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A Role for CDC7 in Repression of Transcription at the Silent Mating-Type Locus *HMR* in *Saccharomyces cerevisiae*

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The mating-type genes at *MAT* in *Saccharomyces cerevisiae* are expressed, whereas the same genes located at *HML* and *HMR* are transcriptionally repressed. The DNA element responsible for repression at *HMR* has been termed a silencer and contains an autonomous replication sequence, a binding site for GRFI/RAPI, and a binding site for ABFI. A double-mutant *HMR-E* silencer that contains single nucleotide substitutions in both the GRFI/RAPI- and ABFI-binding sites no longer binds either factor in vitro, nor represses transcription at *HMR* in vivo. In *MAT* α cells, this derepression of a information results in a nonmating phenotype. Second-site suppressor mutations were isolated that restored the α mating phenotype to *MAT* α cells containing the double-mutant silencer. One of these suppressors, designated *sas1-1*, conferred a temperature-sensitive lethal phenotype to the cell. *SAS1* was found to be identical to *CDC7*, a gene which encodes a protein kinase required for the initiation of DNA replication. This new allele of *CDC7* was designated *cdc7-90*. *cdc7-90* restored the α mating phenotype by restoring silencing. The original allele of *CDC7*, isolated on the basis of the cell cycle phenotype it confers, also restored silencing, and overexpression of CDC7 interfered with silencing. *cdc7-90* did not restore detectable binding of GRFI/RAPI or ABFI to the double-mutant silencer in vitro. These results indicate that a reduced level of CDC7 function restores silencing to a locus defective in binding two factors normally required for silencing.

A large body of circumstantial evidence correlates aspects of DNA replication and changes in chromatin structure with changes in gene expression. The classic example of a correlation of chromatin structure with gene expression is the heterochromatic condensation and associated transcriptional repression of an entire X chromosome in female mammals (12). Alterations in histone gene dosage have been shown to alter transcription in *Saccharomyces cerevisiae* (8), providing a clear demonstration that a change in chromatin structure can cause a change in gene expression. The relationship between DNA replication and gene expression is less clear. In some cases the two processes may simply share the same factors. For example, both transcription and replication can be activated by the CTF/NFI protein (32). In other cases replication may be required to remove the existing chromatin structure from DNA so that a new structure may be established. In support of this idea, it has been shown that cell cycle progress is required for induction of *PHO5* (14). Similarly, the establishment of repression at the silent mating-type loci *HMR* and *HML* in *Saccharomyces cerevisiae* requires passage through the S phase of the cell cycle (33). Several lines of evidence, discussed below, suggest that DNA replication and chromatin structure influence the transcriptional repression of *HML* and *HMR*. Investigation of the mechanism of repression of *HML* and *HMR* should further our understanding of the effects of DNA replication and chromatin structure on gene expression.

The *MAT* locus encodes the master regulators of cell type in *S. cerevisiae*. The two mating types, **a** and α, are determined by the *MAT***a** and *MAT*α alleles, respectively. Conjugation of **a** and α cells results in the formation of the **a**/α diploid cell type, which expresses both *MAT***a** and *MAT*α information and consequently is unable to mate. *MAT***a** and *MAT*α each contain two divergently transcribed genes (**a**1 and **a**2 in *MAT***a**, and α1 and α2 in *MAT*α). The **a** or α genes at the *MAT* locus are the only expressed copies of the mating-type genes. However, in most laboratory strains, repressed copies of the α and the **a** genes are present at the *HML* and *HMR* loci located near the left and right telomeres of chromosome III, respectively. Although the naturally occurring *HML*α and *HMR***a** genes are not expressed, they are identical to their counterparts at *MAT*, containing intact structural genes and promoters. Cells that are unable to maintain *HML* and *HMR* in a transcriptionally repressed state are unable to mate as a result of simultaneous expression of **a** and α genes within the same cell (reviewed in references 19 and 20).

Repression of the silent mating-type loci is mediated by flanking sequences that are approximately 1 kb away from the promoters of these loci. The regulatory sequences on the left of both *HML* and *HMR* are designated *E* elements, and the regulatory sequences on the right of these loci are designated *I* elements (1, 11). *HMR-E* is essential for repression of mating-type information at *HMR*, whereas *HMR-I* is required only for complete repression (1, 24). A 530-bp fragment containing *HMR-E* has the ability to repress transcription at *HMR* in a distance- and orientation-independent manner and therefore has been termed a silencer (4). Mutational analysis of this silencer has revealed three partially redundant elements: any two of the three are sufficient for repression. These three genetically defined elements are coincident with (i) an autonomous replication sequence (ARS), (ii) a binding site for the protein GRFI/RAPI, and (iii) a binding site for the protein ABFI (5, 26). Both GRFI/RAPI and ABFI bind a variety of other sites in the yeast genome. Although GRFI/RAPI and ABFI have been implicated in repression of transcription at *HMR*, in other contexts they can act as transcriptional activators; they thus appear to serve different functions in different contexts (see references 6, 7, and 45 and references therein). Genetic evidence

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suggests that GRFI/RAPI is the protein that actually binds and regulates *HMR* in vivo (16a); however, there is as yet no direct evidence that ABFI acts at *HMR*.

Additional factors that are required in *trans* to repress transcription at *HMR* and *HML* were identified by mutations that result in the derepression of these loci. *SIR1* (silent information regulator) aids in repression, whereas *SIR2*, *SIR3*, and *SIR4* are absolutely required for repression at *HMR* and *HML* (39). The mechanisms by which the SIR proteins act remain unknown, although SIR1 has been shown to aid in the establishment of repression as opposed to the maintenance of the repressed state (37). Changes in the histone components of chromatin also affect repression of *HMR* and *HML*. Deletion of the N terminus of histone H4 results in complete derepression of *HMR* and *HML* (25), and site mutations in the histone H4 N terminus severely compromise repression (23, 31). A role for chromatin structure in the transcriptional repression of *HMR* and *HML* is not surprising, considering the general nature of the inactivation of these loci. For example, the HO endonuclease can cleave its recognition sequence at *MAT*, but cannot cleave the same sequence at *HML* or *HMR* (28, 49). Also, several unrelated genes are transcriptionally repressed when positioned at *HMR* or *HML*, including the *TRP1* and *LEU2* genes (4) and the tRNA gene, *SUP3am*, which is transcribed by RNA polymerase III (43). Thus, transcriptionally inactive chromatin, analogous to heterochromatin, is presumably part of the mechanism by which the silent mating-type loci are repressed.

Several lines of circumstantial evidence suggest that replication also plays a role in the transcriptional silencing of *HMR* and *HML*. First, *HMR-E*, *HMR-I*, *HML-E*, and *HML-I* each contain ARS elements and thus may function as origins of DNA replication. The relative infrequency of ARS elements throughout the genome suggests that a chance occurrence of an ARS at each of these regulatory sites would be statistically unlikely (1, 11). Second, the construction of a synthetic silencer, composed of the three known silencer elements (an ABFI-binding site, a GRFI/RAPI-binding site, and an ARS) but differing from the wild-type *HMR-E* silencer with respect to sequences between and directly flanking the three elements, revealed that both the ARS function and the silencer function of the synthetic silencer depend on the sequences directly flanking the silencer. Flanking sequences that provide ARS function allow silencer function, whereas flanking sequences that do not provide ARS function do not allow silencer function (30a). Third, it has been shown that establishment of repression requires passage through the S phase of the cell cycle (33). Nevertheless, there is no direct evidence that initiation of DNA replication at *HMR* is required for silencing. In fact, it has been shown that the *HML-E* element does not function efficiently as a chromosomal origin of replication in wild-type cells (52).

To learn more about the mechanism of transcriptional silencing and the relative roles of the three elements within the silencer, we isolated mutations that could restore silencing in strains containing a defective *HMR-E* silencer. The silencer was rendered nonfunctional by a combination of two mutations: a single base pair change in the GRFI/RAPI-binding site (GRFI-IIA) and a single base pair change in the ABFI-binding site (ABFI-IA). These mutant binding sites are not recognized by their corresponding factors in vitro. Each of these mutations independently has little or no effect on transcriptional repression, yet, when combined, they lead to almost complete derepression of *HMR***a** (26). We refer to an *HMR* allele containing this double-mutant *HMR-E* silencer as *HMR***a**-*e***.

This study describes the isolation of several suppressors referred to generically as *sas* mutations (for something about silencing). In principle, these suppressor mutations could restore silencing by a number of different mechanisms: (i) restoring the binding of GRFI/RAPI or ABFI to the mutant sites; (ii) altering proteins that mediate the effect of the silencer upon transcription; (iii) bypassing the need for the silencer altogether; or (iv) altering a process that functions through the third silencer domain, the ARS. Further characterization of one of these suppressors revealed that *SAS1* was *CDC7*, a cell division cycle gene whose product is required for cell cycle progress past the G1/S boundary. The *CDC7* product is a Ser-Thr protein kinase (21, 36) that is required for the initiation of DNA replication (17). An analysis of the role of *CDC7* in silencer function is presented.

MATERIALS AND METHODS

Strains, media, and genetic methods. The strains and crosses used in this study are described in Table 1. All media were prepared as described previously (40), except that minimal complete medium was prepared by supplementing SD with 0.5% Casamino Acids and with tryptophan, adenine, and additional supplements for auxotrophic markers at 30 μg/ml. Lactate plates were prepared by replacing the glucose in YPD with 2% lactic acid and adjusting the pH to 6.0 with NaOH. Yeast genetic manipulation, ethyl methanesulfonate mutagenesis, isolation of DNA, and transformation after lithium acetate treatment were performed as described previously (40). Mating types were determined as described previously (39), with mating-type tester strains 227 (**a**) and 70 (α) unless otherwise noted. Strains containing plasmids were grown on minimal complete medium lacking uracil and were replica plated onto a *MAT***a** *ura3* (TD4) or a *MAT*α *ura3* (DBY703) tester lawn to ensure that the mating of only plasmid-bearing cells was measured. The efficiency of mating was determined as described previously (39) with the tester strain TD4 (**a**) or DBY703 (α). Cells containing a temperature-sensitive lethal mutation were grown at 22°C as the permissive temperature and at 34 or 37°C as the nonpermissive temperature.

Genetic test for functional a1 information at *HMR*. The assay for functional **a**1 information was based on the observation that diploids require **a**1 protein to sporulate. Each of the *sas* mutants (*HML*α *MAT*α *HMR***a**-*e*** *sasx*) was mated with JRY79, an *HML*α *MAT*α *HMR*α *HO* strain, and diploids were isolated by selecting for prototrophic growth. JRY79 was able to mate inefficiently as an **a**, presumably owing to transient cleavage at *MAT* by the HO endonuclease. (Lack of expression of α information produces an **a** mating phenotype, since the **a** mating type requires neither **a** nor α gene products.) However, the diploids formed (*HML*α/*HML*α *MAT*α/*MAT*α *HMR*α/*HMR***a**-*e*** *HO*/*ho SASX*/*sasx*) had only one potential source of **a**1 information, located at *HMR***a**-*e***. If an intact **a**1 gene is present at *HMR*, it will be transposed to the *MAT* locus by the HO endonuclease, and at the *MAT* locus, the **a**1 gene will be expressed and will allow the diploid to sporulate. Strains carrying mutations in the **a**1 gene cannot sporulate.

Construction of an *HMR*α-*e* allele.** A strain containing an *HMR*α-*e*** allele was obtained by screening for a meiotic recombination/gene conversion between *HMR*α and *HMR***a**-*e*** (Fig. 1). A *mat***a**1 *HMR***a**-*e*** strain (JRY2612) was

TABLE 1. Strains used in this study

Strain	Genotype[a]	Source[b]
Haploid		
70	*MATα thr3-10*	I. Herskowitz
227	*MATa lys1-1*	I. Herskowitz
DBY703	*MATα his3 trp1 ura3-52 cir°*	D. Botstein
DBY1039	*MATa suc1 his4 ura1*	D. Botstein
DG168	*matα-1613 his4 leu2 trp1 ura3*	D. Giesman and K. Tatchell
DG169	*matα-1617 his4 leu2 trp1 ura3*	D. Giesman and K. Tatchell
RM14-3A	*MATa bar1 his6 leu2-3,112 trp1-289 ura3-52 cdc7-1*	W. Fangman
TD4	*MATa his4-519 leu2-3,112 trp1 ura3-52*	G. Fink
AMR27	*MATa ade2-1 can1-100 his3-11 leu2-3,112 trp1-1 ura3-1 sir1-23.2::LEU2*	R. Sternglanz
JRY63	*matal ade2 leu1 ura3 can1-11 cyh2-21 rme1*	
JRY79	*MATα HMLα HMRα HO ade5 his5 met4 ura4*	
JRY80	*matal HMLα HMRα ade2 leu1 ura3 can1-11 cyh2-21 rme1*	
JRY527	*MATa ade2-101 his3Δ200 lys2-801 ura3-52 Met−*	
JRY528	*MATα ade2-101 his3Δ200 lys2-801 tyr1 ura3-52*	
JRY1303	*MATa ade2-101 his3Δ200 lys2-801 ura3-52 Met− sir3::HIS3*	
JRY2069	*MATα HMRa-e** ade2-101 his3 lys2 tyr1 ura3-52*	
JRY2262	*MATα HMRa-e** ade2-101 his3 lys2 tyr1 ura3-52 cdc7-90*	
JRY2467	*MATα ade2-101 his3Δ200 leu2 lys1 lys2-801 ura3-52 sum1-1 sir4::HIS3*	
JRY2516	*MATα his4*	
JRY2608	*MATα HMRa-e** ade2-101 his3 lys2 tyr1 ura3-52* (*cdc7-90* reverted)	
JRY2611	*MATa HMRa-e** ade2-101 his3 leu2-3,112 trp1Δ*	
JRY2612	*matal HMRa-e** ade2 leu2-3,112 ura3*	
JRY2615	*MATα HMRa-e** ade2 his3 lys2-801 tyr1 cdc7-90*	
JRY2616	*MATα HMRa-e** ade2 his3 leu2-3,112 tyr1 ura3 cdc7-90*	
JRY2619	*matal ade2 his3 lys2-801 tyr1 ura3 cdc7-90*	
JRY2622	*MATα ade2 can1 his3Δ200 leu2-3,112 lys2-801 ura3 cdc7-90*	
JRY2625	*MATα ade2-101 his3Δ200 lys2-801 Met⁻ ura3-52 YIp5::cdc7*(pJR749)	
JRY2630	*MATa ade2-1 can1 his3 leu2-3,112 ura3 cdc7-90*	
JRY2631	*MATα ade2-1 can1 his3 leu2-3,112 ura3 cdc7-90*	
JRY2634	*MATa can1 lys2-801 ura3 cdc7-90*	
JRY2635	*matal HMRα-e** ade2 leu2-3,112 ura3*	
JRY2641	*MATa HMRα-e** ade2 leu2-3,112 ura3*	
JRY2649	*matal HMRα-e** lys2-801 ura3 cdc7-90*	
JRY2653	*MATα HMRa-e** ade2 leu2-3,112 trp1-289 ura3 cdc7-1*	
JRY2822	*MATα ade2 ura3 cdc7-90*	
Diploid		
JRY2617	JRY63 × JRY2615	
JRY2949	JRY2615 × JRY2619	

[a] Unless otherwise noted, all strains were *HMLα* and *HMRa*.
[b] Unless otherwise noted, all strains were laboratory stocks or were constructed in the course of this study.

crossed with a *matal HMRα* strain (JRY80) containing *MATα* on a plasmid. The plasmid allowed JRY80 to mate as an α, and in the diploid it provided the α mating-type information required for sporulation. Upon sporulation of the diploid, random spores were germinated on 5-fluoroorotic acid plates to select against *URA3* expression and, consequently, for plasmid loss (40). Neither *matal HMRa-e*** nor *matal HMRα* segregants could mate with a *MATa* lawn. However, a *matal* strain containing a recombinant *HMRα-e*** allele would express the α information at *HMR* and thus could mate with a *MATa* lawn. A segregant with the α mating type (JRY2635) was isolated, and DNA blot hybridization analysis indicated that an *HMRα-e*** allele was indeed present in this strain (data not shown). Furthermore, 35% recombination between *HMRα-e*** and *matal* was observed, consistent with the previously reported 39% recombination between *MAT* and *HMR* (34). Although JRY2635 exhibited a strong α mating phenotype, it also exhibited a weak **a** mating phenotype (bimating), indicating that the α1 and α2 genes were not fully expressed from *HMRα-e***. A *MATa HMRα-e*** strain (JRY2641) and a *MATal HMRα-e** cdc7-90* strain (JRY2649) were obtained as segregants from a cross between the *matal HMRα-e*** strain (JRY2635) and a *MATa cdc7-90* strain (JRY2634). Neither *MATa HMRα-e*** nor *MATa HMRα-e** cdc7-90* was able to mate as an α, because **a**1 expression from *MAT*, in combination with α2 from *HMRα-e***, causes the nonmating phenotype of an **a**/α diploid (50). However, both strains exhibited strong **a** mating phenotypes, because the α genes were not fully expressed from *HMRα-e***. The presence of the *HMRα-e*** allele in *MATa* strains was determined by DNA blot hybridization analysis.

Plasmids and in vitro DNA manipulations. Plasmid DNA preparation and in vitro DNA manipulations were performed as described previously (30). Plasmids were transformed into *Escherichia coli* TG1, DHI, or DH5αF′ by the procedure of Hanahan (15). Double-stranded plasmid DNA was sequenced with the Sequenase II system (Amersham) as recommended by the manufacturer. DNA blots were transferred to Zetaprobe membrane (Bio-Rad) and hybridized as recommended by the manufacturer. The yeast plasmid library used to clone the *SAS1* gene was a gift from F. Spencer and P. Hieter (48).

The probe used to measure **a**1 mRNA levels was a 473-bp fragment of the **a**1 gene (from the *Fok*I site at the 5′ end of the **a**1 mRNA to the *Bgl*II site at the 3′ end of the mRNA),

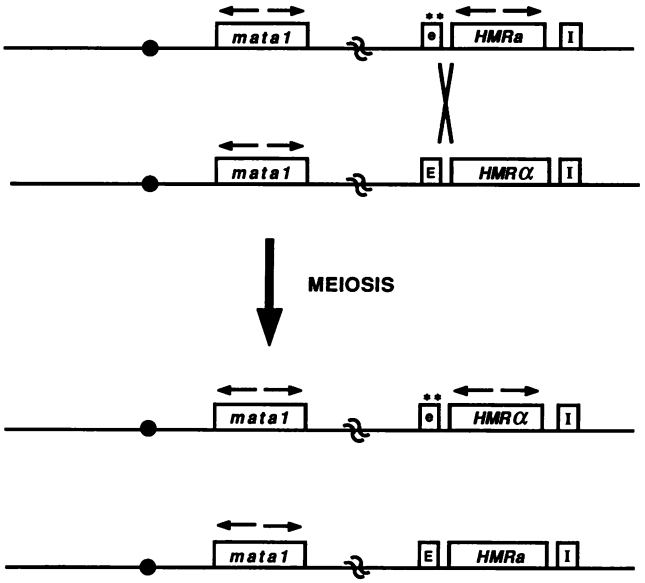

FIG. 1. Schematic representation of a meiotic recombination event between the *HMR-E* silencer and the *HMR* locus. As in the text, ** designates an *E* silencer containing the point mutations in the RAP1/GRF1- and ABF1-binding sites. The recombination event is diagrammed as a reciprocal exchange, although a gene conversion event could also explain the generation of the *HMR*α-*e*** recombinant. Arrows above the locus indicate that the **a** or α genes are transcribed.

which shares no homology with the α1, α2, or **a**2 mRNAs. This fragment was liberated from the replicative form of the M13-**a**1 probe described previously (33) by cleavage at the *Fok*I site in the **a**1 gene and at the *Eco*RI site in the polylinker. To facilitate subsequent fragment purification, the *Fok*I site was filled in, and the fragment was then subcloned between the *Hin*dII and *Eco*RI polylinker sites of pUC118 to form pJR889.

Expression of the **a**1 gene flanked by several different sequences was assayed on centromere-containing plasmids. Both the wild-type silencer and the double-mutant silencer (each as a 490-bp fragment) had been substituted previously into the multicopy plasmid p8ΔE, which contains an *Eco*RI-*Hin*dIII fragment of *HMR***a** deleted for 800 bp including and flanking the *HMR-E* silencer (26). Fragments containing the entire *HMR* sequence were subcloned between the *Eco*RI and *Hin*dIII sites of the single-copy vector pRS316 (47) to form pJR759 (*HMR***a**) and pJR891 (*HMR***a**-*e***). The *MAT***a** locus on a 5-kb *Hin*dIII fragment had been cloned into the *Hin*dIII site of the centromere-containing vector, YCp50, to form pJR156.

DNA mobility shift electrophoresis. DNA mobility shift electrophoretic analysis was performed as described previously (26). The probes were prepared from a plasmid carrying the wild-type (pJR315 [27]) or the double-mutant (pJR628 [26]) silencer. Protein extracts prepared from isogenic *sas1-1* (JRY2262) and *SAS1* (JRY2069) strains grown at 22°C were tested in DNA-binding reactions at 22°C and at 37°C, the nonpermissive temperature for *sas1-1*. Protein extracts prepared from cells incubated at 37°C for 4 h were tested in DNA-binding reactions, which were incubated at 37°C.

Isolation of poly(A)$^+$ RNA and quantitation of a1 mRNA levels. Total nucleic acids were isolated from *S. cerevisiae* by a modification of the method described previously (40), and the poly(A)$^+$ fraction was purified by using oligo(dT)-cellulose type 3 (Collaborative Research) as specified by the manufacturer. The RNA was quantitated by measuring the A_{260}, and samples containing 1, 2, and 4 μg of RNA were prepared. Denatured poly(A)$^+$ RNA samples were applied to a Zetaprobe membrane (Bio-Rad) by slot blotting and hybridized as specified by the manufacturer. The **a**1-specific probe was an *Eco*RI-*Hin*dIII fragment of pJR899 containing 473 bp of **a**1 sequence, and the probe to control for equal loading of total mRNA was a 420-bp internal *Bam*HI-*Bgl*II fragment of the *HMG2* gene (3). The fragments were labeled with the multiprime DNA-labeling system (Amersham) as specified by the manufacturer. The filter was hybridized with an excess of the **a**1 probe, the **a**1 hybridizing fragment was removed, and then the filter was probed with an excess of the *HMG2* fragment. The filters were exposed to Kodak XAR-5 film with a Lightning-Plus screen, and a Kratos SD30000 densitometer was used to calculate the ratio of **a**1 mRNA to *HMG2* mRNA.

RESULTS

Isolation of suppressors of a mutant silencer. In a previous study we constructed an allele of the *HMR-E* silencer with single base pair mutations in the binding sites for both ABFI and GRFI/RAPI. The *HMR* locus containing this double-site mutant silencer, referred to here as *HMR***a**-*e***, is derepressed. In *MAT*α strains, derepression of **a**1 information from *HMR***a**-*e*** results in the nonmating phenotype (26). To identify functions important for silencing *HMR*, we isolated mutations that could suppress the nonmating phenotype caused by the *HMR***a**-*e*** allele. Ten independent cultures of a *MAT*α *HMR***a**-*e*** strain (JRY2069) were mutagenized with ethyl methanesulfonate to between 12 and 41% survival. Of the approximately 120,000 colonies screened, 1 of every 600 was able to mate with an **a** tester lawn at 22°C. Colonies that were able to mate as a result of mutations within the **a**1 gene itself were eliminated by a genetic test for functional **a**1 information at *HMR* (see Materials and Methods). Of the 160 mutants tested, 138 contained an intact **a**1 gene. To characterize the suppressors of *HMR***a**-*e*** further, 63 of the mutants with the strongest α mating phenotype (and with an intact **a**1 gene) were tested for their ability to grow at 37°C. Only one mutant was unable to grow at 37°C, and the mutation in this strain that was responsible for the restored mating was designated *sas1-1*. The mating phenotypes of the parental *MAT*α *HMR***a**-*e*** strain (JRY2069) and the *MAT*α *HMR***a**-*e*** strain containing the *sas1-1* suppressor (JRY2262) are shown in Fig. 2A and B. The other *sas* mutants have been placed into at least four complementation groups (39a) and will be described elsewhere.

The *sas1-1* mutation confers temperature-sensitive growth. The *sas1-1* strain (JRY2262) was unable to grow at 30°C or higher. Two genetic tests were used to determine whether the temperature-sensitive mutation in JRY2262 was identical to the mutation that restored mating ability. First, revertants of the temperature-sensitive phenotype were selected and assayed for loss of mating ability. Four of five temperature-resistant revertants lost the ability to mate, indicating that the temperature-sensitive growth and mating phenotypes frequently coreverted, as expected if both phenotypes resulted from the same mutation. The nonmating phenotypes of one of these revertants and the isogenic parent are shown in Fig. 2. The second test provided additional evidence that

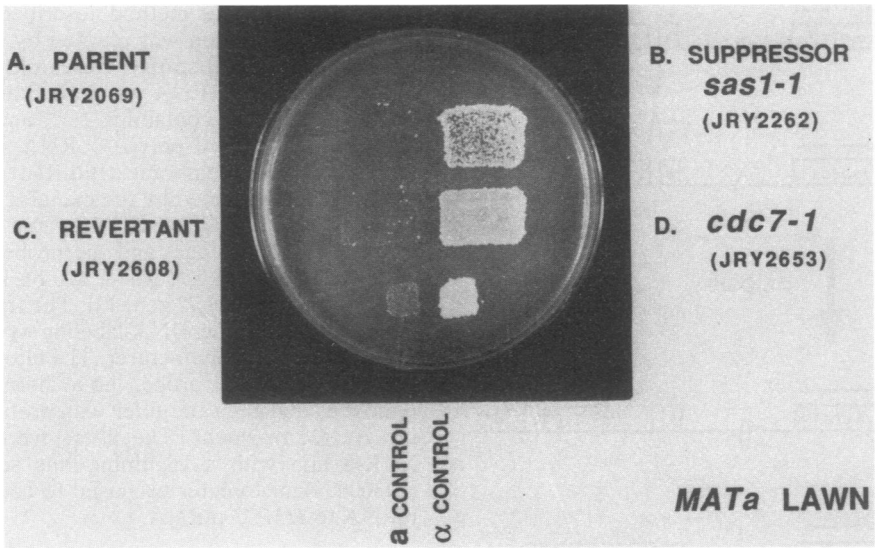

FIG. 2. Mating of *MAT*α *HMR*a-*e*** strains with a lawn of **a** cells (227 cells) at 22°C. (A) *MAT*α *HMR*a-*e*** (JRY2069). (B) *MAT*α *HMR*a-*e*** *sas1-1* (JRY2262). (C) *MAT*α *HMR*a-*e*** *sas1-1* revertant (JRY2608). (D) *MAT*α *HMR*a-*e*** *cdc7-1* (JRY2653).

both phenotypes resulted from the same mutation by demonstrating that both phenotypes cosegregated in meiosis. For this analysis, JRY2262 (*MAT*α *HMR*a-*e*** *sas1-1*) was crossed with JRY2611 (*MAT***a** *HMR*a-*e*** *SAS1*) and segregants from 33 tetrads were assayed for the ability to grow at the restrictive temperature and for the ability to mate with a *MAT***a** tester lawn at the permissive temperature (Table 2). Each tetrad yielded two segregants that could grow at the restrictive temperature and two that could not, as expected for a single genetic lesion. *MAT* also segregated 2:2, yielding two segregants with the **a** mating phenotype and two segregants with the α mating or nonmating phenotype, depending upon whether the segregant contained the suppressor which restored mating ability. All segregants with the α mating phenotype were temperature sensitive for growth. Conversely, all but two of the nonmating segregants were able to grow at the restrictive temperature, indicating that the mating ability and the temperature-sensitive growth were due either to the same mutation or to two closely linked mutations. The two exceptions shared a trait which set them apart from the rest of the segregants: both were respiration deficient, as demonstrated by their inability to grow on medium containing lactate as the sole carbon source. Thus, it is unlikely that these two segregants were bona fide recombinants that separated the mating phenotype from the temperature-sensitive growth. Together, the cosegregation and coreversion of the mating ability and the temperature sensitivity indicated that both phenotypes were caused by the same mutation, *sas1-1*.

TABLE 2. Cosegregation of temperature-sensitive lethality with *sas1-1*

Phenotypes of segregants[a]	% of segregants expected if *ts* and *sas1-1* were:		% of segregants observed (no. of segregants)
	Linked	Unlinked	
a, *ts*	25	25	28 (37)
a, +	25	25	22 (29)
α, *ts*	25	12.5	20.5 (27)
α, +	0	12.5	0 (0)
nm, *ts*	0	12.5	1.5 (2)[b]
nm, +	25	12.5	28 (37)

[a] **a** is the **a** mating phenotype; α is the α mating phenotype; *nm* is the nonmating phenotype; *ts* is temperature-sensitive lethality at 34°C; + is growth at 34°C.

[b] Both of these strains were respiration deficient, which affects the penetrance of the *sas1-1* phenotype.

Quantitation of mating in a *sas1-1* strain. The *sas1-1* allele partially suppressed the mating defect of the *MAT*α *HMR*a-*e*** strain, allowing these cells to mate with *MAT***a** cells. The magnitude of suppression is shown in Table 3. The parental *MAT*α *HMR*a-*e*** strain (JRY2069) mated with 0.0023% of wild-type efficiency. The *sas1-1* mutant derived from this strain (JRY2262) mated with 1.4% efficiency, a 1,000-fold increase. This difference was readily distinguished in a patch mating test (compare Fig. 2A and B). Thus, at the permissive temperature, the *sas1-1* mutation provided substantial, but not complete, restoration of mating ability.

Linkage of *SAS1* and *TRP1*. The *SAS1* gene was shown to be closely linked to *TRP1* both in the cross described in a previous section and in a subsequent cross between JRY2622 and AMR27. The data from these crosses place *SAS1* at approximately 3.2 centimorgans (cM) from *TRP1* (Table 4).

***sas1-1* is recessive.** To determine whether the *sas1-1* allele restored mating and conferred a temperature-sensitive

TABLE 3. Quantitative mating assay[a]

Strain	Relevant genotype	Mating efficiency[b]	Relative mating efficiency (%)
JRY528	*MAT*α	0.9	100
JRY2069	*MAT*α *HMR*a-*e***	2.1×10^{-5}	2.3×10^{-3}
JRY2262	*MAT*α *HMR*a-*e*** *sas1-1*	1.3×10^{-2}	1.4

[a] Quantitative mating assays were performed with tester strain JRY19 as described in Materials and Methods.

[b] The mating efficiency is the fraction of cells that were able to mate and form prototrophic colonies. These numbers are the average of two independent assays.

TABLE 4. Linkage of *SAS1* and *TRP1*

Cross	No. of tetrads of type[a]:			Map distance (cM)
	PD	T	NPD	
JRY2262 × JRY2611	30	3	0	4.5
JRY2622 × AMR27	14	0	0	<3.6
Total	44	3	0	3.2

[a] PD, Parental ditype; T, tetratype; NPD, nonparental ditype.

growth phenotype to the cell through a reduction of function or through a neomorphic function, we evaluated the phenotypes of a *sas1/SAS1* heterozygote. A *MAT*α *HMR***a**-*e*** *sas1-1* segregant (JRY2615) was mated with a *mat***a***1* strain (JRY63), and the diploid JRY2617 (*MAT*α/*mat***a***1 HMR***a**-*e***/*HMR***a** *sas1-1/SAS1*) was isolated. *MAT*α/*mat***a***1* diploids have the α mating type because there is no **a**1 product present (50). However, sufficient **a**1 product was expressed from *HMR***a**-*e*** in the diploid JRY2617 to block mating ability (Fig. 3), indicating that *sas1-1* was recessive. In contrast, the *sas1-1* homozygous diploid JRY2949 (*MAT*α/*mat***a***1 HMR***a**-*e***/*HMR***a** *sas1-1/sas1-1*) was able to mate (Fig. 3). *sas1-1* was also recessive with respect to its temperature-sensitive lethality, as demonstrated by the ability of the diploid JRY2617 to grow at the restrictive temperature. Even at this elevated temperature, *sas1-1* remained recessive and was unable to restore mating ability to the diploid. The recessive nature of *sas1-1* suggested that it was a reduced level of SAS1 function that allowed *MAT*α *HMR***a**-*e*** cells to mate.

***sas1-1* does not restore ABFI or GRFI/RAPI binding to a mutant silencer in vitro.** Mutation of either the ABFI- or the GRFI/RAPI-binding site of *HMR-E* has little or no effect on silencer function, whereas the double-site mutant results in loss of silencer function. Therefore, if binding to either the ABFI-IA or the GRFI-IIA mutant silencer site were restored, the mutant silencer would presumably be functional. In principle, binding could be restored by a change in either ABFI or GRFI/RAPI or by a change in another factor that would allow ABFI or GRFI/RAPI to bind to its mutant site. The temperature-sensitive lethality of *sas1-1* would be consistent with a mutation in *ABFI* or *GRFI/RAPI*, since both are essential genes (10, 46). However, the recessive phenotype of *sas1-1* suggested that it did not code for an ABFI or GRFI/RAPI with altered binding specificity, since such a mutation would probably be dominant. The recessive phenotype of *sas1-1* would, however, be consistent with the mutation of a factor that could modify the binding specificity of ABFI or GRFI/RAPI. In vitro DNA mobility shift assays with extracts prepared from *sas1-1* strains provided no evidence for an altered factor that was able to bind to a mutant site. Proteins in *sas1-1* extracts bound to the wild-type ABFI and GRFI/RAPI sites, but did not bind to the mutant sites under any conditions tested (data not shown). Although it was still possible that binding in vivo could be restored under conditions not effectively measured by this in vitro assay, these results suggested that *sas1-1* suppressed the nonmating phenotype caused by the mutant silencer without restoring the binding of either ABFI or GRFI/RAPI. The cloning of *SAS1* (below) confirmed that *SAS1* was neither *ABFI* nor *GRFI/RAPI*.

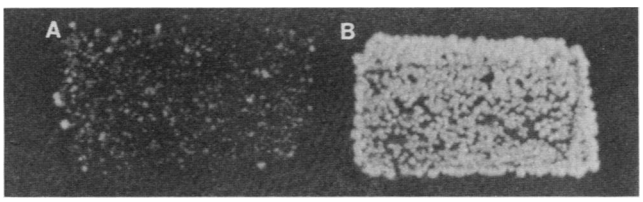

FIG. 3. *sas1-1* is recessive with respect to its ability to confer mating competence on *MAT*α *HMR***a**-*e*** strains. The figure shows mating of a *sas1-1* heterozygous strain, JRY2617 (*MAT*α/*mat***a***1 HMR***a**-*e***/*HMR***a** *sas1-1/SAS1*) (A), and a *sas1-1* homozygous strain JRY2949 (*MAT*α/*mat***a***1 HMR***a**-*e***/*HMR***a** *sas1-1/sas1-1*) (B), with a lawn of **a** cells (227 cells).

Cloning of *SAS1*. The *SAS1* gene was cloned by its ability to complement the temperature-sensitive lethal phenotype of the *sas1-1* mutation. A *sas1-1 leu2* strain (JRY2616) was transformed with a *LEU2 CEN* yeast genomic library (48) at 22°C. Of the 1,000 Leu^+ transformants tested, 2 were able to grow at 37°C. Plasmid DNA was isolated from both and transformed into *E. coli*. Upon retransformation of JRY2616, both plasmids were able to complement *sas1-1*. Restriction analysis of both plasmids revealed that one (pJR731) contained an 8-kb insert that was an internal fragment of a 12-kb insert from the other (pJR732).

To determine whether pJR731 contained the structural gene for *SAS1*, we tested a fragment of this plasmid for its ability to direct integration at the chromosomal *SAS1* locus. A 5-kb *Bam*HI-*Hin*dIII fragment of pJR731 was subcloned into YIp5 (51), an integrating *URA3*-containing vector. The resulting plasmid, pJR749, was linearized at the unique *Sac*I site in the insert, and transformation of this linear fragment into a *SAS1* strain (JRY527) yielded several Ura^+ transformants. One of these (JRY2625) was crossed with a *sas1-1 ura3* strain (JRY2631), and in each of 21 tetrads examined, the integrated *URA3* marker cosegregated with the *SAS1* allele. Therefore, pJR731 contained the *SAS1* structural gene.

Subclones of pJR731 were tested for their ability to complement the temperature-sensitive lethality of JRY2616, and a 2.2-kb subclone, pJR757, was found to be sufficient. A partial DNA sequence of this region was obtained and compared with sequences in the GenBank data base by the FASTN program. This search revealed that *SAS1* was identical to *CDC7* (bp 21 to 117 [36]). Consistent with this interpretation, the restriction maps of *SAS1* and *CDC7* were identical (36); both mapped near *TRP1* (34); and *sas1-1* cells at the nonpermissive temperature arrested as large budded cells, as described previously for a *cdc7* strain (9). Henceforth, *sas1-1* will be referred to as *cdc7-90*.

Reduced CDC7 function suppresses *HMR*a**-*e*****.** The recessive nature of the *cdc7-90* allele suggested that *cdc7-90* resulted in a partial loss of CDC7 function at the permissive temperature. If the partial loss of CDC7 function could suppress *HMR***a**-*e***, then suppression of *HMR***a**-*e*** should be a common property of *CDC7* loss-of-function alleles. *cdc7-1*, the first allele of *CDC7*, which was isolated on the basis of its cell cycle phenotype (17), was tested for its ability to suppress the mating defect caused by the *HMR***a**-*e*** allele in a *MAT*α strain. A *MAT***a** *HMR***a** *cdc7-1 trp1* strain (RM14-3A) was crossed with a *MAT*α *HMR***a**-*e*** *cdc7-90 TRP1* strain (JRY2616). Since *CDC7* and *TRP1* are very closely linked (3.2 cM), tryptophan prototrophy was used to assess which allele of *CDC7* was segregated to each spore. Segregants from 25 tetrads were tested for their ability to mate (Table 5). Each tetrad contained two *MAT***a** segregants which had the **a** mating phenotype and two *MAT*α segregants whose phenotypes were as follows. *MAT*α segregants lack-

TABLE 5. Suppression of *HMRa-e*** by *cdc7-1*

Phenotypes of segregants[a]	% of total segregants expected[b] if:		% of segregants observed (no. of segregants)
	cdc7-1 did not suppress *HMRa-e***	*cdc7-1* did suppress *HMRa-e***	
a Trp^+ or Trp^-	50	50	50 (48)
α (strong) Trp^+ or Trp^-	20	20	24 (23)
α (weak) Trp^+	15	15	18 (17)
α (weak) Trp^-	0	15	7 (7)
nm Trp^+	0	0	0 (0)
nm Trp^-	15	0	1 (1)

[a] Segregants from JRY2616 × JRY2449 (*MAT*α/*MAT***a** *HMR***a**-*e***/*HMR***a** *cdc7-90*/*cdc7-1 TRP1*/*trp1*). **a** indicates the **a** mating phenotype; α (strong) indicates a strong α mating phenotype; α (weak) indicates a weak α mating phenotype; *nm* indicates the nonmating phenotype.

[b] Based on a recombination frequency of 39% between *MAT* and *HMR* (34).

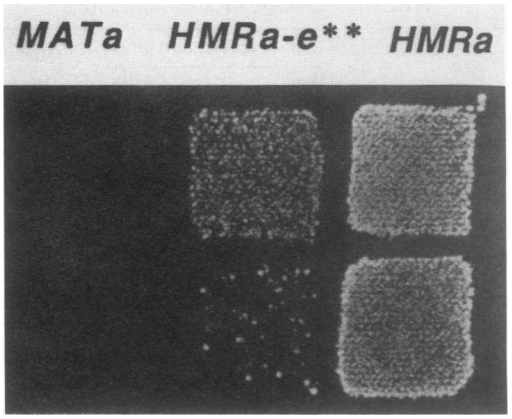

FIG. 4. A *MAT*α *cdc7-90* strain (JRY2822) (top row) and a *MAT*α *CDC7* strain (JRY528) (bottom row) were transformed with single-copy plasmids containing *MAT***a** (pJR156), *HMR***a**-*e*** (pJR891), or *HMR***a** (pJR759). The mating phenotypes of these transformants with a *MAT***a** *ura3* lawn (TD4) demonstrated that *cdc7-90* did not repress *MAT***a**.

ing the *HMR***a**-*e*** allele exhibited a strong α mating phenotype. *MAT*α segregants containing the *HMR***a**-*e*** allele varied somewhat in the levels of α mating they displayed. However, there was no difference between the mating patterns of *cdc7-90* (Trp^+) and *cdc7-1* (Trp^-) segregants, indicating that *cdc7-1* suppressed *HMR***a**-*e*** as efficiently as did *cdc7-90*. No nonmating *MAT*α *HMR***a**-*e*** segregants were recovered among 25 tetrads. The mating phenotype of a representative *MAT*α *HMR***a**-*e*** *cdc7-1* segregant is shown in Fig. 2D. These results demonstrated that suppression of *HMR***a**-*e*** by *CDC7* mutations was not likely to be allele specific, indicating that a reduced level of CDC7 function suppressed *HMR***a**-*e***.

cdc7-90* does not repress the a1 gene at *MAT*.** Mutations in *CDC7* restored mating to a *MAT*α strain containing a defective *HMR-E* silencer, suggesting that reduced CDC7 function restored silencing at *HMRa**. However, there were alternative explanations for the observed suppression. Mating ability could also be restored by interfering with a transcriptional activator of the **a**1 gene or with the manifestation of the nonmating **a**/α phenotype. To determine whether *cdc7-90* suppression of the **a**1 gene at *HMR***a**-*e*** is dependent on the silencer, we assayed the ability of *cdc7-90* to suppress expression of the **a**1 gene at *MAT***a**. The **a**1 promoter and transcript are identical at *HMR* and *MAT*. Two assays were used to determine the effect of *cdc7-90* on the expression of *MAT***a**. First, *cdc7-90* was assayed for its ability to suppress the nonmating phenotype caused by *MAT***a** on a single-copy plasmid in *MAT*α strains. A *MAT*α *cdc7-90* strain (JRY2822) was transformed with a single-copy plasmid carrying the *MAT***a** locus, pJR156, and the transformants were tested for their ability to mate. These transformants were unable to mate, indicating that *cdc7-90* could not suppress the **a**1 gene expressed from *MAT***a** on the plasmid (Fig. 4). Control experiments indicated that *cdc7-90* could suppress *HMR***a**-*e*** on a single-copy plasmid. Both a *MAT*α *cdc7-90* strain (JRY2822) and a *MAT*α *CDC7* strain (JRY528) were transformed with a single-copy plasmid carrying *HMR***a**-*e***, pJR891. The *cdc7-90* transformants mated more efficiently than the *CDC7* transformants did (Fig. 4). As expected, transformants of both strains with a single-copy plasmid carrying a wild-type *HMR***a** locus, pJR759, mated efficiently (Fig. 4). Since transformants with pJR156 (*MAT***a**) were unable to mate, *cdc7-90* did not appear to affect the expression of **a**1 information from *MAT***a**. As a second test, **a**/α diploids homozygous for *cdc7-90* were tested for their ability to mate. *cdc7-90* was unable to suppress the nonmating phenotype of the **a**/α diploid (data not shown).

A point to consider in comparing repression of *MAT***a** with repression of *HMR***a**-*e*** was that the level of **a**1 mRNA expressed from these two loci appeared not to be equal. Comparison of JRY528 transformed with pJR891 (*HMR***a**-*e***) and pJR156 (*MAT***a**) revealed that *MAT***a** completely inhibited mating whereas *HMR***a**-*e*** allowed a very low level of mating (Fig. 4). Thus, **a**1 expression from *HMR***a**-*e*** may be more subject to modifying influences than **a**1 expression from *MAT***a** is. Nevertheless, *cdc7-90* did increase the mating of *MAT*α *HMR***a**-*e*** strains 1,000-fold, whereas there was no detectable effect on *MAT***a**. Since *cdc7-90* did not appear to affect the expression of **a**1 information from *MAT***a**, yet was able to suppress **a**1 information at *HMR***a**-*e***, *cdc7-90* restored silencing. Further evidence that the *cdc7-90* effect depends on the silencer is presented in the next section.

cdc7-90* suppresses the α genes at *HMR*α-*e**.** If *cdc7-90* suppresses the **a**1 gene at *HMR***a**-*e*** by restoring silencing at the locus, *cdc7-90* should also be able to suppress expression of the α genes at *HMR*α-*e***. A strain containing an *HMR*α-*e*** allele was obtained genetically as described in Materials and Methods. A *mat***a**1 *HMR*α-*e*** strain had a strong α mating phenotype as a result of derepression of the α genes at *HMR* (in the absence of **a**1 information), but also had a weak **a** mating phenotype, because *HMR*α-*e*** was not completely derepressed. (The absence of mating-type information results in the **a** mating phenotype.) If *cdc7-90* could suppress *HMR*α-*e***, then the levels of both α1 and α2 would be lower in *cdc7-90 mat***a**1 *HMR*α-*e*** strains than in *CDC7 mat***a**1 *HMR*α-*e*** strains. Since the role of α1 is to activate α-specific genes, strains with lower levels of α1 activity should be worse α maters. Furthermore, since α2 represses **a**-specific genes, strains with less α2 should be better **a** maters. Therefore, if *cdc7-90* could suppress *HMR*α-*e***, then *cdc7-90 mat***a**1 *HMR*α-*e*** strains would be worse α maters and better **a** maters than *CDC7 mat***a**1 *HMR*α-*e*** strains. A *MAT***a** *HMR*α-*e*** *CDC7* strain (JRY2641) was crossed with a *mat***a**1 *HMR*α-*e*** *cdc7-90* strain (JRY2649), and 27 tetrads were analyzed (Table 6).

TABLE 6. Suppression of *HMRα-e*** by *cdc7-90*[a]

Mating phenotype	Growth at 34°C	% (no. observed)	Deduced genotype[b]
a mater	+	23 (25)	*MAT***a** *HMRα-e** CDC7*
	−	27 (29)	*MAT***a** *HMRα-e** cdc7-90*
Bimater[c]			
(i) Stronger mating as α's than as **a**'s	+	26 (28)	*mat***a**1 *HMRα-e** CDC7*
	−	0 (0)	
(ii) Stronger mating as **a**'s than as α's	+	0 (0)	
	−	20 (22)	*mat***a**1 *HMRα-e** cdc7-90*
(iii) Equal mating as **a**'s or α's	+	1 (1)	*mat***a**1 *HMRα-e** CDC7*
	−	3 (3)	*mat***a**1 *HMRα-e** cdc7-90*

[a] Segregants from JRY2641 × JRY2649 (*MAT***a**/*mat***a**1 *HMRα-e***/*HMRα-e** CDC7*/*cdc7-90*). The four possible genotypes, *MAT***a** *HMRα-e** CDC7*, *MAT***a** *HMRα-e** cdc7-90*, *mat***a**1 *HMRα-e** CDC7*, and *mat***a**1 *HMRα-e** cdc7-90*, were expected in equal ratios.

[b] The expected phenotypes for these genotypes were not known a priori but were deduced from the phenotypes of the original *mat***a**1 *HMRα-e** CDC7* strain (JRY2635) and the phenotypes observed in this cross and in a previous cross (see text).

[c] The bimaters were divided into three categories based on their relative abilities to mate with lawns of **a** cells and α cells.

Each tetrad contained two segregants (*MAT***a**) that were able to mate as **a**'s and were unable to mate as α's (see Materials and Methods for an explanation of the mating phenotype of *MAT***a** *HMRα-e*** strains). The other two segregants (*mat***a**1) were bimaters, that is, able to mate with both **a** and α mating-type tester lawns. Three classes of bimaters were observed: (i) strong α maters which also mated weakly as **a**'s; (ii) weak α maters which mated more efficiently as **a**'s; and (iii) segregants that mated equally well as **a**'s or α's. Segregants that mated better as α's than as **a**'s (class 1) were always *CDC7*, whereas segregants that mated less efficiently as α's than as **a**'s (class 2) were *cdc7-90*. Therefore, *cdc7-90* suppressed *HMRα-e***. The magnitude of the difference between the ability of a strain to mate as an α versus an **a** varied among individual segregants, and 4 of the 108 segregants examined (3 *cdc7-90* and 1 *CDC7*) mated equally well with an α lawn and an **a** lawn. However, no *cdc7-90* segregant mated more efficiently as an α, and no *CDC7* segregant mated more efficiently as an **a**. A representative tetrad analyzing *cdc7*-dependent suppression of *HMRα-e*** is shown in Fig. 5. *cdc7-90* made *mat***a**1 *HMRα-e*** segregants worse α maters and better **a** maters, indicating that *cdc7-90* restored repression at *HMRα-e***.

*cdc7-90 MAT*α strains mated as efficiently as *CDC7 MAT*α strains did (data not shown), indicating that *cdc7-90* could not repress the α genes at *MAT* and must therefore suppress *HMRα-e*** by restoring silencing. However, because *HMRα-e*** did not fully derepress the α genes, it might have been easier to repress *HMRα-e*** than to repress *MAT*α. To test this possibility, we assayed *cdc7-90* for its ability to repress a *MAT*α allele which is reduced for the level of α gene expression. Two different point mutations in the GRFI/RAPI-binding site of the *MAT*α promoter, *matα-1613* and *matα-1617*, confer a bimating phenotype on cells, indicating a reduced level of α gene expression from *matα-1613* and *matα-1617* (6, 12a). In fact, DG168 (*matα-1613*) and DG169 (*matα-1617*) were even less efficient α maters than JRY2635 (*mat***a**1 *HMRα-e***). The mating phenotypes of segregants from crosses between the strains containing *matα-1613* or *matα-1617* (DG168 or DG169) and strains containing *cdc7-90* (JRY2630 or JRY2634) revealed that *cdc7-90* was unable to affect the mating phenotypes of *matα-1613* and *matα-1617* strains expressing a low level of α1 and α2 (data not shown). Since *cdc7-90* suppressed α information at *HMRα-e***, but not at *MAT*, *cdc7-90* must restore silencing, and not repress expression of *MAT*.

Overproduction of CDC7 interferes with repression of *HMRα-e.*** Since a reduced level of CDC7 function restored silencing in strains containing the double-mutant silencer, we tested the effect of overexpression of CDC7 on silencing.

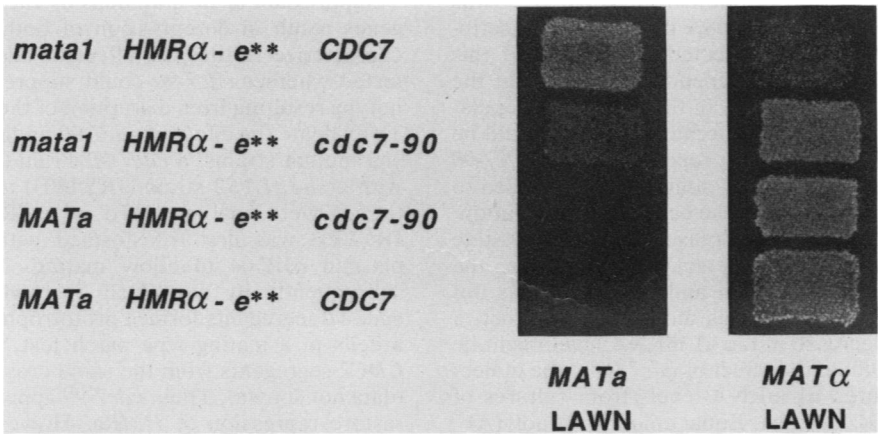

FIG. 5. Mating phenotype of a representative tetrad from the cross between JRY2641 (*MAT*α *HMRα-e***) and JRY2649 (*mat***a**1 *HMRα-e** cdc7-90*). The *mat***a**1 *HMRα-e** CDC7* segregant was a bimater that mated more efficiently with a lawn of **a** cells (227 cells) than with a lawn of α cells (70 cells), whereas the *mat***a**1 *HMRα-e** cdc7-90* segregant was a bimater that mated more efficiently with a lawn of α cells (70 cells) than with a lawn of **a** cells (227 cells).

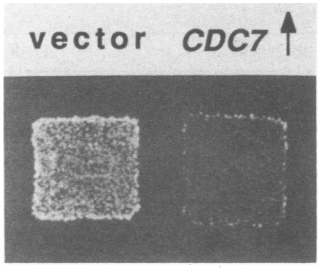

FIG. 6. A *mat***a***1 HMR*α*-e*** strain (JRY2635) was transformed with a multicopy plasmid containing *CDC7* (pRS7 [36]) and with the vector YEp24. The mating phenotypes of these transformants with a *MAT*α *ura3* lawn (JRY412) demonstrated that overexpression of CDC7 interfered with silencing at *HMR*α*-e***.

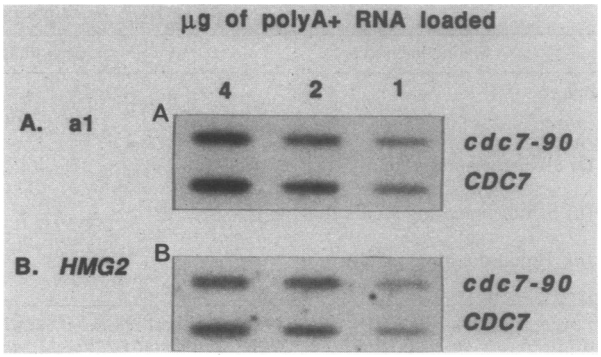

FIG. 7. Comparison of the steady-state levels of **a**1 mRNA expressed from *HMR***a***-e*** in a *CDC7* and a *cdc7-90* strain. Poly(A)$^+$ RNA (1, 2, and 4 μg) from a *cdc7-90* strain (JRY2262) and a *CDC7* strain (JRY2069) was applied to Zetaprobe membrane and hybridized with an excess of **a**1-specific probe (A). The blot was then stripped of the **a**1 probe and hybridized with an excess of *HMG2*-specific probe as a control for loading variation (B). *HMG2* encodes an isozyme of HMG coenzyme A reductase (3) and is not related to either mating-type or cell cycle functions. Densitometric scans of these autoradiograms revealed that, after normalization to the amount of *HMG2* mRNA, there was no significant difference in the amounts of **a**1 mRNA in the *CDC7* and *cdc7-90* strains.

With the bimating *mat***a***1 HMR*α*-e*** strain (JRY2635), we could assay for both restoration of silencing (which would make the strain mate better with a lawn of α cells and worse with a lawn of **a** cells) and loss of silencing (which would make the strain mate worse with a lawn of α cells and better with a lawn of **a** cells). JRY2635 was transformed with a multicopy plasmid containing the *CDC7* gene (pRS7 [36]) and with the YEp24 vector. Transformants containing the *CDC7* multicopy plasmid mated less efficiently with a lawn of α cells than did transformants containing the vector (Fig. 6). JRY2635 mated very efficiently with a lawn of **a** cells, precluding the chance of observing an increase in mating efficiency. Quantitative mating assays showed that transformants containing the *CDC7* multicopy plasmid mated more than 10-fold less efficiently with α cells than did transformants containing the vector (data not shown). These results indicated that overexpression of CDC7 interfered with silencing at *HMR*α*-e***, providing additional evidence that silencing is sensitive to the level of CDC7.

a1 mRNA levels. The genetic analysis described above indicated that *cdc7-90* allowed *MAT*α *HMR***a***-e*** strains to mate approximately 1,000-fold more efficiently by decreasing the expression of **a**1 from *HMR***a***-e***. However, this mating efficiency was still only 2% of the mating efficiency of a wild-type *MAT*α strain (Table 2). There are two extreme models for how *cdc7-90* might restore mating competence in only 2% of the *MAT*α *HMR***a***-e*** cells. One view would be that in 2% of the cells transcription from *HMR***a***-e*** was completely repressed, whereas in 98% of the cells transcription from *HMR***a***-e*** was not affected by *cdc7-90*. If this model were correct, a *cdc7-90*-dependent decrease in the steady-state level of the **a**1 mRNA in the entire cell population would be undetectable. An alternative model would be that transcription of *HMR***a***-e*** is repressed in all *cdc7-90* cells, but the level of **a**1 mRNA is sufficiently decreased to allow mating in only a minority of the cells in the population. If this model were correct, a decrease in the steady-state level of **a**1 mRNA might be detectable. However, the relationship between mRNA level and phenotype has not been established. Therefore, if this model were correct, a *cdc7-90*-dependent decrease in the **a**1 mRNA level might be undetectable. To evaluate the effect of *cdc7-90* at the molecular level, we measured **a**1 mRNA levels from cultures of both *cdc7-90* and *CDC7* strains. Equal amounts of poly(A)$^+$ RNAs from a *MAT*α *HMR***a***-e*** *CDC7* strain (JRY2069) and a *MAT*α *HMR***a***-e*** *cdc7-90* strain (JRY2262) were applied to a charged nylon membrane and probed with a fragment of the **a**1 gene. This fragment did not hybridize to poly(A)$^+$ RNA from a *MAT*α strain on the same filter (data not shown). Little or no difference in the level of **a**1 mRNA was observed between JRY2069 (*CDC7*) and JRY2262 (*cdc7-90*) (Fig. 7A). To control for loading variation, the blot was stripped of hybridizing probe and rehybridized with a fragment of the *HMG2* gene (Fig. 7B). Densitometric analysis of exposures of the blot showed that after normalization to *HMG2* mRNA levels, there was less than a 1.5-fold difference between the levels of **a**1 mRNA in the *CDC7* and *cdc7-90* strains. Therefore, *cdc7-90* either may restore repression at *HMR***a***-e*** in only 2% of the cells or may decrease the amount of **a**1 mRNA expressed from *HMR***a***-e*** in all cells by an unmeasurable amount. In the latter case, the relationship between the **a**1 mRNA level and the mating phenotype must be such that a small decrease in **a**1 mRNA can have a significant effect on the mating phenotype.

Suppression of *sir* mutations by *cdc7-90*. Mutations in *SIR* genes result in derepression of both *HML* and *HMR*. To characterize further the effect of *cdc7-90* on silencing, we tested whether *cdc7-90* could suppress the nonmating phenotype resulting from disruption of the *SIR3* and *SIR4* genes. To evaluate the effect of *cdc7-90* on the mating phenotype of *sir3* mutant strains, a *cdc7-90* strain (JRY2622) was crossed with a *sir3*::*LYS2* strain (JRY1303) and segregants from 33 tetrads were analyzed. (To mate JRY1303 with JRY2622, JRY1303 was first transformed with the *SIR3*-containing plasmid pJR104 to allow mating. The plasmid was lost subsequently by nonselective growth.) *MAT*α *sir3*::*LYS2* *cdc7-90* segregants formed prototrophic papillae on a lawn of **a** cells in a mating-type patch test, but *MAT*α *sir3*::*LYS2* *CDC7* segregants from the same cross were unable to do so (data not shown). Thus, *cdc7-90* appeared marginally able to restore repression of *HMR***a**. However, *MAT***a** *sir3*::*LYS2* *cdc7-90* segregants did not form prototrophic papillae on a lawn of α cells, indicating that *cdc7-90* does not restore repression of *HML*α in *sir3* mutants. *cdc7-90* did not restore detectable repression of *HMR***a** or *HML*α in *sir4*::*HIS3*

segregants from a cross between a *cdc7-90* strain (JRY2630) and a *sir4*::*HIS3* strain (JRY2467) (19 tetrads [data not shown]; the **a** mating-type tester strain for this cross was DBY1039). Since *cdc7-90* could not suppress the nonmating phenotype of *sir4*::*HIS3* strains, the ability of *cdc7-90* to repress the *HMR* locus containing the double-mutant silencer must depend on SIR4 function.

DISCUSSION

Suppressors of a defective silencer. The silent mating-type locus *HMR***a** is derepressed by a combination of two point mutations in the *HMR-E* silencer. This double-mutant silencer is unable to bind GRFI/RAPI or ABFI in vitro, but still contains a functional ARS element (26). In *MAT*α cells, derepression of **a** information from *HMR***a**-*e*** results in a nonmating phenotype. We have shown that the α mating phenotype can be restored to these *MAT*α *HMR***a**-*e*** cells by several suppressor mutations and that at least one of these, *sas1-1*, restores silencing.

Cloning and characterization of *sas1-1*, a mutation in *CDC7*. *sas1-1* both partially restored the α mating phenotype to *MAT*α *HMR***a**-*e*** cells and conferred a temperature-sensitive lethal phenotype on the cell, as indicated by the cosegregation and coreversion of the two phenotypes. By the criteria of terminal phenotype, map position, and sequence identity, *sas1-1* was found to be an allele of *CDC7* which was designated *cdc7-90*. It is noted here that *cdc7-90* is identical to *cdc7-7* (28a). In addition, *cdc7-1* (17) restored mating to *MAT*α *HMR***a**-*e*** strains, indicating that suppression was not likely to require allele-specific interactions. Since *cdc7-90* was recessive with respect to its ability to restore repression, and since *cdc7-1* also restored repression, it appeared that a reduction of CDC7 function, rather than a neomorphic CDC7 function, was responsible for the restoration of repression at *HMR*. Silencing was also sensitive to increased levels of CDC7. Overexpression of CDC7 interfered with repression at *HMR*α-*e*** . It has also been shown that CDC7 overexpression inhibits silencing in cells with a wild-type silencer using an *hmr*Δ::*SUP3 am* allele (36a). Thus, CDC7 function normally inhibits repression at *HMR*.

Reduced CDC7 function restores silencing at *HMR*. In principle, *cdc7-90* could have restored mating competence to *MAT*α strains containing *HMR***a**-*e*** in at least three ways: (i) suppressing the nonmating phenotype of cells that simultaneously express both **a**- and α-encoded functions, resulting in the α mating phenotype as the default phenotype, as has been suggested for *aar1* (16); (ii) reducing transcription from the **a**1 promoter per se; and (iii) restoring silencing of genes adjacent to the mutant silencer. The first two possibilities were rejected because *cdc7-90* had no effect on the nonmating phenotype of either **a**/α diploids or *MAT*α strains containing *MAT***a** on a plasmid. Also inconsistent with the first two possibilities, *cdc7-90* was able to restore repression of α1 and α2 at the *HMR*α-*e*** locus. The inability of *cdc7-90* to repress *MAT*α genes whose expression was reduced by a mutation in the GRFI/RAPI-binding site in the *MAT*α upstream activation sequence (6, 12a) indicated that the inability of *cdc7-90* to repress *MAT* was not merely due to quantitative differences in the level of expression of genes at *MAT*α versus *HMR*α-*e*** . All of the data were consistent with the third possibility, that *cdc7-90* restored silencing.

Since the mobility shift experiment suggested that *cdc7-90* did not restore the binding of GRFI/RAPI or ABFI to their mutant sites, it was possible that the effect of *cdc7-90* was mediated through the remaining function of the double-site mutant silencer, the ARS. Alternatively, *cdc7-90* might have bypassed the need for the *HMR-E* silencer, perhaps by enhancing the silencing capability of the *HMR-I* element. It was unlikely that *cdc7-90* restored repression through an entirely new mechanism, since the repression restored in *cdc7-90* strains was dependent upon *SIR4*.

Does the effect of *CDC7* on silencing reflect its role in DNA replication? An association between late replication and repression of *HMR* would not be without precedent. In general, active genes are replicated early during the S phase, whereas repressed genes are replicated late (2, 13, 22). For example, genes that are expressed in a tissue-specific manner are often replicated earlier in the expressing cell type than in the repressing cell type (18). Similarly, the transcriptionally inactive X chromosome in female mammals is late replicating. This correlation is extended by the observation that a reactivated locus on the inactive X chromosome is early replicating (42) and by a model for the fragile X syndrome of humans suggesting that the fragile site of the active X chromosome is transcriptionally repressed and late replicating (29).

Since CDC7 is required for the initiation of mitotic DNA replication (17), one possible mechanism whereby a reduction of *CDC7* function may restore silencing at *HMR* is by delaying the replication of *HMR*. *HMR* is normally replicated near the end of S phase (38). *cdc7* mutations may cause *HMR* to be replicated even later. Similarly, if the double-site mutation at *HMR* results in the early replication of *HMR*, *cdc7* mutations may restore late replication of *HMR*. In this regard, *HMR* appears to have a more stringent demand on CDC7 function for its replication than does the rest of chromosome III (38). Further studies of the effect of *cdc7* on repression of *HMR* may establish a causal relationship between late replication and transcriptional repression.

Another model for the effect of *cdc7* mutations on silencing is suggested by the possibility that initiation of DNA replication at the *HMR-E* ARS is required for the establishment of repression at *HMR*. As described in the Introduction, several lines of circumstantial evidence suggest that DNA replication plays a role in silencing. In particular, there is an ARS at both E and both I elements and an S-phase requirement for the establishment of repression. Although use of the *HMR-E* origin in the chromosome has not been determined, two-dimensional gel analysis techniques have failed to detect the frequent use of *HML-E* as a replication origin (52). However, this observation does not eliminate a role for the initiation of DNA replication at the silencer since the available evidence implicates DNA replication in the establishment of transcriptional repression at *HML*, and not in its maintenance (33). The establishment event may be relatively rare, since the repressed state is mitotically stable once established (37). Thus, replication initiation at the *HML-E* silencer may be required only for the establishment of repression and hence would occur in only a small fraction of cells and would not have been detected by existing techniques. If initiation of DNA replication at *HMR-E* is required for the establishment of repression, *cdc7* mutations may restore silencing by increasing the frequency at which the *HMR-E* origin is used.

Other explanations for the role of *CDC7* in *HMR* repression should also be considered. The CDC7 kinase (21, 36) may have multiple substrates, as suggested by the existence of multiple roles for CDC7 (35, 41, 44). An alternative model for the effect of *cdc7* on repression of *HMR* would be that CDC7 phosphorylates a protein that is important for the silencing of *HMR*. CDC7 may phosphorylate one of the SIR

proteins or a structural component of chromatin, since chromatin structure has been implicated in the repression of *HMR* and *HML*. It is also possible that the multiple deficiencies of *cdc7* mutants are the result of a change in chromatin structure. We propose that a reduced level of CDC7 function restores silencing at the mutant *HMRa-e*** locus either through its effect on replication or through an uncharacterized role for *CDC7* in the control of chromatin structure.

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