also defective in silencing of the *HMR* locus (25). Another collection of *rap1* mutants (*rap1^s*) that are unaffected in apparently essential activation functions, but are defective in silencing at an *HMR* locus containing a deletion of the ARS consensus sequence at the silencer have been isolated (44). The *rap1^s* mutants all map to a short region of the C terminus of the protein and are possibly defective in recruiting another protein (RIF1) to the silencer (17). In addition to affecting silencing, the *rap1^s* mutants result in elongation of the poly($C_{1-3}A$) sequences at telomeres (44). These two phenomena seem to be related, because the strength of the silencing defect in the four different *rap1^s* alleles is correlated with the extent of telomere elongation.

Two *rap1^s* alleles (*rap1-11* and *rap1-14*) appear to have only a weak effect on silencing. For example, populations of mutant cells containing a *hmrΔA::TRP1* locus (a silencer deleted for the ARS consensus sequence, with the *TRP1* gene replacing the normal *a1* and *a2* genes) grow poorly in the absence of tryptophan, suggesting only slight derepression of the silenced *TRP1* gene. However, when assayed at the level of individual cells, a fraction of cells within a culture appear to be completely derepressed (and hence able to form colonies on medium lacking tryptophan), whereas most cells fail to grow at all. This behavior of the *hmrΔA::TRP1* locus in the *rap1^s* strains is reminiscent of effects seen at telomeres (16) and at the *HML* silent locus in *sir1* strains or strains containing *cis* silencer mutations (29, 36).

Here we examine this phenomenon in more detail by placing the *ADE2* gene at the silent *HMR* locus. Cells lacking *ADE2* activity (either as a result of mutation or silencing of the gene) accumulate a pigment and give rise to pink colonies, whereas Ade$^+$ colonies are white. This property of *ADE2* has allowed us to assay silencer function at the level of individual colonies, as has recently been accomplished for telomeric position effect (2). Cells containing either *rap1^s* mutations or a deletion of the ARS consensus sequence give rise to sectored pink and white colonies, indicating switching between repressed and derepressed states at the *hmr::ADE2* locus. In diploid cells containing two copies of *hmr::ADE2*, the expression of the two loci appears to be correlated, because these diploid cells also give rise to sectored colonies indistinguishable from those of isogenic haploids. These results suggest that the ARS consensus element and the RAP1 protein are important in the establishment of the silenced state but may not be required for its maintenance. In contrast, the activity of the *SIR* genes appears to be required for the maintenance of silencing, since mutations in the *SIR* genes tested result in complete derepression of *hmr::ADE2*. However, the establishment of silencing, as measured in strains containing *rap1^s* mutations, is extremely sensitive to the gene dosage of two *SIR* genes, *SIR4* and *SIR1*. Finally, the *ADE2* colony color assay has allowed us to identify new genes potentially involved in the establishment of repression. We describe a screen for *rap1^s* suppressors that has led to the isolation of a large number of unlinked recessive mutations that restore metastable repression in *rap1^s hmrΔA* strains.

MATERIALS AND METHODS

Yeast strains and media. The yeast strains used in this study and their genotypes are listed in Table 1. All yeast strains were derived from W303-1B (39). Growth and manipulation of yeast strains was done according to standard procedures (40). For the color assays, strains were plated on rich medium (YEPD) at 30°C for 2 or 3 days and then shifted to 4°C overnight before being photographed.

***hmr::ADE2* strain constructions.** To construct the *hmr::ADE2* locus, an *Eco*RI-to-*Hin*dIII fragment of each *HMRa* silencer derivative (wild type, Δ*A*, Δ*B*, Δ*E*, and Δ*E*Δ*B*) was subcloned into pUC18. These plasmids were then digested with *Xba*I and end filled with the Klenow fragment of DNA polymerase I, and *Bgl*II linkers were added. The DNAs were then digested with *Bgl*II and religated to delete a large internal fragment of *HMRa* including all of the *a2* gene and most of the *a1* gene. A 3.6-kb *Bam*HI fragment of *ADE2* (16) was then ligated into the *HMR Bgl*II deletion derivatives that had been digested with *Bgl*II and treated with calf alkaline phosphatase. The *ADE2* gene was inserted in both orientations with respect to the E silencer. (The promoter distal orientation is indicated in Table 1 by writing the *ADE2* gene name backwards). The series of

TABLE 1. Yeast strains used in this study

Strain	Genotype	Source[a] or reference
YDS 2	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein
YDS 3	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	R. Rothstein
YDS38	YDS3; *hmrΔ77-144::SUP4-o*	6
YLS404	YDS38; *hmrΔA* (*Δ358-352*[b])::*ADE2*	
YLS405	YDS38; *hmrΔA* (*Δ358-352*[b])::*2EDA*[c]	
YLS407	YDS38; *hmrΔEΔB* (*Δ331-324 Δ274-256*[b])::*ADE2*	
YLS409	YDS38; *HMR::ADE2*	
YLS410	YDS38; *HMR::2EDA*[c]	
YLS413	YLS404; *rap1-11::LEU2*	
YLS419	YLS404; *rap1-12::LEU2*	
YLS421	YLS404; *rap1-13::LEU2*	
YLS423	YLS404; *rap1-14::LEU2*	
YLS438	YLS409; *rap1-12::LEU2*	
YLS440	YLS409; *rap1-13::LEU2*	
YLS454	YLS409; *rap1-11::LEU2*	
YLS460	YLS409; *rap1-14::LEU2*	
YLS526	YLS404; *RAP1::URA3 MATa*	
YLS532	YLS526 × YLS567	
YLS556	YLS409, *sir1::LEU2*	
YLS567	YLS405; *RAP1::HIS3*	
YLS575	YLS421; *HIS3::SIR4*	
YLS577	YLS404; *HIS3::SIR4*	
YLS579	YLS419; *HIS3::SIR4*	
YLS586	YDS38; *hmrΔB* (*Δ274-256*[b])::*ADE2*	
YLS588	YDS38; *hmrΔE* (*Δ331-324*[b])::*ADE2*	
YLS590	YLS404; *sir1::LEU2*	
YLS592	YLS409; *sir3::LEU2*	
YLS594	YLS410; *sir4::LEU2*	
YLS595	YLS526 × YLS590	
YLS596	YLS526 × YLS594	

[a] Where no source is given, see Materials and Methods.
[b] The *HMR* deletion nomenclature is from Brand et al. (7).
[c] The designation *2EDA* is meant to indicate that the *ADE2* gene has been placed in a promoter-distal orientation with respect to the E silencer.

hmr::ADE2 plasmids, designated LSD270 and 271 (*hmr::ADE2* and *hmr::2EDA*), LSD272 and 273 (*hmrΔA::ADE2* and *hmrΔA::2EDA*), LSD396 and 400 (*hmrΔB::ADE2* and *hmrΔB::2EDA*), and LSD398 and 402 (*hmrΔE::ADE2* and *hmrΔE::2EDA*), were used to replace the normal chromosomal *HMR* locus as described previously (7), except that lithium acetate transformation rather than spheroplast transformation was used.

***rap1[s] hmr::ADE2* strain constructions.** Each of the *rap1[s]* alleles was introduced into the series of *RAP1 hmr::ADE2* strains through a genetic cross. To easily distinguish between the *RAP1* and *rap1[s]* alleles, the *rap1[s]* alleles were first tagged with the *LEU2* gene. A *Sac*I-to-*Bgl*II fragment containing sequences lying upstream of the *RAP1* gene was isolated from the *RAP1* λgt 11.8 clone (41). This fragment was subcloned into *Bam*HI-*Sac*I-digested pRS306, an integrating *LEU2* plasmid, destroying the *Bam*HI site in the process. This entire plasmid was then targeted for integration at the *RAP1* locus by digestion with *Bam*HI to create a double-stranded break at a unique site upstream of the *RAP1* gene. The digested plasmid was then introduced into the appropriate strains by lithium acetate transformation, and the correct integration was confirmed by Southern blotting. The resulting strains were crossed to the series of *RAP1 hmr::ADE2* strains, sporulated, and dissected to obtain the appropriate segregants. YLS526 and YLS567 were created by tagging the *RAP1* gene with *URA3* and *HIS3*, respectively. We employed the same strategy described above, using either pRS306 (*URA3*) or pRS305 (*HIS3*) as the integrating vectors.

Mutant isolation. Yeast cells (relevant genotype, *rap1[s]::MARKER1 hmrΔA::ADE2*) were mutagenized with 3% ethyl methanesulfonate to 40% lethality as previously described (3). Serial dilutions of the mutagenized cells were plated on solid YPD medium and incubated for 3 to 4 days at 30°C. Following incubation, the plates were stored at 4°C for 2 to 3 days to enhance the pigmentation of Ade$^-$ cells. Potential mutants that displayed any pink coloring were isolated from the original YPD plate and colony purified. Each mutant that displayed colonies with pink and white sectors upon retesting was analyzed further. Mutants that appeared to produce completely pink colonies with no evidence of white sectors were presumed to contain mutations in the *ADE2* gene at *HMR* or other genes in the adenine biosynthetic pathway and were not pursued further. To test for dominance the potential mutants were crossed to a *rap1[s]::MARKER2 hmrΔA::TRP1* strain, and expression of the *TRP1* and *ADE2* genes at *HMR* was monitored in the resulting diploid. Linkage to *HMR* or *RAP1* was examined by sporulation and tetrad analysis of these diploids. Segregants containing a recessive suppressor mutation unlinked to either *RAP1* or *HMR* were chosen for further analysis. Using the *hmrΔA::TRP1* locus as a reporter, pairwise crosses of the suppressor strains were performed to define complementation groups. A primary group of mutants was used to define 21 different complementation groups (termed *SDS1-21*, for suppressor of defective silencing), the validity of which was confirmed by segregation analysis of the diploids. Additional alleles were identified by failure to complement a mutant from 1 of the 21 original complementation groups. Further details of this analysis will be presented elsewhere.

RESULTS

A colony color assay reveals epigenetic switching between repressed and derepressed states at *HMR* in the absence of an ARS element at the silencer. We noted previously that cells containing either of two weaker *rap1[s]* alleles (*rap1-11* and *rap1-14*) appear to exist in two different states when assayed for expression of the *TRP1* gene placed at the *HMR* silent locus (*hmrΔA::TRP1*) (44). A small fraction of these genetically identical cells grow on medium lacking tryptophan, and thus are expressing the *TRP1* gene, whereas most cells fail to grow under selection, indicating that the *hmrΔA::TRP1* gene is being silenced. To determine if this phenotypic variation is due to switching between two distinct transcriptional states, we have constructed a series of *hmr::ADE2* strains. In these strains, *ADE2* expression can be monitored nonselectively at the clonal level by colony color (2, 18, and references therein). This is made possible by the fact that Ade$^+$ cells produce white colonies, whereas Ade$^-$ cells yield pink colonies. In principle, this assay allows one to visualize the existence of two populations of cells, each representing a different expression state, by the appearance of sectored colonies. Those cells in a stable on expression state will give rise to white sectors while cells in an off expression state will be represented in a pink sector. Alternatively, if partial derepression of the *hmr::ADE2* silent locus is due to an intermediate level of expression in every cell in the population, these cells would produce uniformly light-pink colonies.

To first determine if *ADE2* expression is subject to silenc-

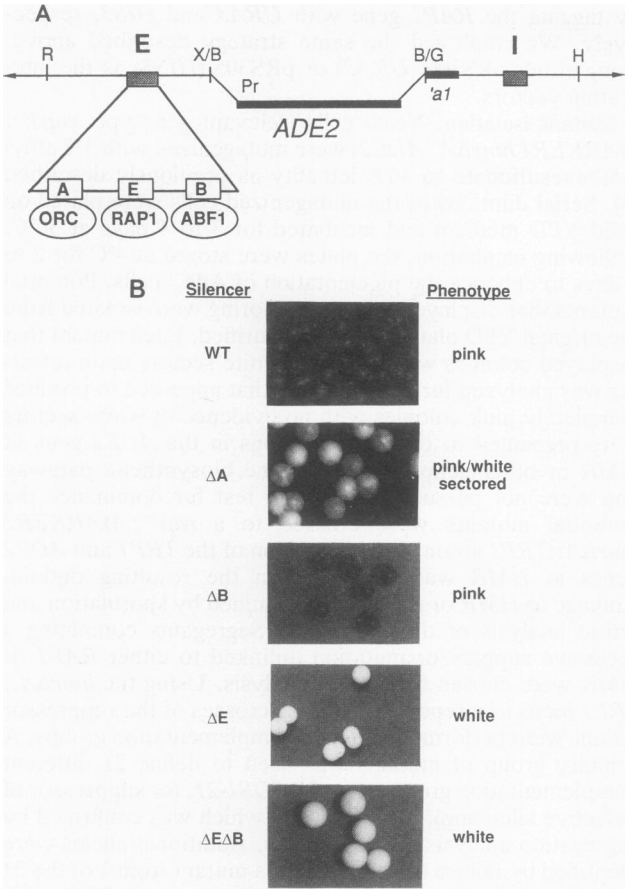

FIG. 1. Phenotypes of *HMR*::*ADE2* and *hmr*Δ::*ADE2* strains. (A) Schematic diagram of the *HMR*::*ADE2* locus. (R, *Eco*RI; X, *Xho*I; B, *Bam*HI; G, *Bgl*II; H, *Hin*dIII; Pr, promoter). (B) Colony phenotypes of *HMR*::*ADE2* and four different *hmr*Δ::*ADE2* strains. Cultures were grown overnight in YEPD medium before plating on YEPD plates. Colonies were grown for 2 to 3 days at 30°C. Plates were then placed at 4°C for at least 1 day before being photographed. All five strains are isogenic to each other; the *HMR-E* silencer is the only variable. The *HMR-E* silencer genotype is indicated to the left of the photographs, while the colony color phenotype is indicated to the right. WT, wild type.

er-mediated repression, we placed a *Bam*HI fragment containing the *ADE2* gene into a large deletion of the *HMR***a** silent locus (Fig. 1A; see Material and Methods for details). We then assayed *ADE2* expression in the context of a wild-type *HMR-E* silencer element, and various mutated silencers, by simply examining colony color. The mutated silencers examined contain linker substitution mutations in either the ARS consensus sequence (Δ*A*), the RAP1 binding site (Δ*E*), or the ABF1 binding site (Δ*B*) or a double mutation (Δ*E*Δ*B*) removing both silencer factor binding sites (7). The results are shown in Fig. 1B. As expected, strains in which the *ADE2* gene is in the context of a wild-type or Δ*B* silencer give rise to pink colonies, which are indistinguishable from the parent strain (which contains the *ade2-1* allele, an ochre mutation in the chromosomal gene and no additional copy at *HMR*), suggesting that the gene is completely repressed in these contexts. In contrast, the *ADE2* gene at *HMR* appears to be fully expressed when the silencer element is deleted for both the RAP1 and ABF1 binding sites because these *hmr*Δ*E*Δ*B*::*ADE2* cells form homogeneous white colonies.

However, when *ADE2* is placed in the context of an *hmr*Δ*A* silencer, pink- and white-sectored colonies are observed, indicating that repression is unstable in the context of this silencer. All previous assays have indicated that the *hmr*Δ*A* silencer is completely functional: no steady-state *a1* transcript can be detected by S1 analysis, and *hmr*Δ*A*::*TRP1* expression is repressed as indicated by a failure to form colonies on medium lacking tryptophan (7, 44). Apparently the *ADE2* gene differs from *a1* and *TRP1* in some way that allows it to occasionally escape repression by the *hmr*Δ*A* silencer. Once this happens, the derepressed state is relatively stable, as indicated by the size of the white sectors within the *hmr*Δ*A*::*ADE2* colonies. Although there are two clearly distinct phenotypic states in the *hmr*Δ*A*::*ADE2* mutant strain with respect to *ADE2* expression, we cannot be certain that they correspond to full repression or derepression. In fact, we often find that the pink sectors have a lighter appearance than uniformly repressed colonies. This may indicate a slight leakiness in the repressed state, in which occasional transcription of *ADE2* might occur. Alternatively, a rather high rate of switching in these colonies may be producing visually undetectable microsectors within large pink sectors that lead to a uniformly light-pink appearance.

Finally, we assayed *ADE2* expression in the context of a silencer deleted for the RAP1 binding site (E element). A deletion of this element has previously been shown to result in partial derepression of the locus both by quantitative S1 nuclease protection assays of *a1* mRNA levels and by measuring the ability of *hmr*Δ*E*::*TRP1* strains to grow on SC-Trp medium (7, 44). By using the colony color assay, the *hmr*Δ*E*::*ADE2* strain appears to be completely derepressed, giving rise to homogeneous white colonies.

For the wild-type and mutant silencers described above, we have constructed and examined strains with both orientations of the *ADE2* gene with respect to the E silencer. (The strains depicted in Fig. 1B all contain the *ADE2* gene with its promoter proximal to the silencer). We never observed a reproducible difference between the two orientations. In particular, the repressed or derepressed strains are invariant regardless of *ADE2* orientation, and the two sectoring *hmr*Δ*A* strains are indistinguishable. Most subsequent experiments were performed with the promoter-proximal constructions.

***rap1*^{s} mutants cause metastable repression of the *hmr*::*ADE2* locus.** *rap1*^{s} mutants have no effect on a wild-type *HMR* locus, but result in derepression when the ARS consensus element is deleted at the *HMR-E* silencer. However, the observation that *ADE2* expression at *HMR* appears to switch on and off when the ARS consensus element is deleted at *HMR-E*, a mutation that has no effect on *a1* or *TRP1* expression, prompted us to ask whether *rap1*^{s} mutations in a wild-type silencer background might also lead to metastable repression of *ADE2*. *rap1*^{s} mutations were introduced into strains containing an *HMR*::*ADE2* locus through a genetic cross, and the appropriate segregants were assayed for their colony color phenotype. The results are summarized in Table 2, and two representative *rap1*^{s} alleles are shown in Fig. 2. Consistent with the previous analysis of steady-state *a1* mRNA levels, neither of the weaker *rap1*^{s} alleles (*rap1-11* and *rap1-14*) allow derepression of the *HMR*::*ADE2* strain. However, *rap1-12* and *rap1-13 HMR*::*ADE2* strains display a low level of derepression revealed by colony sectoring. The majority of cells in the population are repressed, producing pink colonies, but a small yet significant percentage of sectored pink and white colonies is also evident. The appearance of sectored *rap1-12 HMR*::*ADE2*

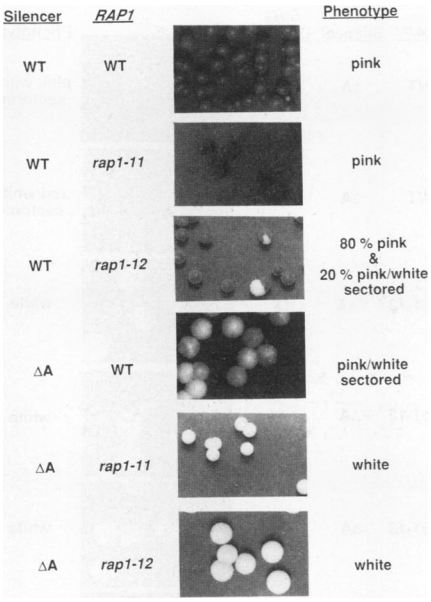

FIG. 2. The strong *rap1*^{s} mutation *rap1-12* causes derepression of *HMR*::*ADE2*. The phenotypes of representative *RAP1*$^+$ and *rap1*^{s} strains are presented here: wild-type (WT) (*RAP1*), weak *rap1*^{s} (*rap1-11*), and strong *rap1*^{s} (*rap1-12*) in both *HMR*::*ADE2* and *hmrΔA*::*ADE2* backgrounds.

colonies is qualitatively different from that of *RAP1 hmrΔA*::*ADE2* strains. The former produce fewer white (derepressed) sectors, yet these white sectors are generally larger and rarely switch back to the pink (repressed) state. These results suggest that the rates of switching between the two expression states differ between the two mutant strains. In the *rap1*^{s} strains, switching to either the repressed or derepressed state appears to occur at a lower rate than in the *hmrΔA*::*ADE2* strain.

We also tested the *rap1*^{s} mutants in an *hmrΔA*::*ADE2* background. The two strongest *rap1*^{s} alleles, *rap1-12* and *rap1-13*, completely derepress an *hmrΔA*::*ADE2* silencer, producing uniformly white colonies 100% of the time (Table 2; Fig. 2). The weaker alleles, *rap1-11* and *rap1-14*, give rise to a mixed population; most of the colonies are homogeneously white, but pink- and white-sectored colonies occasionally arise (Table 2). As in the experiments involving

TABLE 2. Summary of *rap1*^{s} phenotypes in *hmr*::*ADE2* and *hmrΔA*::*ADE2* backgrounds

Relevant genotype		Colony color[a] (%)
hmr::*ADE2*	*RAP1*	
wt[b]	wt	Pink (100)
wt	*rap1-11*	Pink (100)
wt	*rap1-12*	Pink (80) and p/w sectors (20)
wt	*rap1-13*	Pink (90) and p/w sectors (10)
wt	*rap1-14*	Pink (100)
ΔA	wt	P/w sectors
ΔA	*rap1-11*	White (90) and p/w sectors (10)
ΔA	*rap1-12*	White (100)
ΔA	*rap1-13*	White (100)
ΔA	*rap1-14*	White (90) and p/w sectors (10)

[a] p/w, pink and white.
[b] wt, wild type.

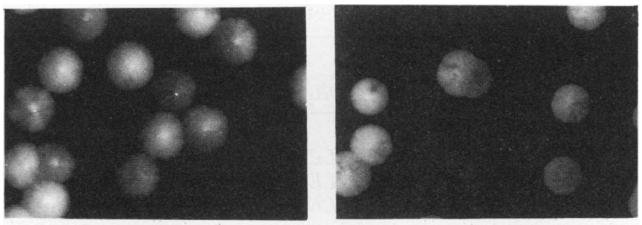

FIG. 3. The colony-sectoring phenotype of *hmrΔA*::*ADE2* diploid cells resembles that of isogenic haploid cells. The left panel shows a haploid *RAP1 hmrΔA*::*ADE2* strain, and the right panel shows an isogenic diploid strain.

single mutations either in the ARS consensus element or *RAP1*, the *ADE2* color assay appears to be more sensitive to the various double-mutant combinations than is either *a1* or *TRP1* expression. However, the relative levels of derepression observed amongst the *rap1*^{s} alleles in either the *HMR*::*ADE2* or *hmrΔA*::*ADE2* strains is consistent with assays of both *a1* and *TRP1* at *HMR*.

Two copies of *HMR* in a diploid cell appear to behave identically. A haploid *RAP1 hmrΔA*::*ADE2* strain gives rise to sectored pink and white colonies, indicating that the locus can exist in two relatively stable transcriptional states, allowing the formation of large sectors of cells with a given phenotype. We reasoned that a diploid strain containing a second copy of *hmrΔA*::*ADE2* could have two distinct phenotypes, depending on how the establishment of repression was controlled (36). If a change in the transcriptional state at *HMR* is a property of the locus, each copy of *hmrΔA*::*ADE2* would switch independently. This would double the probability of a switching event occurring in an individual cell as well as allowing each of the loci to exist simultaneously in a different transcriptional state. This effect should result in a marked decrease in the occurrence of pink sectors, which in our strain backgrounds would require the simultaneous repression of both *hmrΔA*::*ADE2* loci. If the switching event reflects a property of the cell, rather than a specific event at the affected silent locus, then both *hmr*::*ADE2* loci would be expected to exist in the same state, with switching occurring simultaneously. In this scenario, the sectoring phenotype of a diploid should be identical to that of the isogenic haploid strain.

As shown in Fig. 3, the degree of sectoring in a homozygous *hmrΔA*::*ADE2* diploid strain is indistinguishable from its isogenic haploid parent, with no detectable variation in pigment accumulation. Therefore, although the switching event at *HMR* would appear to be random, two different *HMR* loci in the same cell behave in a concerted fashion. The same behavior has been observed for cells containing two copies of *HML* and a *sir1* mutation (36).

Silencing of *hmr*::*ADE2* expression is *SIR* dependent and extremely sensitive to *SIR4* and *SIR1* gene dosage. The above analysis indicates that *ADE2* expression is regulated by the *HMR* silencer when the gene is inserted in place of the normal *a1* and *a2* genes. However, a novel expression state is observed when the ARS consensus element is deleted. In this case, genetically identical cells switch between Ade$^+$ (white) and Ade$^-$ (pink) phenotypes. To confirm that the transcriptional regulation of *hmr*::*ADE2* is *SIR* dependent, and therefore working via a mechanism similar to that which regulates the normal *HMRa* locus, we disrupted several of the *SIR* genes in the *hmr*::*ADE2* strains (21). The results are summarized in Table 3. As expected of *SIR*-dependent

TABLE 3. Phenotypes of *sir hmr::ADE2* strains

Relevant genotype[a]		Colony color
hmr::ADE2	*SIR*	
wt	*SIR*$^+$	Pink
wt	*sir1::LEU2*	White
wt	*sir3::LEU2*	White
wt	*sir4::LEU2*	White

[a] wt, wild type.

control, deletions of either *SIR3* or *SIR4* result in complete derepression of the locus, allowing expression of the *ADE2* gene and producing white colonies. We also constructed strains that were deleted for *SIR1*. The establishment of silencing at *HML* is impaired in *sir1* mutant strains, resulting in epigenetic switching between repressed and derepressed states. With the *hmr::ADE2* reporter assay, however, we detect complete derepression in the *sir1::LEU2* strains, resulting in white colonies.

In the course of constructing strains carrying extra copies of the *SIR* genes, we noticed that two chromosomal copies of *SIR4* in a haploid strain can suppress the defective *hmrΔEΔB* silencer, restoring nearly complete repression of a linked *TRP1* gene. To study this phenomenon further, we integrated an extra copy of *SIR4* into an *hmrΔA::ADE2* haploid strain. As described previously, an *hmrΔA::ADE2* strain is partially derepressed and produces sectored pink and white colonies. However, as shown in Fig. 4A, a second integrated copy of *SIR4* significantly improves the repression of this locus to produce a strain that forms predominantly red colonies containing some white sectors. The fact that we see both decreased sectoring and enhanced color pigmentation suggests that increased *SIR4* dosage affects both the establishment of silencing and the absolute level of repression of the off state. We also tested the ability of a duplication of *SIR4* to suppress the *rap1-12* or *rap1-13* defect in an *HMR::ADE2* background. As shown previously, each of these *rap1*^{s} alleles leads to a low level of depression of this silencer. The introduction of an additional copy of *SIR4* into these strains, however, appears to improve silencing (data not shown). We then asked whether just two copies of *SIR4* in a haploid strain was sufficient to suppress the *rap1*^{s} *hmrΔA::ADE2* double mutant. A *rap1-12* or a *rap1-13* allele was crossed into a haploid strain containing two integrated copies of *SIR4* and an *hmrΔA::ADE2* locus. As shown in Fig. 4A, two copies of *SIR4* are partially able to restore repression of this locus in a *rap1-13* strain but not in the more defective *rap1-12* strain. These results indicate that small increases in the dosage of *SIR4* can suppress two different mutations (ARS consensus deletion and *rap1*^{s}) that affect the establishment of repression.

To determine if the duplication of other *trans*-acting factors known to be involved in silencing could also restore repression of this locus, a second copy of either *RAP1* or one of the other *SIR* genes was introduced into the *hmrΔA::ADE2* strain, either by integration into the chromosome or by transformation with a centromere-containing (*CEN*) plasmid carrying the appropriate gene. Additional integrated copies of *RAP1*, *SIR1*, *SIR2*, or *SIR3* had little or no effect on the repression of the *hmrΔA::ADE2* locus. However, a copy of *SIR1* introduced on a *CEN* plasmid (which may be present at more than one copy per cell) did appear to increase repression of the *hmrΔA::ADE2* locus (data not shown).

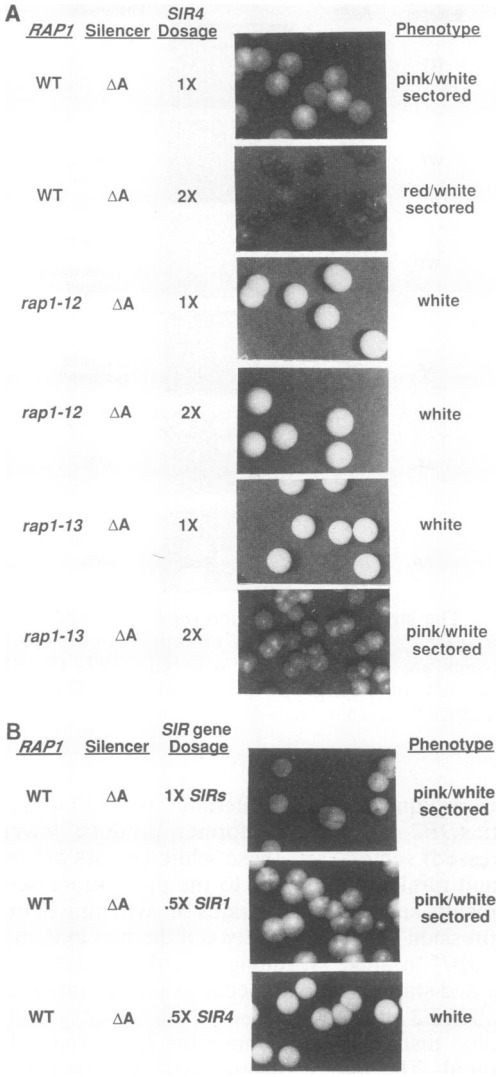

FIG. 4. *SIR4* gene dosage affects repression of *hmrΔA::ADE2*. (A) Effects of a single extra copy of *SIR4* in several *RAP1* *hmrΔA::ADE2* strains. Each strain shown is haploid with either the normal dosage of *SIR4* (1×) or an additional copy of *SIR4* integrated at the *HIS3* locus (2×). (B) The effect on *hmrΔA::ADE2* expression in strains containing half the normal complement of *SIR4* or *SIR1* genes. Each strain shown is a diploid containing either two copies of each of the *SIR* genes (1×), only one copy of *SIR1* (5×), or only a single copy of *SIR4* (.5×), WT, wild type.

Because small increases in both *SIR4* and *SIR1* gene dosage appear to affect silencing at *HMR*, we wanted to determine if a decrease in the normal dosage of these two genes also had an effect on silencing. To this end, diploid strains containing two *hmrΔA::ADE2* loci and only a single copy of either *SIR1* or *SIR4* were constructed. As shown in Fig. 4B, in a *sir1::LEU2/SIR1* diploid there is little or no change in silencing. At this level of analysis we are unable to detect a consistent phenotypic difference between the heterozygote and its *SIR1/SIR1* homozygote parent. Alternatively, the diploid strain containing only a single copy of *SIR4* is completely derepressed, indicating that the repression system is quite sensitive to the gene dosage of *SIR4*. At present, we cannot determine whether this effect results from a defect in maintenance of the repressed state, a

complete failure to establish repression, or a weakening of both functions.

A screen for new genes affecting the establishment of silencing. To identify factors that interact either directly or indirectly with RAP1 at the *HMR-E* silencer, we sought extragenic suppressors of the *rap1^s* mutants. The *hmrΔA*::*ADE2* colony color assay described above provided a sensitive means to screen for such suppressors. We carried out our screen in strains containing either of the stronger *rap1^s* mutant alleles (*rap1-12* or *rap1-13*) and the *hmrΔA*::*ADE2* silent locus. These strains produce uniformly white colonies. Extragenic suppressors that are able to compensate for the *rap1^s* defect should restore repression of the *hmrΔA*::*ADE2* locus to produce colonies with pink and white sectors, the phenotype observed in *RAP1 hmrΔA*::*ADE* strains. The mutant screen is outlined in Fig. 5A. From this type of analysis, we expect three general classes of mutants to arise: (i) *rap1^s* revertants or intragenic suppressors, (ii) mutations in the *ADE2* gene at *HMR* (or other genes in the adenine biosynthetic pathway), and (iii) extragenic suppressors of the *rap1^s* mutation, the ARS consensus mutation, or both defects. Mutations in the *ADE2* gene should be easily identified, as they would give rise to homogeneous pink colonies rather than sectored pink and white colonies. Revertants or intragenic suppressors can be distinguished from extragenic suppressor mutations by performing linkage analysis.

We first used this strategy to search for genes that when present in high copy number are able to partially suppress the *rap1^s hmrΔA* defect and give rise to sectored colonies. Direct experiments had shown that elevated gene dosage of either *SIR1* or *SIR4* can suppress *rap1^s* defects in both *hmrΔA*::*TRP1* strains (44) and *hmrΔA*::*ADE2* strains (see above). We hoped that by screening libraries of yeast genomic sequences, we might identify additional genes with this property. To this end, we introduced a multicopy (YEp24) library (a generous gift of M. Carlson) into the *rap1^s hmrΔA*::*ADE2* strain and looked for sectored colonies amongst the transformants, indicative of suppression. Approximately 33,000 transformants were examined. As expected, both *SIR1* and *SIR4* were isolated repeatedly from this screen (4 and 11 times, respectively), as were 9 isolates of *RAP1* (data not shown). We failed to identify any additional genes that could suppress the *rap1^s hmrΔA* defect when present on the multicopy plasmid. It is worth noting that we isolated several partial clones of *SIR4* in this screen that contained only a carboxy-terminal segment of the gene. Overexpression of the C-terminal portion of *SIR4* has previously been associated with an anti-SIR (derepression) effect (30). However, in the *rap1^s* strains, elevated dosage of *SIR4* or a C-terminal domain of *SIR4* has an opposite effect, resulting in restoration of repression.

We next attempted to isolate chromosomal mutations that would suppress the silencing defect of the *rap1^s hmrΔA* mutants. *rap1-12* or *rap1-13 hmrΔA*::*ADE2* strains were chemically mutagenized with ethyl methanesulfonate, plated on rich medium, and screened for the presence of pink- and white-sectored colonies. From approximately 60,000 colonies screened, >500 sectored colonies were identified from both *rap1^s* strains. Further genetic analysis (see Materials and Methods) revealed that 57 of these colonies arose from cells containing recessive mutations in single genes, unlinked to either *RAP1* or *hmrΔA*::*ADE2*, that were responsible for the colony-sectoring phenotype. The effect of these suppressor mutations is not specific to the *ADE2* gene, since they all restore repression in *rap1^s hmrΔA*::*TRP1* strains

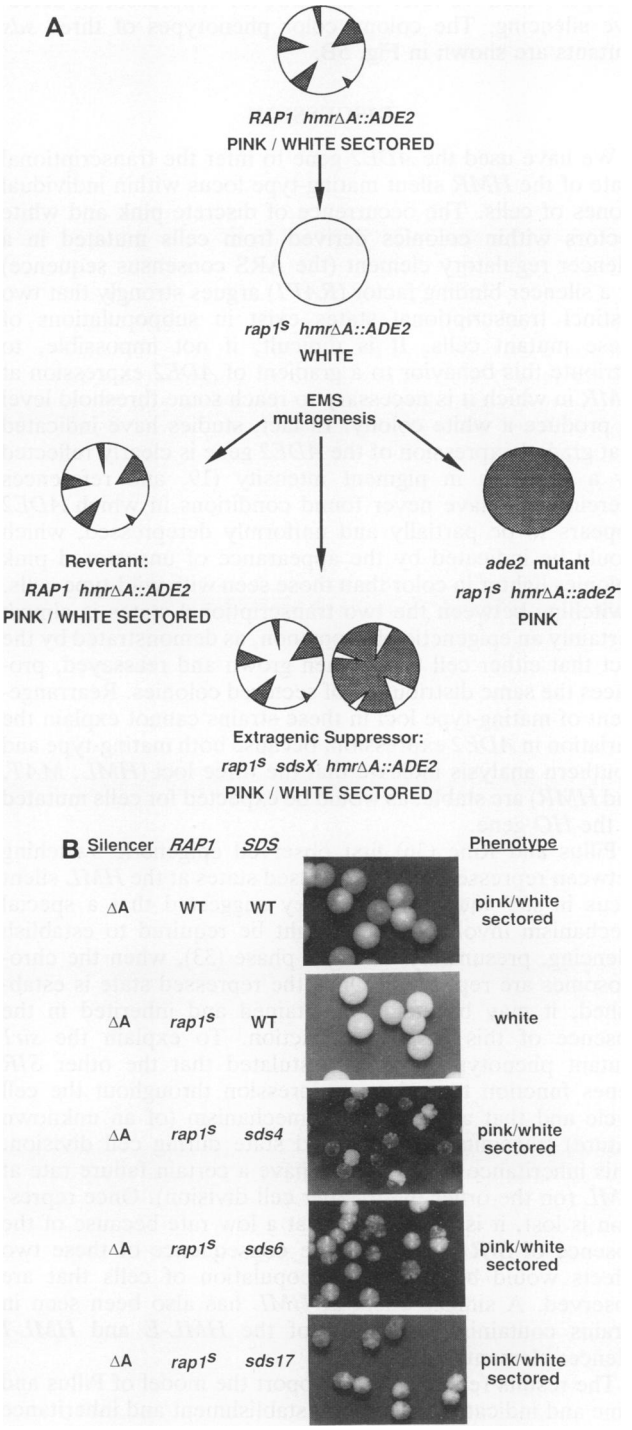

FIG. 5. Isolation of extragenic suppressors (*sds* mutants) in a silencing-defective *rap1^s hmrΔA*::*ADE2* strain by a colony color screen. (A) Diagram of the selection scheme for *sds* mutants. The predominant sectoring phenotype of suppressors might be expected to vary depending upon the strength of the allele and the ability to suppress both the *rap1^s* and *hmrΔA* mutations. (B) Colony color phenotypes of three different *sds* mutants, with parent strains for comparison. WT, wild type.

(data not shown). Complementation studies showed that these 57 mutants define 21 different complementation groups, which we refer to as *SDS*, for suppressor of defective silencing. The colony color phenotypes of three *sds* mutants are shown in Fig. 5B.

DISCUSSION

We have used the *ADE2* gene to infer the transcriptional state of the *HMR* silent mating-type locus within individual clones of cells. The occurrence of discrete pink and white sectors within colonies derived from cells mutated in a silencer regulatory element (the ARS consensus sequence) or a silencer binding factor (*RAP1*) argues strongly that two distinct transcriptional states exist in subpopulations of these mutant cells. It is difficult, if not impossible, to attribute this behavior to a gradient of *ADE2* expression at *HMR* in which it is necessary to reach some threshold level to produce a white colony. In fact, studies have indicated that graded expression of the *ADE2* gene is clearly reflected by a variation in pigment intensity (19, and references therein). We have never found conditions in which *ADE2* appears to be partially and uniformly derepressed, which would be indicated by the appearance of unsectored pink colonies lighter in color than those seen with wild-type cells. Switching between the two transcriptional states is almost certainly an epigenetic phenomenon, as demonstrated by the fact that either cell type, when grown and reassayed, produces the same distribution of sectored colonies. Rearrangement of mating-type loci in these strains cannot explain the variation in *ADE2* expression, because both mating-type and Southern analysis indicate that the three loci (*HML*, *MAT*, and *HMR*) are stable, as would be expected for cells mutated in the *HO* gene.

Pillus and Rine (36) first observed epigenetic switching between repressed and derepressed states at the *HML* silent locus in *sir1* mutant cells. They suggested that a special mechanism involving SIR1 might be required to establish silencing, presumably during S phase (33), when the chromosomes are replicated. Once the repressed state is established, it may be stably maintained and inherited in the absence of this assembly function. To explain the *sir1* mutant phenotype, it was postulated that the other *SIR* genes function to maintain repression throughout the cell cycle and that an inheritance mechanism (of an unknown nature) transmits the repressed state during cell division. This inheritance process must have a certain failure rate at *HML* (on the order of 10^{-3} per cell division). Once repression is lost, it is reestablished at a low rate because of the absence of *SIR1* function. The consequence of these two effects would be the mixed population of cells that are observed. A similar effect at *HML* has also been seen in strains containing mutations of the *HML-E* and *HML-I* silencer elements (29).

The results reported here support the model of Pillus and Rine and indicate that similar establishment and inheritance functions operate at the *HMR* locus. Viewed in this light, the sectoring phenotypes of both ARS consensus and *rap1^s* mutations suggest that they are defective in the establishment (or more precisely, reestablishment) of silencing. Heritable silencing is observed in these mutants, but when lost is reestablished at a low rate. The role of RAP1 or the ARS consensus element in the maintenance or inheritance of silencing remains to be tested directly. The involvement of the ARS consensus element in the establishment of silencing is consistent with earlier observations of Miller and Nasmyth (33), who showed that the reestablishment of silencing after a temperature shift in a *sir3^{ts}* strain requires progression through the S phase of the cell cycle. More recently, Rivier and Rine (38) showed that the *HMR-E* silencer functions in vivo as an origin of DNA replication. Moreover, they observed a correlation between origin and silencer function in different mutant silencer strains and proposed that initiation of DNA replication at *HMR-E* is required for the establishment of silencing. If this model is correct, one might predict that both *hmrΔA::ADE2* and *rap1^s HMR::ADE2* strains have reduced origin function at the *HMR-E* silencer.

Although the parallels between *HMR* and *HML* are striking, one significant difference to be noted is the fact that *SIR1* appears to be required for maintenance of repression at *hmr::ADE2*, whereas at *HML* it appears to be required only for establishment of silencing. One explanation of these results is that *SIR1* plays different roles at *HML* and *HMR*. We prefer another explanation which suggests a varying requirement for the establishment function at every S phase, depending upon the silencer element and repressed gene. Because the repressed state (whether it involves a covalent modification of the DNA or a change in chromatin structure) must be replicated during S phase, it is easy to imagine that repression is particularly sensitive to disruption at this stage of the cell cycle. The efficiency with which the repressed state is inherited during chromosome replication may vary depending upon several factors, such as the ability of activator proteins to compete for binding to the promoter of the silenced gene, or the inherent ability of the silenced DNA to be folded into a condensed conformation. The differences that we observe between silencing of *ADE2* and either *a1* or *TRP1* may be explained by poor inheritance of the repressed state of *HMR::ADE2* and thus a more stringent requirement for reestablishment functions at S phase to allow continuous *ADE2* repression. We have noted previously that other genes (e.g., *LEU2*, *URA3*, and *HIS3*) also appear to be less susceptible to repression than are *a1* or *TRP1* when placed at *HMR* (44a). It will be interesting to determine whether these differences can be traced to particular promoter or UAS elements at these individual genes.

Mutations in the ARS consensus sequence at the *HMR-E* silencer and *rap1^s* mutations have revealed a sensitivity of the silencer to the gene dosage of both *SIR4* and *SIR1*. The case of *SIR4* is particularly striking, because both lower and higher than normal gene dosages can affect silencing. Previous studies have indicated that overexpression of carboxy-terminal fragments of SIR4 disrupts silencing (21, 30). Studies reported here, however, demonstrate that both the rate of establishment and the degree of silencing can be improved by only a single extra copy of the *SIR4* gene in both ARS consensus sequence and *rap1^s* mutants. In addition, SIR4 activity is limiting in diploids containing only a single copy of the gene. Gene dosage effects have been observed for a number of protein assemblies (11, 14, 31), leading to the suggestion that SIR4 participates in forming a complex at the silencer involving RAP1 and the origin recognition complex (ORC [5]). When the ARS consensus element or RAP1 is mutated, more SIR4 may be required to assemble a functional silencer complex. Stabilization of this hypothetical complex might occur through a direct interaction with RAP1, the ORC, or SIR3, whose overexpression can partially counteract the derepressing effect of the SIR4 carboxy terminus (30). The fact that silencing depends so critically on the proper *SIR4* gene dosage raises the possibility that the level and/or activity of SIR4 protein plays an important role in the switch between repressed and derepressed states.

Perhaps the simultaneous switching of both copies of *hmrΔA::ADE2* observed in diploids is related to the activity of SIR4 in these cells. *SIR1* gene dosage does not show the same critical concentration effect that is seen with *SIR4*, but instead simply appears to be limiting in various mutant conditions, including but not limited to mutations in RAP1, its silencer binding site, and the ARS consensus element (43, 44). With this in mind, one might imagine that SIR1 functions as an assembly factor for establishment of repression but is not itself a component of a protein complex at the silencer.

An important benefit of the *ADE2* colony color assay for silencing is that it has provided us with an effective new screen to identify genes involved in silencing. A potential advantage of this method is that it may be more sensitive than screens based on mating. The screen described here appears to have identified genes whose wild-type function is inhibitory to the establishment of silencing. Related screens using the *ADE2* colony color assay should allow the identification of new genes that promote the establishment of silencing. The identification of 21 different *SDS* genes, mutations in which are suppressors of *rap1^s hmrΔA* mutants, suggests that a complex set of factors may influence the establishment of silencing at *HMR*. Many *SDS* genes have probably been missed in previous genetic screens that favored the identification of factors required for the maintenance of silencing. It will be interesting to determine whether any of the *SDS* gene products interact directly with *rap1^s* or the mutated *hmrΔA* silencer element or whether they function indirectly, perhaps by generating chromatin structures permissive to transcription which the silencing machinery must counteract. Alternatively, the *sds* mutants might affect the function of the silencer-associated ARS in such a way as to improve the chances of reestablishing repression during S phase. It is worth noting in this regard that a temperature-sensitive allele of *CDC7*, a serine-threonine kinase required for the initiation of DNA replication, was identified as a suppressor of a double point mutant silencer, lacking both the RAP1 and ABF1 binding sites (4).

In conclusion, the results reported here support the generality of a silencer-mediated function involved in the establishment of repression (36) and provide the first molecular characterization of this phenomenon at the *HMR* silent mating-type locus. In particular, we have demonstrated a direct role for the silencer binding protein RAP1 and the ARS consensus sequence at the *HMR-E* silencer in the establishment of repression. The efficiency of this establishment function at *HMR* was further shown to be extremely sensitive to the dosage of the *SIR4* gene and, to a lesser extent, *SIR1*. Finally, we have shown that the *ADE2* colony color assay, applied previously to the study of unstable telomeric silencing (2), can be used in a genetic screen to identify genes with possible roles in the establishment of repression at the normally stably repressed *HMR* locus. Further study of the *SDS* genes promises to provide insights into the molecular mechanisms underlying the assembly of repressed chromatin in *S. cerevisiae*.

ACKNOWLEDGMENTS

We thank K. Huang for assistance and advice in photography, D. Gottschling for providing us with the yeast *ADE2* gene, M. Carlson for the YEp24 yeast genomic library, members of the Shore laboratory for useful discussions, and H. Young and R. Sternglanz for helpful comments on the manuscript.

This work was supported by grants from the NIH (GM40094), the American Cancer Society (JFRA-231 and MV-534), the Searle Scholars Fund/Chicago Community Trust, and the Irma T. Hirschl Charitable Trust to D.S. and by an ACS Institutional Research Grant (IRG-177A) to the Comprehensive Cancer Center at Columbia University. L.S. and D.V. were predoctoral trainees on an NIH Cancer Biology grant (CA09503-0).

REFERENCES

1. **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.
2. **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.
3. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1987. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
4. **Axelrod, A., and J. Rine.** 1991. A role for *CDC7* in repression of transcription at the silent mating-type locus *HMR* in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **11:**1080–1091.
5. **Bell, S. P., and B. Stillman.** 1992. ATP-dependent recognition of eukaryotic origins of DNA replication by a multiprotein complex. Nature (London) **357:**128–134.
6. **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.
7. **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.
8. **Buchman, A. R., W. J. Kimmerly, J. Rhine, 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.
9. **Buchman, A. R., N. F. Lue, and R. D. Kornberg.** 1988. Connections between transcriptional activators, silencers, and telomeres as revealed by functional analysis of a yeast DNA-binding protein. Mol. Cell. Biol. **8:**5086–5099.
10. **Chambers, A., J. S. H. Tsang, C. Stanway, A. J. Kingsman, and S. M. Kingsman.** 1989. Transcriptional control of the *Saccharomyces cerevisiae PGK* gene by RAP1. Mol. Cell. Biol. **9:**5516–5524.
11. **Clark, A. C. D., D. Norris, M. A. Osley, J. S. Fassler, and F. Winston.** 1988. Changes in histone gene dosage alter transcription in yeast. Genes Dev. **2:**150–159.
12. **Dubey, D. D., L. R. David, S. A. Greenfeder, L. Y. Ong, J. Zho, J. R. Broach, C. S. Newton, and J. A. Huberman.** 1991. Evidence suggesting that the *ARS* elements associated with silencers of the yeast mating-type locus *HML* do not function as chromosomal DNA replication origins. Mol. Cell. Biol. **11:** 5346–5355.
13. **Feldman, J. B., J. B. Hicks, and J. R. Broach.** 1984. Identification of sites required for repression of a silent mating-type locus in yeast. J. Mol. Biol. **178:**815–834.
14. **Floor, E.** 1970. Interaction of morphogenetic genes of bacteriophage T4. J. Mol. Biol. **47:**293–306.
15. **Giesman, D., L. Best, and K. Tatchell.** 1991. The role of RAP1 in the regulation of the *MATα* locus. Mol. Cell. Biol. **11:**1069–1079.
16. **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.
17. **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.
18. **Hieter, P., C. Mann, M. Snyder, and R. W. Davis.** 1985. Mitotic stability of yeast chromosomes: a colony color assay that measures nondisjunction and chromosome loss. Cell **40:**381–392.
19. **Hieter, P., D. Pridmore, J. H. Hegemann, M. Thomas, R. W. Davis, and P. Philippsen.** 1985. Functional selection and analysis of yeast centromeric DNA. Cell **42:**913–921.
20. **Huet, J., P. Cottrelle, M. Cool, M.-L. Vignais, D. Theile, C. Marck, J.-M. Buhler, A. Sentenac, and P. Fromageot.** 1985. A

general upstream binding factor for genes of the yeast translational apparatus. EMBO J. **4**:3539–3547.
21. **Ivy, J. M., A. J. S. Klar, and J. B. Hicks.** 1986. Cloning and characterization of four *SIR* genes from *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6**:688–702.
22. **Kayne, P. S., U. J. Kim, M. Han, J. R. Mullen, F. Yoshizaki, and M. Grunstein.** 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.
23. **Kimmerly, W., A. Buchman, R. Kornberg, and J. Rhine.** 1988. Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J. **7**:2241–2253.
24. **Kimmerly, W. J., and J. Rine.** 1987. Replication and segregation of plasmids containing cis-acting regulatory sites of silent mating-type genes in *Saccharomyces cerevisiae* are controlled by the SIR genes. Mol. Cell. Biol. **7**:4225–4237.
25. **Kurtz S., and D. Shore.** 1991. The RAP1 protein activates and silences transcription of mating-type genes in yeast. Genes Dev. **5**:616–628.
26. **Laurenson, P., and J. Rine.** 1992. Silencers, silencing, and heritable transcriptional states. Microbiol. Rev. **56**:543–560.
27. **Longtine, M. S., N. M. Wilson, M. E. Petracek, and J. Berman.** 1989. A yeast telomere binding activity binds to two related telomere sequence motifs and is indistinguishable from RAP1. Curr. Genet. **16**:225–240.
28. **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.
29. **Mahoney, D. J., R. Marquardt, G.-J. Shei, A. B. Rose, and J. R. Broach.** 1991. Mutations in the *HML* E silencer of *Saccharomyces cerevisiae* yield metastable inheritance of transcriptional repression. Genes Dev. **5**:605–615.
30. **Marshall, M., D. Mahoney, A. Rose, J. B. Hicks, and J. R. Broach.** 1987. Functional domains of *SIR4*, a gene required for position effect regulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7**:4441–4452.
31. **Meeks-Wagner, D., and L. Hartwell.** 1986. Normal stoichiometry of histone dimer sets is necessary for high fidelity of mitotic chromosome transmission. Cell **44**:43–52.
32. **Megee, P. C., B. A. Morgan, B. A. Mittman, and M. M. Smith.** 1990. Genetic analysis of histone H4: essential role of lysines subject to reversible acetylation. Science **247**:841–845.
33. **Miller, A. M., and K. A. Nasmyth.** 1984. Role of DNA replication in the repression of silent mating-type loci in yeast. Nature (London) **312**:247–251.
34. **Mullen, J. R., P. S. Kayne, R. P. Moerschell, S. Tsunasawa, M. Gribskov, S. M. Colavito, M. Grunstein, F. Sherman, and R. Sternglanz.** 1989. Identification and characterization of genes and mutants for an N-terminal acetyltransferase from yeast. EMBO J. **8**:2067–2075.
35. **Park, E.-C., and J. W. Szostak.** 1990. Point mutations in the yeast histone H4 gene prevent silencing of the silent mating type locus *HML*. Mol. Cell. Biol. **10**:4932–4934.
36. **Pillus, L., and J. Rine.** 1989. Epigenetic inheritance of transcriptional states in S. cerevisiae. Cell **59**:637–647.
37. **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.
38. **Rivier, D. H., and J. Rine.** 1992. An origin of DNA replication and a transcriptional silencer require a common element. Science **256**:659–663.
39. **Rothstein, R.** 1983. One-step gene disruption in yeast. Methods Enzymol. **101**:202–211.
40. **Sherman, F., G. R. Fink, and J. B. Hicks.** 1983. Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
41. **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.
42. **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.
43. **Stone, E. M., M. J. Swanson, A. M. Romeo, J. B. Hicks, and R. Sternglanz.** 1991. The SIR1 gene of *Saccharomyces cerevisiae* and its role as an extragenic suppressor of several mating-defective mutants. Mol. Cell. Biol. **11**:2253–2262.
44. **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.
44a. **Sussel, L., and D. Shore.** Unpublished data.
45. **Whiteway, M., R. Freeman, S. Van Arsdell, J. W. Szostak, and J. Thorner.** 1987. The yeast *ARD1* gene product is required for repression of cryptic mating-type information at the *HML* locus. Mol. Cell. Biol. **7**:3713–3722.