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Specific Repression of the Yeast Silent Mating Locus *HMR* by an Adjacent Telomere

JEFFREY S. THOMPSON, LIANNA M. JOHNSON, AND MICHAEL GRUNSTEIN*

Molecular Biology Institute and Department of Biology, University of California, Los Angeles, California 90024

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The yeast silent mating loci *HML* and *HMR* are located at opposite ends of chromosome III adjacent to the telomeres. Mutations in the N terminus of histone H4 have been previously found to derepress the yeast silent mating locus *HML* to a much greater extent than *HMR*. Although differences in the a and α mating-type regulatory genes and in the *cis*-acting silencer elements do not appear to strongly influence the level of derepression at *HMR*, we have found that the differential between the two silent cassettes is largely due to the position of the *HMR* cassette relative to the telomere on chromosome III. While *HML* is derepressed to roughly the same extent by mutations in histone H4 regardless of its chromosomal location, *HMR* is affected to different extents depending upon its chromosomal positioning. We have found that *HMR* is more severely derepressed by histone H4 mutations when positioned far from the telomere (*cdc14* locus on chromosome VI) but is only minimally affected by the same mutations when integrated immediately adjacent to another telomere (*ADH4* locus on chromosome VII). These data indicate that the degree of silencing at *HMR* is regulated in part by its neighboring telomere over a distance of at least 23 kb and that this form of regulation is unique for *HMR* and not present at *HML*. These data also indicate that histone H4 plays an important role in regulating the silenced state at both *HML* and *HMR*.

Chromatin structure plays an important role in the regulation of gene expression in the eukaryotic cell. Active genes are generally located in euchromatic regions of the genome, while genes found in the condensed heterochromatic regions tend to be inactive (70). Position effects are observed when active genes are translocated to heterochromatic regions, resulting in either complete repression or variegated expression (reviewed in references 16 and 28). While the precise mechanism of heterochromatic condensation and repression is unknown, heterochromatin is found to spread along the chromosome and is regulated by boundaries that separate euchromatic and heterochromatic regions. These boundaries may create chromatin domains that establish a transcriptional state for the genes contained within the region (20).

The yeast *Saccharomyces cerevisiae* does not have visibly different forms of chromatin, but position effects are observed in yeast chromosomes near the telomeres and at the silent mating loci (reviewed in reference 41). Yeasts are composed of three cell types: **a** and α, which are haploids, and **a**/α diploids. Haploid cells are able to mate with other haploids of the opposite mating type to form **a**/α diploids. Diploid cells are inhibited from mating because of the expressions of both **a** and α regulatory information at the *MAT* locus, which act together to repress haploid cell functions (23). Mating type in haploids is dependent on the proper regulation of the silent copies of the mating-type genes. Copies of the α and **a** mating-type regulatory genes are encoded at the silent mating loci *HML* and *HMR*, respectively, located near the ends of chromosome III (Fig. 1). *HML* is located about 12 kb from the left telomere, and *HMR* is about 23 kb from the right telomere (55). Although the coding information and promoters of the mating-type genes at the silent loci are identical to those found at the expressed *MAT* locus (3), they are maintained in a permanently repressed, or silenced, state (54). Proper silencing of these genes is critical for the mating efficiency of a haploid cell, since derepression of the silent loci will lead to the simultaneous expression of **a** and α information in the cell, resulting in a nonmating phenotype (29).

A variety of *trans*-acting genes have been previously identified that are required for the repression of the silent mating loci (reviewed in reference 41). *SIR1*, *SIR2*, *SIR3*, and *SIR4* have been shown to be required for silencing in both **a** and α cells (60). Mutations in any of these genes cause derepression of both silent loci, although *sir1* mutations produce lower levels of transcription from the silent cassettes than do mutations in the other *SIR* genes (32). Other genes, such as *NAT1* (52), *ARD1* (71), and *HHF* (encoding histone H4) (33–35, 49, 56) are also important for the regulation of the silent mating loci, but mutations in these genes have been found to preferentially affect mating in **a** strains over mating in α strains. Other factors involved in repression that have been identified include RAP1 (discussed below), RIF1 (26), and ORC2 (41).

The silent mating loci are also regulated by two *cis*-acting regions that flank the two loci, referred to as E (for essential) and I (for important) (1). Silencing at *HML* is dependent on either E or I in the chromosome, as deletion of either region has no effect on transcription at *HML* (45). *HMR*, on the other hand, is exclusively dependent on the E region, since deletions of *HMR* E (but not *HMR* I) affect the level of repression at *HMR* (4). The E and I regions are composed of various regulatory elements, including ARS (autonomously replicating sequence) consensus sequences (17), RAP1 consensus binding sites, and ABF1 consensus binding sites (Fig. 1) (5, 7). ARS sequences confer replicating capacity to plasmids (1, 37) and are considered potential origins of replication in the yeast genome (6). RAP1 and ABF1 are both highly abundant, essential proteins (14, 64) that have

* Corresponding author. Mailing address: Molecular Biology Institute, University of California, 405 Hilgard Ave., Los Angeles, CA 90024. Phone: (310) 825-8106. Fax: (310) 206-9073. Electronic mail address: mg@wald.mbi.ucla.edu.

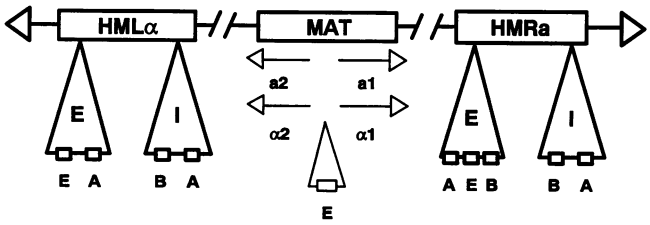


FIG. 1. Schematic representation of the yeast mating loci on chromosome III. The mating type of the cell is determined by the genes expressed at the *MAT* locus. Both sets of genes (**a** and α) are expressed from divergent promoters, as indicated by the arrows. Silent copies of the α and **a** genes are located at *HML* and *HMR*, respectively. Each silent locus is flanked by regulatory regions (E and I) that contain the various consensus sequences indicated (A, ARS consensus sequence; B, ABF1 consensus binding site; E, RAP1 consensus binding site). *HML* is located about 12 kb from the left telomere, and *HMR* is about 23 kb from the right telomere.

been implicated in gene activation (8, 9, 13, 19) and repression (25, 36, 38, 67). RAP1 also functions in the regulation of telomere length and stability (12, 39, 44), as well as playing a direct role in repression at *HMR* (68), *HML*, and telomeres (40). Although both sets of E and I regions are composed of virtually the same elements, *HMR* E possesses an ABF1 binding site (in addition to an ARS consensus sequence and a RAP1 binding site) that is not present at *HML* E.

Telomeres have also been identified as regions that cause position effects in yeast. Gottschling et al. (22) have demonstrated that a number of genes placed directly adjacent to telomeres are silenced in a manner similar to that at the silent mating loci. Telomeric silencing is dependent on *SIR2*, *SIR3*, *SIR4*, *NAT1*, *ARD1*, and *HHF* (histone H4) gene products (2), as well as RAP1 (40), but it is distinguished from mating-type silencing by a lack of dependence on *SIR1*. Genes silenced at the telomere exhibit a low-frequency switching from the silenced to the expressed state similar to the switching of the transcriptional state observed at the silent mating loci in *sir1*$^-$ strains (57), *HML* E and *HMR* E mutant strains, and *rap1*^{s} mutant strains (46, 68). Telomeric silencing spreads out continuously from the end of the chromosome, affecting genes as far away as 3 to 4 kb from the telomere (58). Furthermore, the degree of spreading can be extended to at least 22 kb from the telomere by overexpression of the *SIR3* gene.

As noted above, mutations in a number of genes involved in silencing have a much stronger effect on **a** strains than α strains. Mutations in *NAT1* and *ARD1* result in a small decrease in mating efficiency in **a** strains but have virtually no effect in α strains (52, 71). It has generally been accepted that this is a reflection of differences in the levels of derepression of the two silent mating loci. We have demonstrated previously that deletion of the N terminus of histone H4 causes a complete loss of mating in **a** strains but only a partial loss of mating in α strains, suggesting that this mutation has only a weak effect at *HMR***a** (35). This has been further demonstrated in strains carrying amino acid substitutions in the H4 N terminus that specifically affect yeast mating efficiency, in which complete derepression of *HML*α but no derepression of *HMR***a** is observed (33, 56). While functional redundancy of the elements at *HMR* E has been suggested as an explanation for this observation (52), no direct evidence that accounts for the differences in the two mating loci has been shown.

We have attempted to identify the factors influencing the level of derepression at the silent loci in H4 mutant strains by examining a number of parameters that distinguish the two loci. We have examined differences in the mating-type genes occupying the two silent mating loci, variations in the *cis*-acting regulatory sequences occupying the E regions, and the role of chromosomal location in the regulation of the silent mating loci. While the differences in the mating-type genes and the E-region sites do not appear to play major roles in the level of derepression of *HMR* in H4 mutant strains, repression at *HMR* is strongly influenced by its chromosomal location. This position effect is a result of a neighboring telomere that maintains the silenced state at *HMR* in the presence of histone H4 mutations. Since *HML* is not affected differently depending on its chromosomal location, we conclude that the telomere plays a unique regulatory role at *HMR* in the establishment and/or maintenance of the silenced state.

MATERIALS AND METHODS

Plasmid construction. Plasmids were constructed for the integration of the silent mating loci at the *cdc14* locus. A 1.5-kb *Stu*I-*Xba*I fragment of *cdc14* from pJWC100 (69) was subcloned into the *Nhe*I-*Nru*I sites of the integrating plasmid YIP5. This plasmid is referred to as pJT102. The 6.0-kb *Hin*dIII fragment of *HML* from pJR742 was subcloned into the *Hin*dIII site of pJT102, creating pJT103. The 5.0-kb *Hin*dIII fragment of *HMR* from pJR82 was subcloned into the *Hin*dIII site of pJT102, creating pJT104. Both plasmids are constructed such that the E regions of *HML* and *HMR* are distal to the *cdc14* fragment in the plasmid.

Plasmids were constructed for integration at the *ADH4* locus, utilizing the integrating plasmid pADHUCA-IV, kindly provided by D. Gottschling. This vector integrates at the *ADH4* locus, placing the *URA3* marker adjacent to *ADH4*, and creating a de novo telomere adjacent to *URA3* (related plasmids are described in reference 22). The *Hin*dIII fragments of either *HML* or *HMR* were subcloned into the *Hin*dIII site of pADHUCA-IV, between the *ADH4* fragment and the *URA3* marker. The plasmid containing *HML* is pJT105, and the plasmid containing *HMR* is pJT106. The fragments containing *HML* and *HMR* were oriented such that the E regions are distal to the *URA3* marker and the telomeric $C_{1-3}A$ repeat sequence.

A *SIR3* wild-type plasmid with a *TRP1* marker was created from pLJ87 (34). An *Ssp*I-*Stu*I fragment from YRP17 containing the *TRP1* gene was isolated and subcloned into the *Eco*RV site of pLJ87 (within the *URA3* marker). The *TRP1* gene is oriented such that its transcription is directed toward the adjacent *SIR3* gene. This plasmid was named pJT87T.

Plasmid pLJ87L was constructed for disruption of *SIR3* in LJY412I. pLJ87 was digested with *Cla*I and *Xho*I (to delete the region encoding amino acids 163 to 945), and a *Nar*I-*Sal*I fragment encoding *LEU2* was inserted to make plasmid pLJ87L.

Strain construction. A partial strain list is shown in Table 1. The genotypes listed in the constructions below indicate only the relevant markers.

LJY412I (*MAT***a** *hhf-1*::*HIS3* H4gln16-*TRP1*) was constructed analogously to LJY438I, as previously described (34). All three mating loci in LJY412I were converted to **a** by using pGAL-HO as described previously (34). This strain was named JTY111P. *SIR3* was disrupted in LJY412I by transformation with a *Sal*I-*Nco*I restriction fragment from pLJ87L. The *sir3* disruption was confirmed by Southern

TABLE 1. Strains used in this study

Strain	Genotype
JTY111P	*HMLa MATa HMRa hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY222P	*HMLα MATα HMRα hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY112P	*HMLa MATa HMRα hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY211P	*HMLα MATa HMRa hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY221P	*HMLα MATα HMRa hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY122P	*HMLa MATα HMRα hhf1::HIS3 hhf2gln16-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY111H	*HMLa MATa HMRa hhf1::HIS3 hhf2Δ4-19-TRP1 ade2-101 his3-201 leu2-3,112 lys2-801 trp1-901 ura3-52*
JTY222H	*HMLα MATα HMRα hhf1::HIS3 hhf2Δ4-19-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY112H	*HMLa MATa HMRα hhf1::HIS3 hhf2Δ4-19-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY211H	*HMLα MATa HMRa hhf1::HIS3 hhf2Δ4-19-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY221H	*HMLα MATα HMRa hhf1::HIS3 hhf2Δ4-19-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY122H	*HMLa MATα HMRα hhf1::HIS3 hhf2Δ4-19-TRP1 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY111S	*HMLa MATa HMRa sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY222S	*HMLα MATα HMRα sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY112S	*HMLa MATa HMRα hhf1::HIS3 sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY211S	*HMLα MATa HMRa hhf1::HIS3 sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY221S	*HMLα MATα HMRa sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY122S	*HMLa MATα HMRα sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY111W	*HMLa MATa HMRa hhf1::HIS3 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY222W	*HMLα MATα HMRα hhf1::HIS3 ade2 leu2-3,112 lys2-801 trp1 ura3-52*
JTY130	*MATα hhf1::HIS3 hhf2Δ4-19-TRP1 hmreΔa ade2 leu2-3,112 trp1 ura3*
JTY131	*MATα hhf1::HIS3 hhf2Δ4-19-TRP1 hmreΔb ade2 leu2-3,112 trp1 ura3*
JTY132	*MATα hhf1::HIS3 hhf2Δ4-19-TRP1 hmreΔe ade2 leu2-3,112 trp1 ura3*
JTY135	*MATα hhf1::HIS3 hhf2gln16-TRP1 hmre*Δa *sir3::LEU2 ade2 his3 leu2-3,112 trp1 ura3* with plasmid pLJ87(*SIR3-URA3*)
JTY136	*MATα hhf1::HIS3 hhf2gln16-TRP1 hmre*Δb sir3::*LEU2 ade2 his3 leu2-3,112 trp1 ura3* with plasmid pLJ87(*SIR3-URA3*)
JTY137	*MATα hhf1::HIS3 hhf2gln16-TRP1 hmre*Δe sir3::*LEU2 ade2 his3 leu2-3,112 trp1 ura3* with plasmid pLJ87(*SIR3-URA3*)
I series	See Materials and Methods
T series	See Materials and Methods
D series	See Materials and Methods
LJY412I	*MATa hhf1::HIS3 hhf2gln16-TRP1 ade2-101 his3-201 leu2-3,112 lys2-801 trp1-901 ura3-52*
LJY512I	*MATa hhf1::HIS3 hhf2gln16-TRP1 sir3::LEU2 ade2-101 his3-101 his3-201 leu2-3,112 lys2-801 trp1-901 ura3-52*
LJY653	*MATα hhf1::HIS3 ade2-101 his3-201 leu2-3,112 lys2-801 trp1-901 ura3-52*
D585-11c	*MATa lys1*
D587-4b	*MATα his1*
YDS36[a]	*MATα hmre*Δa *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3*
YDS37[a]	*MATα hmre*Δb *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3*
YDS39[a]	*MATα hmre*Δe *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3*

[a] Provided by D. Shore.

analysis, and the strain was named LJY512I. This strain was used in the construction of JTY135, JTY136, and JTY137 (see the description below). All other *sir3*$^-$ strains are derived from AB18-11B *sir3::LEU2* (*HMLα MATa HMRα sir3::LEU2 ade2 leu2-3,112 lys2-801 trp1 ura3-52*) (J. Broach laboratory). All three mating loci in AB18-11B *sir3::LEU2* were converted to α by using pGAL-HO to yield strain JTY222S.

Strains for switching the **a** and α information at the silent mating loci were created as follows. JTY111H (*HMLa MATa HMRa* H4Δ4-19-*TRP1 hhf-1::HIS3*) (analogous to JTY111P described above, except derived from LJY438I [34]) was mated with JTY222S (*HMLα MATα HMRα sir3::LEU2*). The resultant diploids were sporulated and dissected, and spores that were Trp$^+$ His$^+$ Leu$^-$ (expressing only H4Δ4-19) were identified. The presence of **a** or α information at the three mating loci was determined by Southern analysis as previously described (24). Disruption of *HHF-1* (encoding wild-type H4) was also verified by Southern analysis. Strains possessing all combinations of **a** and α genes were identified and are listed in Table 1. They are identified by the suffix H (for H4 mutant). Leu$^+$ Trp$^-$ spores were identified from the same cross to create the same **a** and α configurations in a *sir3::LEU2* background. Candidate spores were screened by Southern analysis as described above and are indicated in the strain list with the suffix S (for *SIR3* mutant).

Similar constructs containing the H4gln16 mutation were constructed by mating JTY111P (*HMLa MATa HMRa* H4gln16-*TRP1 hhf-1::HIS3*) with JTY222S (*HMLα MATα HMRα sir3::LEU2*) and analyzed as described above. All **a** and α combinations were identified as above and are indicated in the strain list with the suffix P (for point mutation). Trp$^-$ Leu$^-$ spores were identified from the same cross and screened by Southern analysis of the mating loci to create a similar series of wild-type strains. Two wild-type strains that possess **a** information at all three mating loci (JTY111W) or α information at the three mating loci (JTY222W) were created.

Strains that contain H4 mutations in combination with a deletion of each of the three regulatory elements in *HMR* E were constructed. H4Δ4-19 was combined with these deletions by mating JTY112H (*HMLa MATa HMRα* H4Δ4-19-*TRP1 HHF-1::HIS3*) with YDS36 (*MATα hmre*Δa), YDS37 (*MATα hmre*Δb), and YDS39 (*MATα hmre*Δe), kindly pro-

vided by D. Shore. Diploids were sporulated and dissected, and Trp^+ His^+ spores were identified. The mating loci were identified by Southern analysis as described above. Candidates were identified that were *HMLα MATα HMR***a** (the E-region deletion is linked to *HMR***a**). The resultant strains are JTY130, JTY131, and JTY132.

To construct a similar set of strains with the H4gln16 mutation, LJY512I (*HMLα MAT***a** *HMR***a** H4gln16-*TRP1 HHF-1*::*HIS3 sir3*::*LEU2*) was mated with YDS36, YDS37, and YDS39. LJY512I contained pLJ88, encoding a *sir3* suppressor of H4gln16 (34), to allow this strain to mate. Diploids were sporulated and dissected, and Trp^+ His^+ Leu^+ spores were identified. Candidates were screened by Southern analysis, as described above, to identify *HMLα MATα HMR***a** strains. Genomic DNA from these candidates was digested with *Hin*dIII and *Xho*I and probed with a *Hin*dIII-*Xba*I fragment of *HMR* to verify the presence of the E-region deletions (deletions were created with *Xho*I linkers). Subsequent candidates were verified to have lost the *sir3* suppressor plasmid and were retransformed with pLJ87 (wild-type *SIR3*). The resultant strains are JTY135, JTY136, and JTY137.

Strains containing *HML* and *HMR* integrated at the *cdc14* locus were created by integrating pJT103 and pJT104. Plasmids were digested with *Kpn*I, which cuts at a unique site in *cdc14*. Plasmids were purified by agarose gel electrophoresis and electroeluted by using an International Biotechnologies, Inc., Analytical Electroeluter. Linearized pJT103 (*HMLα*) was transformed into JTY111W, JTY111P, JTY111H, and JTY111S (**a** information at all mating loci) to create the strains JTY111WI, JTY111PI, JTY111HI, and JTY111SI. Linearized pJT104 (*HMR***a**) was transformed into JTY222W, JTY222P, JTY222H, and JTY222S (α information at all mating loci) to create the strains JTY222WI, JTY222PI, JTY222HI, and JTY222SI. Ura^+ candidates were isolated and screened by Southern analysis. Genomic DNA was digested with *Hin*dIII and *Xho*I and probed with an *Xho*I-*Stu*I fragment of *cdc14* (not included on the integrating plasmids) to verify an integration at *cdc14*. Strains were screened with the *MAT***a** probe to verify the presence of the integrated copy of *HML* or *HMR*.

Strains containing *HML* and *HMR* integrated at the *ADH4* locus were created by integrating pJT105 and pJT106. These plasmids were linearized by digestion with *Not*I, which cuts at a unique site at the end of the $C_{1-3}A$ telomeric repeats. Linearized plasmids were purified by agarose gel electrophoresis and electroelution. pJT105 (*HMLα*) was integrated into JTY111P, JTY111H, and JTY111S (**a** information at all mating loci) to create the strains JTY111PT, JTY111HT, and JTY111ST. pJT106 (*HMR***a**) was integrated into JTY222P, JTY222H, and JTY222S (α information at all mating loci) to create the strains JTY222PT, JTY222HT, and JTY222ST. Ura^+ candidates were identified and screened by Southern analysis. Genomic DNA was digested with *Eco*RI and probed with a *Hin*dIII-*Sal*I fragment of *ADH4* to verify an integration event at the *ADH4* locus. Transformants of pJT105 were then probed sequentially with *Hin*dIII-*Bam*HI of *HML*, *Hin*dIII-*Pvu*II of *HML*, and *Hin*dIII-*Bam*HI of *URA3* to verify the constructs as they are displayed in Fig. 3. Transformants of pJT106 were similarly probed with *Hin*dIII-*Eco*RI of *HMR*, *Hin*dIII-*Xho*I of *HMR*, and *Hin*dIII-*Bam*HI of *URA3*. A genomic *Hin*dIII digest was probed again with *URA3* to verify the presence of the telomeric $C_{1-3}A$ repeats at the end of the *URA3* gene. Strains were also probed with the *MAT***a** probe to verify the integration of either *HML* or *HMR*. We were unable to obtain any integrations into the wild-type strains (JTY111W and JTY222W). Wild-type strains were constructed by transforming JTY111ST and JTY222ST (*sir3*::*LEU2*) with pJT87T (wt*SIR3*). The transformed strains are named JTY111WT and JTY222WT.

Transformants with pJT106 were also screened for potential integration at the *HMR* locus, in order to delete the end of chromosome III from the right end of *HMR* to the telomere. Ura^+ candidates that failed to show integration at *ADH4* were screened by probing with *MAT***a** and detecting a conversion from *HMR*α to *HMR***a** (the original parent strains are *HMR*α). Candidates that gave the conversion were further screened by Southern analysis, as described above for the pJT106 plasmid, including the detection of the adjacent telomere with the *URA3* probe. The strains created are JTY221PD, JTY221HD, and JTY221SD. No integrations were obtained from the wild-type strain, so a wild-type strain was constructed by transforming JTY221SD (*sir3*::*LEU2*) with pJT87T (wt *SIR3*) and was named JTY221WD.

LJY153 (*MAT***a** *ade2-101 his3-200 leu2-3,112 lys2-801 trp1-901 ura3-52 hhf1*::*HIS3 HHF2*$^+$) was isolated from a cross of strain PKD2-5C with UKY412 (34). The *MAT* locus was switched to α by using pGAL-HO to yield strain LJY653 (confirmed by Southern analysis of the mating loci).

Quantitative mating assay and yeast transformation. Quantitative matings were done as previously described (35), with D585-11c (*MAT***a** *lys1*) as the **a** tester strain and D587-4b (*MATα his1*) as the α tester strain. All mating efficiency values presented have been normalized to an appropriate wild-type control strain and are the averages of four mating experiments. For strains with *HML* and *HMR* integrated at the *cdc14* locus, corrections were made to the mating efficiencies to adjust for low-level instability of the integrated constructs (excision of the integrated silent mating locus would revert a potential nonmater to a mater, producing an artificially high mating efficiency). A minimum of 20 diploids produced from each mating experiment were picked and analyzed by genomic Southern analysis (as described for the construction of these strains) to determine the presence or absence of the integrated silent mating locus at *cdc14*. The percentage of diploids that had mated because of loss of the integration was determined, and these maters which were falsely positive due to excision were subtracted from the total number of maters. For example, a strain with a mating efficiency of 1×10^{-4} (100 maters of 10^6 total cells) and a frequency of mating events due to excision of 85% would have only 15 true maters (100 minus 85%), resulting in a mating efficiency of 1.5×10^{-5} (15 of 10^6). For the same strain with an excision frequency of 100%, none of the maters would be considered true maters and the mating efficiency is reported as less than the inverse of the total number of cells plated (for this example, 10^6 total cells, mating efficiency $<1.0 \times 10^{-6}$).

All transformations were done by the lithium acetate method as previously described (63).

RESULTS

Greater derepression of *HML* versus *HMR* in histone H4 mutant strains is not gene dependent. One factor that could account for the different effect of H4 mutations on **a** and α strains is that each silent mating locus contains a different set of mating-type genes. In wild-type yeast strains, α1 and α2 genes reside at *HML*, while **a**1 and **a**2 genes are located at *HMR* (Fig. 1). The promoter regulating the α genes possesses a RAP1 binding site, while the promoter regulating the **a** genes is not known to be regulated by any transcrip-

TABLE 2. Quantitative mating analysis of strains expressing **a** or α genes from the silent mating loci[a]

Silent mating locus	Mating efficiency[b]		
	H4gln16[c]	H4Δ4-19[d]	*sir3*$^-$[e]
*HML***a**	1.2×10^{-5}	4.0×10^{-6}	2.1×10^{-6}
*HML*α	3.0×10^{-7}	1.6×10^{-6}	$\leq 1.8 \times 10^{-7}$
*HMR***a**	0.37	1.7×10^{-2}	6.6×10^{-6}
*HMR*α	0.10	7.5×10^{-4}	$\leq 2.5 \times 10^{-6}$

[a] All strain constructs are described in the text. Strains were constructed so that mating efficiency was dependent on silencing of the indicated locus.
[b] Mating efficiencies were determined by quantitative mating assay as described in Materials and Methods (wild type, 1.0).
[c] P series strains, normalized to either JTY111P or JTY222P.
[d] H series strains, normalized to either JTY111H or JTY222H.
[e] S series strains, normalized to either JTY111S or JTY222S.

TABLE 3. Quantitative mating analysis of H4 mutant strains possessing deletions in *HMR* E

HMR E mutation[a]	Mating efficiency[b]		
	WT	H4gln16	H4Δ4-19
+	1.0	0.37	0.017
ΔA	1.0	1.2×10^{-6}	7.8×10^{-6}
ΔE	0.78	1.2×10^{-6}	1.9×10^{-6}
ΔB	1.2	0.015	2.2×10^{-4}

[a] *HMR* with deletion of the indicated consensus region. +, no deletion; A, ARS; E, RAP1 binding site; B, ABF1 binding site.
[b] Mating efficiency was determined by quantitative mating assay, as described in Materials and Methods. All values were normalized to wild-type (WT) strain LJY653 (WT, 1.0).

tional activators (65). Differences in the level of expression of the two gene promoters and in the threshold levels of **a** and α transcripts needed to affect the mating phenotype could account for the differential effects that H4 mutations have on **a** and α strains.

To determine whether the difference in mating efficiencies of **a** and α strains expressing the H4 mutations is due to differences in the mating-type genes or is a characteristic of the silent loci themselves, strains were constructed that have either **a** or α genes at both of the silent loci in various mutant backgrounds. In order to examine expression of α genes at *HML*, we placed **a** information at the other two mating loci (*MAT***a** and *HMR***a**). Likewise, in order to examine the expression of **a** genes at *HML*, the other two loci contained α information (*MAT*α and *HMR*α). The same scheme was used for examining **a** and α genes at *HMR*. In all constructs, only the coding sequences and the corresponding promoter regions were altered; the flanking E and I regulatory regions were unchanged. The mating efficiencies of these strains were determined by a quantitative mating assay, and the data are presented in Table 2. As shown in the first column, the histone H4gln16 mutation (Lys-to-Gln substitution at amino acid 16) caused a major defect in mating, as determined when *HML* was examined, regardless of whether **a** or α genes were present there. However, this mutation had little effect on derepression of *HMR* in the presence of either **a** or α genes. We believe that the slight decrease in mating efficiency in these strains probably reflects a very weak disruption in the silenced state at *HMR*, resulting in a small percentage of cells that become nonmaters, similar to that observed in *sir1*$^-$ strains (57). The histone H4 N-terminal deletion mutation H4Δ4-19 similarly had a strong effect at *HML* but only a moderate effect at *HMR* (100- to 1,000-fold decrease in mating), as previously observed (35). The *sir3*$^-$ null mutation caused a complete loss of mating capacity regardless of the genes present at each locus. There was a difference in the levels of mating efficiency on the basis of the derepression of **a** versus α genes at *HMR* (about 4-fold in the H4gln16 mutant and about 20-fold in the H4Δ4-19 mutant), but the difference was small in comparison with the 10^3- to 10^6-fold difference in mating efficiencies on the basis of the derepression of *HML* versus *HMR*. From this, we conclude that while H4 mutations may derepress α genes slightly more than **a** genes, the difference observed in H4 mutant strains is primarily a result of differences between the flanking regulatory regions of *HML* and *HMR* and not differences between the genes encoded at each locus.

To verify that the measured mating efficiencies were a reasonable assay for the levels of derepression of the silent mating cassettes, we measured transcript levels of the **a** and α genes by an RNase protection assay in the same strains described above. For both **a** and α transcripts, normal levels of transcription were observed when transcripts were measured at *HML* in H4 mutant strains but were at low or undetectable levels when they were measured at *HMR* (data not shown), consistent with the observed mating phenotype. Although the mating assay does not appear to be a perfectly linear measure of transcription from the silent cassettes, decreases in mating efficiency do consistently reflect an increase in the level of expression. There have been reports of significant increases in transcription from the silent mating loci without a major decrease in mating efficiency (67), but all strains reported here with mating efficiencies of >0.1 produced extremely low to undetectable levels of transcription from the silent mating loci. Furthermore, full levels of expression from the silent cassettes were consistently observed in strains with mating efficiencies of $\sim 10^{-4}$ or lower, while strains that had mating efficiencies between 0.1 and 10^{-4} produced intermediate levels of transcription. Despite the nonlinearity of the assay, we feel confident that the mating assay is a sensitive indicator of transcription from the silent mating loci. Additionally, since this experiment demonstrates that the differences observed between *HML* and *HMR* are not a property of the resident genes, the use of the quantitative mating assay should be sufficient for comparing repression levels at these two loci.

The ABF1 binding site at *HMR E* only partially accounts for the differences in derepression between *HML* and *HMR*. The three silencer elements present at *HMR* E (A, E, and B) have been shown previously to function redundantly, requiring only two of the three sites to establish the silenced state (5). Deletion of any one of the sites has little to no effect on mating efficiency, while deletion of any two results in a complete loss of mating capacity in α strains. *HMR* E is distinguished from *HML* E by the presence of the B site (ABF1 consensus binding site) which is absent at *HML* E. In order to determine whether the functional redundancy and the additional B site at *HMR* E were responsible for the weak effect of H4 mutations on derepression of *HMR*, strains that combine deletions of each of the three E region elements with H4 mutations were constructed.

As shown in Table 3, deletion of any one of the E region elements alone had very little effect on mating, consistent with previous observations (5). When the ARS deletion (ΔA) or the RAP1 binding site deletion (ΔE) were combined with either the H4gln16 mutation or the H4Δ4-19 mutation, a complete loss of mating ability was observed. Unlike the first two deletions, however, combining the ABF1 binding site

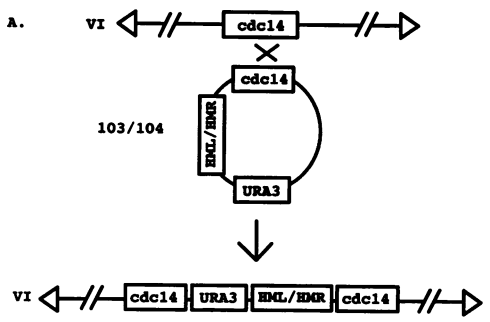
deletion (ΔB) with the H4 mutations resulted in only a partial loss of mating capacity. In combination with the H4gln16 mutation, mating was reduced about 100-fold (about 25-fold lower than H4gln16 alone). Mating was reduced about 10^4-fold in the H4Δ4-19 mutation-ABF1 binding site deletion strain, but this is only about 100-fold lower than the effect of this H4 mutation by itself on *HMR*.

These data indicate that just as the three E-region elements function synergistically, histone H4 also functions synergistically with the ARS consensus sequence and the RAP1 binding site. Deletion or mutation of any one of these three elements has little effect on mating, but mutation of any two results in the complete loss of mating capacity. In contrast, combination of H4 mutations with an ABF1 binding site deletion does not lead to as large a decrease in mating efficiency, suggesting that the ABF1 binding site, which is the element that distinguishes *HMR* E from *HML* E, is not solely responsible for the resistance of *HMR* to derepression by H4 mutations. While the ABF1 binding site may contribute to the function of *HMR* E, its presence does not fully explain the differences between *HML* and *HMR*.

Integration of the silent mating loci at the *cdc14* locus minimizes differences in the levels of derepression in H4 mutant strains. Since neither the differences in the mating-type genes nor those in the known regulatory elements flanking *HMR* fully accounted for the differences between *HML* and *HMR*, we decided to examine the chromosomal location of the silent mating loci as a possible explanation for the observed differences. To determine the importance of chromosomal location in the regulation of the silent mating loci, *Hin*dIII fragments containing either *HML* or *HMR* were integrated at the *cdc14* locus, located approximately halfway between the centromere and the telomere of the right arm of chromosome VI (51). The fragments of *HML* and *HMR* possess the entire mating locus plus several kilobases of flanking sequence (including the E and I regions). *HML*α was integrated into strains that contained only **a** genes at the three native mating loci on chromosome III, and *HMR***a** was integrated into strains that have only α genes at the three native mating loci. In this manner, mating efficiency could only be affected by derepression of the integrated silent locus.

As a result of the duplication of the *cdc14* locus upon integration, the integrated cassettes were found to be reexcised from the genome at a frequency of about 10^{-3} (data not shown), consistent with a similar effect observed previously (62). Mating efficiencies in this section have been corrected (as described in Materials and Methods) to adjust for this instability. We have integrated stable constructs of the silent mating loci at *cdc14* into some of the same parent strains and found mating efficiencies very similar to the corrected values reported here (data not shown).

The mating efficiencies of strains containing *HML* and *HMR* at the *cdc14* locus are shown in Fig. 2. In contrast to the degree of derepression of *HML* and *HMR* at their normal locations, both cassettes were significantly derepressed by mutations in histone H4. All mutant strains mated at low levels, with efficiencies ranging from 10^{-4} to 10^{-7}, regardless of whether the integrated cassette was *HML* or *HMR*. *HML* was derepressed by the H4gln16 mutation somewhat more than *HMR* (9-fold lower mating efficiency), but the difference was small in comparison with the 10^3-fold decrease in mating due to expression of *HMR* at *cdc14* rather than at its normal location. The H4Δ4-19 deletion did not produce as striking a decrease in mating efficiency (about 200-fold lower than H4Δ4-19 at the normal *HMR*), but both histone H4



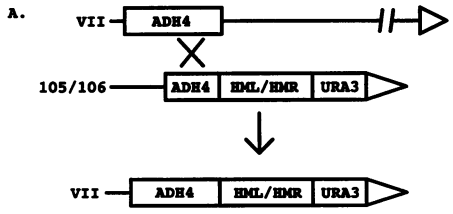
B.

Strain	@cdc14	Mutation	Mating Eff.	% Mating Events Due to Excision	Corrected M.E.
JTY111WI	HMLα	wt	0.58	0	0.58
JTY222WI	HMRa	wt	0.38	0	0.38
JTY111PI	HMLα	H4gln16	3.2×10^{-4}	90	3.2×10^{-5}
JTY222PI	HMRa	H4gln16	2.9×10^{-4}	5	2.8×10^{-4}
JTY111HI	HMLα	H4Δ4-19	4.4×10^{-4}	100	$<1.9 \times 10^{-7}$
JTY222HI	HMRa	H4Δ4-19	4.9×10^{-4}	84	7.8×10^{-5}
JTY111SI	HMLα	sir3-	2.9×10^{-4}	100	$<1.6 \times 10^{-7}$
JTY222SI	HMRa	sir3-	3.0×10^{-4}	100	$<3.1 \times 10^{-7}$

FIG. 2. (A) Integration of *HML* and *HMR* at the *cdc14* locus on chromosome VI. Plasmid pJT103 contains *HML*α, and pJT104 contains *HMR***a**. X indicates the site of integration; the arrow points to the result of the crossover event. (B) Mating efficiency (Eff.) was determined for strains possessing a copy of either *HML*α or *HMR***a** integrated at the *cdc14* locus near the middle of chromosome VI. Mating efficiency was determined by quantitative mating assay, as described in the Materials and Methods. The percent mating events due to excision is the percentage of maters from a particular quantitative mating experiment that had mated as a result of the loss of the integrated locus. Corrected mating efficiencies (M.E.) were calculated as described in Materials and Methods, compensating for the false-positive mater frequency. All *MAT***a** strains (numbered 111) were normalized to JTY111W, and all *MAT*α strains (numbered 222) were normalized to JTY222W (wild-type mating efficiency, 1.0). wt, wild type.

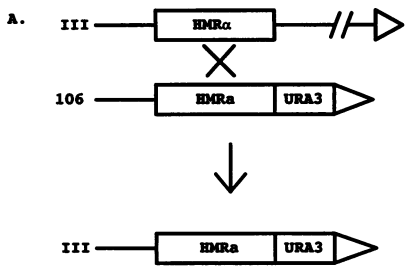
mutations resulted in similar levels of derepression at *HMR*. All mutant strains produced levels of transcription from the integrated cassettes similar to the levels produced in *sir3*$^-$ strains, as assayed by RNase protection (data not shown). Wild-type strains mated at near wild-type levels, although RNase protection revealed extremely low levels of transcription from *HML*, indicating that complete repression of *HML* was not achieved at *cdc14*.

As a control to insure that the *cdc14* locus itself was not responsible for higher levels of derepression of *HMR*, we integrated the silent mating loci at the *URA3* locus on the left arm of chromosome V in the same parent strains. As observed with the integrations at *cdc14*, mutations in histone H4 caused a strong mating defect for strains possessing an integrated copy of either *HML* or *HMR* at *URA3* (data not shown). Since the silent mating cassettes behaved similarly at both neutral loci, we believe that the ability of histone H4 mutations to derepress *HMR* at *cdc14* and *URA3* is due to the loss of a repressive element at the *HMR* locus on chromosome III. Although mutations in histone H4 do not result in a complete loss in mating capacity in this neutral context, we believe that the strong increase in expression of *HMR* at *cdc14* indicates that *HMR* is influenced by a



B. Strain	@ADH4	Mutation	Mating Eff.
JTY111WT	HMLα	wt	0.82
JTY222WT	HMRa	wt	0.62
JTY111PT	HMLα	H4gln16	4.6×10^{-7}
JTY222PT	HMRa	H4gln16	0.76
JTY111HT	HMLα	H4Δ4-19	8.9×10^{-6}
JTY222HT	HMRa	H4Δ4-19	0.29
JTY111ST	HMLα	sir3-	3.4×10^{-6}
JTY222ST	HMRa	sir3-	7.6×10^{-6}

FIG. 3. (A) Integration of *HML* and *HMR* next to a telomere at the *ADH4* locus, utilizing plasmids that possess telomeric $C_{1-3}A$ repeats. Integration at *ADH4* results in the deletion of the left end of chromosome VII and the generation of a de novo telomere next to the site of integration. Plasmid pJT105 contains *HML*α and pJT106 contains *HMR***a**. X indicates the site of integration; the arrow points to the result of the crossover event. (B) Mating efficiency (Eff.) determined for strains with *HML*α or *HMR***a** integrated immediately adjacent to a de novo telomere at the *ADH4* locus on the left arm of chromosome VII. Mating efficiency was determined by the quantitative mating assay described in Materials and Methods. All *MAT***a** strains (numbered 111) were normalized to JTY111W, and all *MAT*α strains (numbered 222) were normalized to JTY222W (wild type [wt], 1.0).



B. Strain	Mutation	Mating Eff.
JTY221WD	wt	0.26
JTY221PD	H4gln16	1.15
JTY221HD	H4Δ4-19	0.25
JTY221SD	sir3-	1.5×10^{-6}

FIG. 4. Deletion of the region between *HMR* and the telomere on chromosome III. (A) Integration of a plasmid containing *HMR***a** and a telomeric $C_{1-3}A$ repeat, resulting in the deletion of the right end of chromosome III from *HMR* to the telomere and creation of a de novo telomere immediately adjacent to *HMR*. X indicates the site of integration; the arrow points to the result of the crossover event. (B) Mating efficiency (Eff.) was determined for strains in which the region from *HMR* to the right telomere of chromosome III was deleted. Mating efficiency was determined by quantitative mating assay, as described in Materials and Methods. All mating efficiencies were normalized to JTY222W (wild type [wt], 1.0).

chromosomal position effect either due to elements flanking *HMR* on chromosome III or due to the close proximity of the telomere.

An adjacent telomere specifically represses *HMR* in an H4 mutant background. To distinguish between the importance of adjacent sequences at the *HMR* locus (outside of the 5-kb *Hin*dIII fragment) versus the proximity of the telomere (23 kb from *HMR*), the *Hin*dIII fragments containing *HML* and *HMR* were integrated at the *ADH4* locus on chromosome VII. The fragments were integrated by using a vector that contains $C_{1-3}A$ telomeric repeats, so that upon integration at *ADH4*, the end of chromosome VII is deleted and a de novo telomere is created adjacent to the site of integration (Fig. 3) (22). The de novo telomere is a generic telomere, composed of only the $C_{1-3}A$ repeats. Integration places the *HML* and *HMR* fragments roughly 1 kb from the telomere. The silent loci were integrated into the same parent strains used for the integrations at *cdc14*, so that any effect on mating would be due to expression of the silent mating locus integrated at *ADH4*. Both silent loci were integrated with the E region oriented away from the telomere as *HMR* is normally oriented on chromosome III.

As shown in Fig. 3, the H4 mutations caused a similar difference in derepression of *HML* and *HMR* as when the loci are at their normal positions on chromosome III. H4 mutant strains containing an integrated copy of *HML* were nonmaters, indicating derepression of *HML*, but *HMR* was barely affected by either mutation. The *sir3*$^-$ mutation caused a complete loss of mating regardless of which locus was integrated. This suggests that it is the proximity of a telomere to *HMR* rather than the sequences immediately adjacent to *HMR* on chromosome III that prevents complete derepression by H4 mutations. It is of interest that the H4Δ4-19 mutation had an even weaker effect on *HMR* in this context than it did on *HMR* at its normal location.

To support these observations, the same integrating vector was used to delete the end of chromosome III from *HMR* to the telomere (Fig. 4). These strains possessed a normal copy of *HMR***a**, but the remaining end of chromosome III was deleted and the normal telomere was replaced with the $C_{1-3}A$ telomeric repeat from the integrating vector. As expected, deleting the end of chromosome III did not cause *HMR* to become derepressed by H4 mutations. The *sir3*$^-$ mutation still completely abolished mating, but the H4 mutations had virtually no effect. These results demonstrate that the sequences between *HMR* and the right telomere do not influence derepression at *HMR* by H4 mutations. As observed with the integrations at *ADH4*, the H4Δ4-19 mutation had a weaker effect with the telomere moved closer to *HMR* (4-fold versus 100-fold for *HMR* on chromosome III). Based on the comparison with the integrations at the *cdc14* locus, these data support the hypothesis that derepression of *HMR* by histone H4 mutations is counteracted by neighboring telomeres.

DISCUSSION

We have shown that repression of *HMR* is uniquely influenced by the presence of a neighboring telomere. As shown previously, histone H4 mutations preferentially derepress *HML* over *HMR* (33, 35, 56). We have shown here that

this is a result of differences between *HML* and *HMR* themselves and not because of differences between the **a** and α genes that regulate the yeast mating type. Furthermore, the presence of an additional regulatory binding site at *HMR* E (an ABF1 binding site) only partially contributes to the differences observed between *HML* and *HMR*. Eliminating this site did not drastically derepress *HMR* in the presence of an H4 mutation, suggesting that the ABF1 binding site only weakly contributes to the observed differences. The difference between the two loci was significantly reduced, however, by relocating *HML* and *HMR* away from the telomere at the *cdc14* locus on chromosome VI. In this environment, both loci were strongly derepressed by H4 mutations, suggesting that *HMR* is influenced by a position effect at its normal location. We determined that this position effect is a result of a neighboring telomere. We found that by integrating *HML* and *HMR* near the end of chromosome VII with a de novo telomere immediately adjacent to the silent loci, *HML* was fully derepressed by H4 mutations but *HMR* was not affected at all. To support this finding, the end of chromosome III from *HMR* to the telomere was deleted and once again the H4 mutations had no effect on *HMR*. In both of these constructs, the H4Δ4-19 mutation, which normally causes a 100-fold decrease in mating in these strains due to partial derepression of *HMR*, caused very little derepression of *HMR* (<4-fold decrease in mating). We believe that these findings indicate that silencing at *HMR* is enhanced by an adjacent telomere.

These results identify a novel role for the telomere in transcriptional regulation that is distinct from general telomere position effects. While previous work has identified a telomere position effect extending 3 to 4 kb from the end of the chromosome (22, 58), we have found that the telomere imposes regulatory effects on *HMR* at least 23 kb from the end of chromosome III, an effect that appears to strengthen when the distance between the two loci is decreased. Unlike the general telomere position effect, which indiscriminate represses any genes placed adjacent to the telomere, the role of the telomere in mating-type repression is specific for *HMR*. Although repression of *HML* is weakened very slightly by positioning away from the telomere, moving *HML* immediately adjacent to the telomere did not facilitate repression at all. Additionally, general telomere position effect is dependent on histone H4 (2), while the interaction between *HMR* and the telomere alleviates the requirement for a fully functional H4 N terminus. We do not know whether a continuous domain of repression is established between *HMR* and the telomere. Only one genetic locus has been mapped in this region (*MAL2* [10]), but because this locus is duplicated five times in the yeast genome, it is not clear whether this locus is transcriptionally active.

The mechanism for the effect of the telomere on *HMR* is unclear. One possibility is that the telomere functions synergistically with the other elements at *HMR* E to create a high level of repression. As shown previously, *HMR* E is functionally redundant, possessing three repressive elements, only two of which are absolutely required for maintaining the repressed state (5). Just as these three elements (in conjunction with the histone H4 N terminus) function synergistically, the telomere may function as an additional synergistic repressive element to aid in the establishment and maintenance of repression at *HMR*. Although moving *HMR* away from the telomere does not significantly inhibit its repression (reference 42 and this paper), combining the loss of the telomere with other weak defects in repression (like mutations in histone H4) results in a strong loss of silencer function. Since the telomere itself can function as a repressor element, presumably utilizing a mechanism similar to that used at the silent mating loci, it is plausible that the telomere simply functions by interacting combinatorially with the other silencer elements present at *HMR*. Whatever the nature of the synergistic interactions is, it must require elements that are unique to *HMR*, since *HML* is unable to achieve such an interaction. It is possible that other sequences at *HMR* E (aside from the A, E, and B elements) may be important for this interaction, since a synthetic silencer possessing only the A, E, and B elements is a weaker silencer than the endogenous *HMR* E (48). The weakened state of the synthetic silencer could be due to the loss of the interaction between *HMR* and the telomere.

In keeping with the idea of a synergistic interaction between the telomere and *HMR*, it is reasonable to speculate that RAP1 could play a role in the establishment of the supersilenced state at *HMR*. In addition to RAP1 being bound at *HMR* E, RAP1 also binds extensively to telomeric repeat sequences in vivo (7, 43) and has been shown to play a direct role in repression at *HMR* (67, 68), *HML*, and telomeres (40). RAP1 bound at the telomere could facilitate silencing at *HMR* by interacting directly with other silencing elements bound at *HMR* E. Models which implicate looping between the E and I regions of the silent mating loci have been suggested (30); similarly, the telomere could fold back on the chromosome to permit direct contact between telomerically bound RAP1 and silencer proteins bound at *HMR*. Interestingly, mutations have been identified in RAP1 that cause derepression at *HMR* specifically when the RAP1 binding site at *HMR* E is absent (25). It is possible that these mutations might specifically disrupt the function of telomeric RAP1 in establishing contacts with *HMR*. It should be noted that RAP1 also plays a role in silencing at *HML*, suggesting that RAP1 would have to function differently at *HMR* than at *HML* if in fact RAP1 is the element that mediates the repressive effect of the telomere on *HMR*.

Alternatively, the telomere might function by regulating, through replication, the ability of the *HMR* E silencer to establish the silenced state. Replication has been shown to be required for the establishment of silencing (50), and more recent studies utilizing a synthetic silencer indicate that replication initiated from the ARS at *HMR* E is correlated with repression of *HMR* (48, 61). The telomere could regulate the function of replication from *HMR* E, perhaps by influencing the time of replication. A correlation between late replication and gene repression in higher eukaryotes has been previously demonstrated (20, 27, 31), and this correlation holds true for the silent mating loci and telomeres in yeasts (47, 59). Additionally, telomeres have been shown to cause late replication of an origin in yeasts (18). In this capacity, the neighboring telomere could function by causing late replication of the *HMR* E origin, which could facilitate the efficient assembly of silencing components. In the case of a histone H4 mutation, the mutations in the N terminus might destabilize (but not completely disrupt) a repressive chromatin structure. However, with the late replicating function of *HMR* E efficiently regulated by its neighboring telomere, *HMR* E would be able to reestablish the repressive chromatin structure, perhaps at each cell cycle, before the defects in histone H4 would allow the chromatin structure to disassemble. *HML*, which lacks an ARS that functions in the genome (15), would be unable to defend against such defects in chromatin structure; thus, the adjacent telomere could not influence the degree of repression at *HML*.

In addition to the importance of these results in terms of

understanding repression of the silent mating loci and the function of telomeres in transcriptional regulation, these results also indicate the importance of histones, particularly the role of histone H4 in transcriptional repression. Chromatin structure has been envisioned as a mechanism through which silencing is achieved (11, 21, 53, 66), and the discovery of mutations in the histone proteins themselves seems to solidify that picture. Nonetheless, it has been difficult to envision how repression at one locus could depend strongly on the function of the H4 N terminus while another locus seemed impervious to it. The results presented here demonstrate that the H4 N terminus plays a central role in repression. *HMR*, while not normally affected by H4 mutations, is strongly derepressed by H4 mutations when the silencing function is weakened, either by eliminating one of the silencer elements at *HMR* E or by translocating the locus to a nontelomeric site. We believe that these results indicate that, although defects in chromatin structure can be compensated for by redundant levels of silencing, chromatin structure and particularly the function of the histone H4 N terminus are essential and central components of the silencing machinery.

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