attenuate the postinjury process of degeneration [E. Assia, M. Rosner, M. Belkin, A. Solomon, M. Schwartz, *Brain Res.* **476**, 205 (1988)]. The combined treatment of low-energy laser irradiation with local injection of soluble substances into injured optic nerves promotes axonal growth in the rabbit optic nerve model (*12*).

15. Rats were deeply anesthetized and placed in a small stereotaxic instrument. In each animal two holes were drilled in the skull, through which two electrodes were implanted, while the dura were kept intact to minimize cortical damage. The electrodes were gold contact pins (Wire-Pro) soldered to screws, which were screwed into the holes and cemented to the skull with acrylic cement. The electrode inserted through the hole drilled in the nasal bone was used as a reference point. The second hole was in area OC1, with coordinates bregma 8 mm and lateral 3 mm. The field potential evoked by the left eye, recorded in the right visual cortex before and after injury, was evoked by stroboscopic light stimulation after implantation of the electrodes. The stroboscopic light had the following characteristics: xenon flash tube (4 W/s, 1- to 2-ms duration, 0.3 Hz) amplified 1000 times (AM Systems, microelectrode AC amplifier, model 1800) and digitized (12 bits, 5000 samples per second) (National Instruments, board NB-MIO16-9 and LabView 2.2.1 data acquisition and analysis software). Unless otherwise indicated, the right eye was covered by black tape during recording of the VEP response to avoid the possibility of a VEP response originating from it.
16. Rat optic nerves were excised and fixed by immersion in 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 2.5 mM $CaCl_2$. The nerves were cut with a razor blade into segments 1 mm long, which were postfixed for 60 min with 1% osmium tetroxide containing 1% potassium ferricyanide in cacodylate buffer, stained en bloc in uranyl acetate (2% in water) for 30 min, and dehydrated by immersion in increasing concentrations of ethanol and then in propylene oxide. Nerve segments were infiltrated with Polybed (Polysciences, Warrington, PA) and cured for 3 days at 60°C. Ultrathin sections were placed on 300-mesh or on formvar-coated one-slot copper grids, stained with lead citrate and saturated uranyl acetate, and examined with a Philips 410 electron microscope. Axons were classified as myelinated if their axoplasm was lucent, their microtubules and neurofilaments were aligned, and their myelin was well preserved. Axons were classified as unmyelinated if they contained aligned microtubules and neurofilaments, lucent cytoplasm, and occasional vesicles.
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Silencers and Domains of Generalized Repression

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Gene expression can be affected by the chromosomal position of the gene. An example of this position effect is silencing of the *HML* and *HMR* mating-type loci of *Saccharomyces cerevisiae*. An in vitro assay revealed that silencing induced a transcription-independent general occlusion of the DNA at *HMR* from sequence-specific interactions of proteins with DNA. The minimum boundaries of the silenced chromatin structure were determined, as were the contributions of the E and I silencers to the size of the silenced domain. Examination of endonuclease-sensitive sites provided evidence that neither the integrity of the chromosomal duplex nor covalent linkage of the silencers to *HMR* was important for maintenance of the silenced structure in vitro.

The eukaryotic genome is arranged into functional domains that influence states of gene expression. Euchromatic regions are highly transcribed, whereas heterochromatic regions are not. Transcriptionally quiet regions are often close to chromosomal landmarks, such as centromeres and telomeres in *Drosophila melanogaster* (*1*, *2*), telomeres in *Saccharomyces cerevisiae* (*3*), and centromeres in mouse (*4*) and *Schizosaccharomyces pombe* (*5*). Genes transposed next to heterochromatin acquire a lower level of expression. The mechanisms by which these position effects operate are not gene specific and may represent a general inactivation of chromosomal segments.

An example of a position effect in *S. cerevisiae* is the transcriptional repression of genes at the mating-type loci *HML*α and *HMR***a**. This block to gene expression shares several characteristics with heterochromatic repression and has been named silencing. Mating-type in *S. cerevisiae* is determined by the information encoded at the *MAT* locus. The *MAT***a** and *MAT*α alleles encode the regulatory proteins that impart the **a** and α mating phenotypes of the haploid cell types and the nonmating, sporulation-proficient phenotypes of the **a**/α diploid (*6*). Most strains of *S. cerevisiae* also have copies of both the α and **a** genes at the transcriptionally repressed *HML*α and *HMR***a** loci. These copies of the α and **a** genes are not expressed unless they are transposed to the *MAT* locus during mating-type switching. Mating-type switching is catalyzed by HO endonuclease cleavage of *MAT*. HO endonuclease cannot cleave its recognition sequences at *HML*α or *HMR***a** (*7*); however, mutations that allow transcription of *HML*α and *HMR***a** at their native chromosomal locations also allow cleavage by HO endonuclease (*8*). Thus, *HML* and *HMR* contain all the sequences necessary for successful transcription, but their transcription is blocked by a combination of proteins and sites that mediate silencing.

Several proteins that are important for silencing have been identified by genetic criteria (*9*). Inactivation of *SIR2*, *SIR3*, or *SIR4* results in complete derepression of *HML* and *HMR*, as well as loss of the telomeric position effect, with few other phenotypes. Mutations in the NH_2-terminus of histone H4 also derepress *HML* and *HMR* (*10–13*). Additional insights into the role of chromatin structure have been obtained from the observations that *SIR2* overexpression results in hypoacetylation of histones (*14*), *SIR3* and histone H4 interact genetically (*11*), and a *SIR3* mutation affects the conformation of a histone H3 variant bound at *HMR* (*15*).

Sequence elements, known as silencers, flanking *HML* and *HMR* are required for repression. *HML* is flanked by the *HML*-E and *HML*-I silencers, and *HMR* is similarly flanked by *HMR*-E and *HMR*-I (*16–18*). The silencers are complex and resemble yeast autonomous replication sequence (ARS) elements, and several observations suggest a role for DNA replication in silencing (*16*, *19–23*). The arrangement of one silencer on each side of *HML* and *HMR* suggests that it is important that both sides of the region under control are flanked; however, in some contexts, single silencers are fully capable of repression (*18*, *24*).

We developed an in vitro assay that mimicked the dependence of the silenced region on both *SIR* function and the presence of the silencers. Isolated yeast nuclei were assayed with purified HO endonuclease to determine whether the DNA at the silent mating-type loci remained in a repressed state. Cleavage by the HO endonuclease in vivo occurs only at *MAT* in wild-type cells; however, in *sir* strains, *HML* and *HMR* are also cleaved by HO endonuclease, allowing mating-type switching at any of the three loci (*7*, *8*). *SIR*-dependent HO endonuclease cleavage was detected in isolated nuclei (Fig. 1A). In wild-type nuclei, only the *MAT* locus was cleaved by HO endonuclease. In contrast, both *MAT* and *HMR* were cleaved in nuclei from a *sir4* strain. Similar results were observed with nuclei from *sir2* and *sir3* strains and for the *HML* locus (see below). Furthermore, the amounts of *MAT* and *HMR* cleaved in nuclei from *sir* strains

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were approximately equal, consistent with their equivalent switching efficiencies in the absence of silencing (*25*).

Restricted HO accessibility could reflect a general blockage of protein–nucleic acid interactions in silenced chromatin. Alternatively, the HO endonuclease may be sensitive to a specific feature of *SIR*-dependent chromatin structure. Indeed, the only obvious *SIR*-dependent change in sensitivity to deoxyribonuclease I at *HML* and *HMR* is at or very near the site at which HO endonuclease cleaves in vivo (*26*). To test the generality of *SIR*-dependent protection, we assayed other sites in and around *HMR* with bacterial restriction endonucleases. The restriction endonuclease Ava II was substituted for HO endonuclease in the assay for silenced DNA. *HMR* was cleaved by Ava II in nuclei isolated from *sir2*, *sir3*, and *sir4* strains, but not in nuclei from a wild-type strain (Fig. 1B). This experiment was repeated with 12 additional restriction endonucleases with similar results (see below), thus greatly extending the generality of the effect of silencing on DNA accessibility and ruling out specific interactions between HO endonuclease and components of the silencing mechanism.

Because *HMR* is transcribed in *sir* strains but repressed in wild-type cells, it is possible that transcription in vivo, prior to the isolation of nuclei, is required for endonuclease access to the DNA in vitro. To test this possibility, we constructed a transcriptionally defective *HMR* allele by substituting its promoter with the pUC18 multiple cloning site. No transcript was detected from this mutant allele in a *MATα sir3* strain. Cleavage of the untranscribed *HMR* locus by HO endonuclease remained under *SIR* control (Fig. 1C) and was qualitatively similar to cleavage at the transcribed *HMR* (Fig. 1A) and *HML* loci, although a slight decrease in sensitivity was noted. Thus, transcription did not greatly influence the accessibility of *HMR* to HO or restriction endonucleases (see below).

To determine the extent of the *SIR*-dependent repressed domain, we assayed sites within a region encompassing all of *HMR* and several kilobase pairs (kb) of flanking DNA with a battery of restriction endonucleases. For these experiments, we used the promoter-deleted *HMR* allele with the pUC18 multiple cloning site inserted between the **a***1* and **a***2* genes; thus, the restriction sites at the multiple cloning site within the silenced region served as convenient positive controls for a *SIR*-dependent effect. Restriction sites whose accessibility was affected by SIR function were detected at least as far as 0.8 kb centromere-proximal of *HMR*-E and 0.4 kb centromere-distal of *HMR*-I (Fig. 2).

The contribution of *HMR*-I to silencing at *HMR* has been measured most thoroughly by the mating efficiency of *MATα* strains. By this assay, *HMR*-I alone is not capable of silencing the **a** genes; its contribution to repression is detectable only in the presence of *HMR*-E (*16*). However, the mating assay indirectly measures only mRNA from the **a***1* gene at *HMR* and may not reveal other *SIR*-dependent influences on nearby chromatin structure. The protection of restriction sites permitted a more rigorous test of whether the *HMR*-I silencer can act autonomously. Specifically, if *HMR*-I alone can bring about repression, some sites would be protected in the absence of *HMR*-E. Several restriction sites were tested for *SIR*-dependent protection in a strain with an inactivating deletion of *HMR*-E (*27*). This deletion (*hmr***a**Δe358–303) removes the ARS consensus sequence and the RAP1 binding site that form the essential part of the *HMR*-E silencer and abolishes silencing at *HMR*. Sites were tested at the extreme limits of the protected domain and none showed a *SIR*-dependent difference in accessibility, indicating that

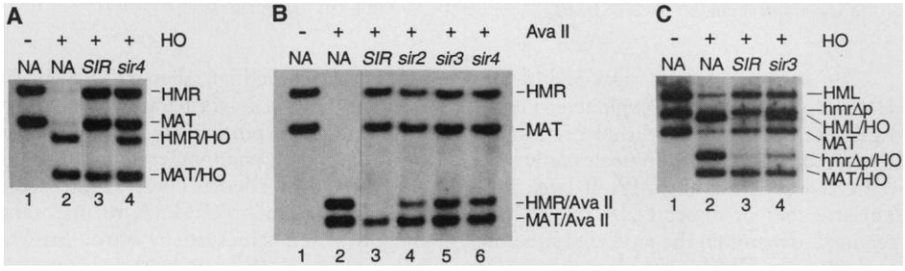

Fig. 1. (**A**) *SIR*-dependent protection of *HMR***a** from HO endonuclease in isolated nuclei (*42*). Lanes 1 and 2, naked DNA (NA) digested with Hind III alone or with both Hind III and HO endonuclease, respectively. Lanes 3 and 4, DNA isolated from *SIR* (W303-1A) (*43*) and *sir4* (JRY3411) nuclei, respectively. (**B**) *SIR*-dependent protection of *HMR***a** from Ava II in isolated nuclei. Lanes 1 and 2, naked DNA digested with Hind III alone or with both Hind III and Ava II, respectively. Lanes 3 to 6, DNA isolated from *MAT***a** *SIR, sir2, sir3,* and *sir4* nuclei (W303-1A, JRY3433, RS862, and JRY3411), respectively. The Ava II site tested was located within the **a***2* gene at *HMR*. (**C**) *SIR*-dependent protection of *HMLα* and *hmr***a**Δp in isolated nuclei in the absence of in vivo transcription from *hmr***a**Δp, a transcriptionally defective allele of *HMR***a**. Lanes 1 and 2, naked DNA digested with Hind III alone or with both Hind III and HO endonuclease, respectively. Lanes 3 and 4, DNA isolated from *SIR* (JRY3528, *HMLα MATα hmr***a**Δp *SIR*) and *sir3* (JRY3525, *HMLα MATα hmr***a**Δp *sir3::TRP1*) nuclei, respectively. In (A) and (B), **a***1* sequence was used to detect *MAT***a** and *HMR***a** on DNA blots; in (C), **a***2* sequence was used to detect *HMLα*, *MATα*, and *hmr***a**Δp (**a***2* and *α2* sequences are identical).

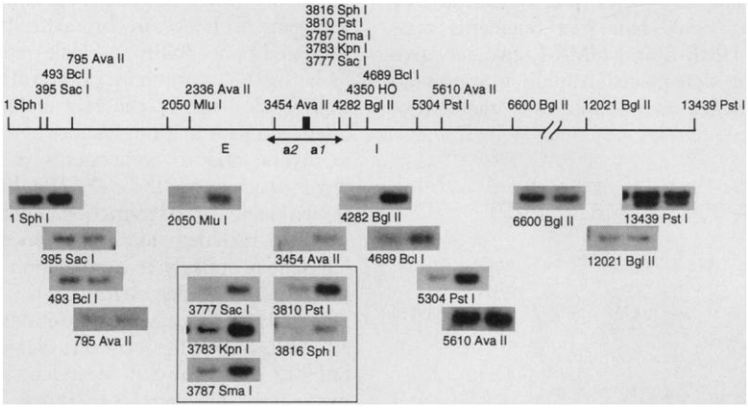

Fig. 2. The linear extent of the protected region at *HMR***a** as measured by restriction endonuclease accessibility. Protection was detected at and between the Mlu I (position 2050) and Pst I (position 5304) sites. Data are from two isogenic strains: *MATα hmr***a**Δp *SIR* (JRY3528) and *MATα hmr***a**Δp *sir3::TRP1* (JRY3525). For each site, the bands corresponding to *HMR***a** cut with the assay endonucleases are shown, with *sir3* on the right. For sites at which differences were not detected, the same sample of DNA was used to confirm that protection was present at another site recognized by the same endonuclease; the multiple cloning site introduced in place of the *HMR***a** promoter often served as this positive control (positions 3777 to 3816, boxed sites). The lower of the two bands shown for Pst I (position 13,439) is the relevant cleavage product; the upper band is the full-length fragment. The Ava II site at position 2336 was always refractory for reasons that are unknown; this was the only site that behaved in this way. The positions of the **a***1* and **a***2* genes and the E and I silencers are indicated.

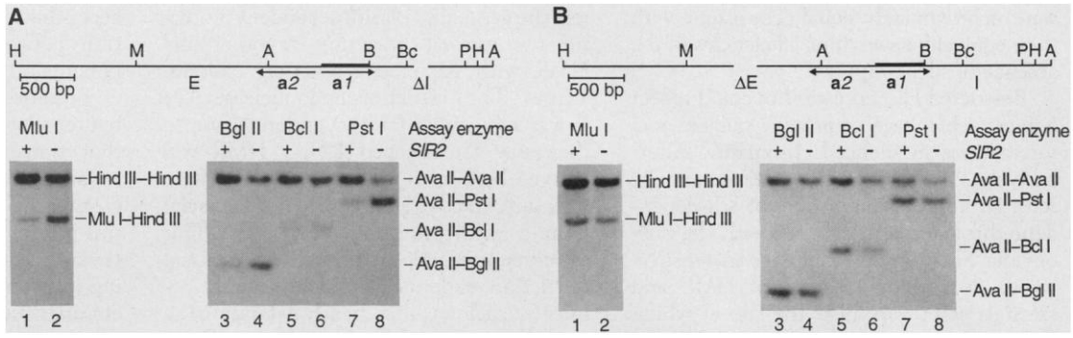

Fig. 3. (**A**) Effect of inactivating deletions of *HMR*-I (**A**) and *HMR*-E (**B**) on the extent of the protected region at *HMR***a**. Nuclei were isolated from a *MATα hmr***a**Δ*I SIR* strain (JRY3842) and a *MATα hmr***a**Δ*I sir2::HIS3* strain (JRY3843) (A) and from a *MATα hmr***a**Δe358–303 *SIR* strain (YAB62) and a *MATα hmr***a**Δe358–303 *sir2::HIS3* strain (JRY3846) (B). In both panels, the Mlu I, Bgl II, Bcl I, and Pst I sites indicated on the restriction maps were tested. Lanes 1 and 2, DNA digested with Hind III before electrophoresis; lanes 3 to 8, DNA digested with Ava II before electrophoresis. An **a***1* probe (thick bars) was used to detect *HMR***a** sequence. Not all sites for these endonucleases are shown. Restriction endonuclease abbreviations are H, Hind III; M, Mlu I; A, Ava II; B, Bgl II; Bc, Bcl I; P, Pst I. bp, base pairs.

HMR-I alone did not induce any detectable change in chromatin structure (Fig. 3B).

In contrast with the deletion of *HMR*-E, the extent of the protected region was similar in the absence or presence of *HMR*-I. In a comparison between a wild-type strain and a *sir2* strain, both of which bore deletions of the entire *HMR*-I element (base pairs 4762 to 5067 of Fig. 2), protection was observed at all restriction endonuclease sites tested (Fig. 3A). Because the sites tested were near the limits of the protected region, *HMR*-I is unlikely to be a major determinant of the linear extent of repression.

In the course of this study, two highly sensitive restriction sites were discovered at *HMR*. When isolated nuclei were incubated in the presence of Dra I, a ~2-kb Dra I–Dra I fragment was efficiently generated (Fig. 4, lanes 1 and 2). This fragment is defined by the Dra I sites indicated as immediately flanking *HMR*. Because the origin recognition complex (ORC) may occupy ARS elements throughout the cell cycle (*28, 29*), we suspect that the sensitivity of these Dra I sites was attributable to ORC bound to ARS elements at *HMR*-E and *HMR*-I.

The sensitivity of the Dra I sites near *HMR*-E and *HMR*-I was exploited to investigate the importance of the silencers and of chromosomal integrity in maintaining the protected structure at *HMR*. If large (>2-kb) stretches of intact DNA duplex were required to maintain the protected structure, then cleaving *HMR* at the two sensitive Dra I sites might remove protection from the fragment in vitro. Nuclei isolated from wild-type and *sir3* strains were treated with Dra I until most of *HMR* was released as the 2-kb Dra I–Dra I fragment (Fig. 4, lanes 1 and 2). After this initial reaction was complete, a second endonuclease was added. The fragments remained refractory to the second endonuclease (Fig. 4, lanes 3 to 8), indicating that the integrity of the chromosomal duplex at *HMR* was not important for the maintenance of the protected structure. *HMR* was modified in this experiment to allow cleavage of the two silencers from the intervening DNA by Dra I. Specifically, by inverting ~300–base pair fragments containing *HMR*-E and *HMR*-I, two sensitive Dra I sites were placed in an orientation such that cleavage resulted in a 2-kb fragment of *HMR* that lacked all silencer sequences. Because the Dra I–Dra I fragment retained *SIR*-dependent protection from cleavage by the second endonuclease, neither of the two *HMR* silencers was required on the same fragment of DNA to maintain the protected structure in vitro. Strains bearing the *HMR* locus without inverted silencers gave indistinguishable results when tested in this manner.

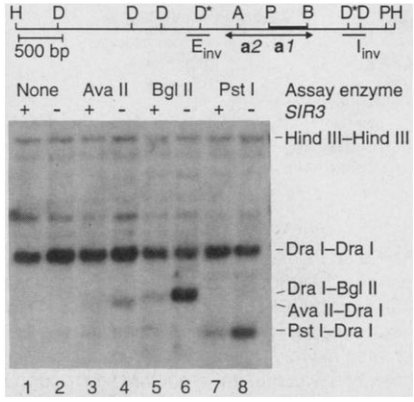

Fig. 4. Maintenance of *SIR*-dependent protection from restriction endonuclease cleavage on a Dra I–Dra I fragment of *HMR***a** cleaved from the chromosomal duplex and lacking both silencers. Nuclei from *MATα hmr***a**Δp and silencers-inverted *SIR* (JRY4006) and *MATα hmr***a**Δp and silencers-inverted *sir3::TRP1* (JRY4009) strains were incubated in the presence of Dra I at 25°C for 1 hour, after which time most *HMR* had been released as a 2-kb Dra I–Dra I fragment (lanes 1 and 2). The remaining sample was divided into three portions and Ava II, Bgl II, or Pst I was added to each. After incubation for 1 hour at 25°C, DNA was extracted from these reactions and used to assess whether *SIR*-dependent protection from the second restriction endonuclease was maintained after cleavage by Dra I (lanes 3 to 8). DNA in even-numbered lanes was slightly overloaded; nevertheless, depletion of the *HMR***a** Dra I fragment was observed in most *sir3* lanes and served as confirmation that protection existed on the *HMR***a** Dra I–Dra I fragment. The Dra I (D) sites indicated with asterisks delimit the 2-kb fragment detected. An **a***1* probe (thick bar) was used to detect *HMR***a** sequence. Bars indicate the locations of the silencer-containing fragments that were inverted, base pairs 2672 to 3047 and 4689 to 5006 of Fig. 2.

We have described the isolation of nuclei from *S. cerevisiae* in a manner that preserves the silenced state at the *HML* and *HMR* mating-type loci. The biological relevance of this state is demonstrated by the exclusion of HO endonuclease from its cleavage sites at *HML* and *HMR* in a *SIR*- and silencer-dependent manner. The silencing of *HML* and *HMR* is thought to be mediated by a general inactivation of these chromosomal regions and not by a process that is specific to the mating-type genes normally resident at these loci (*9*). That silencing is associated with general changes in chromatin structure is suggested by its ability to block transcription by both RNA polymerases II and III (*30*), as well as cleavage by the HO endonuclease. Silenced DNA is also refractory to a bacterial methylase and to components of a DNA repair mechanism (*31–34*). We have now shown that bacterial restriction endonucleases are also excluded from the silenced *HMR* locus. It is unlikely that protection from restriction endonuclease cleavage was attributable to shifts in nucleosome positions unrelated to silencing. Micrococcal nuclease analysis of *HMR* reveals no *SIR*-dependent change in nucleosome phasing (*26*). Simple shifts in some nucleosome positions might have coincidentally exposed sites that were otherwise masked in wild-type nuclei, but they should also mask some sites in nuclei isolated from *sir* strains. Despite the large number of sites tested, such regions were not observed.

In one model of how the size of an inactive domain is limited, heterochromatin assembly is terminated by specific sequence elements along the route of assembly (*35*). In support of this hypothesis, scs

and scs' elements in *D. melanogaster* can insulate a reporter gene from the influences of nearby heterochromatin (*36*). *HMR*-E and *HMR*-I are the only sequences known to influence expression states at the silent mating-type loci and thus they are currently the only candidates for cis-limiting components of repression. We have identified restriction sites that are differentially sensitive outside the limits defined by the *HMR*-E and *HMR*-I silencers, suggesting that silencers do not act like the scs and scs' elements, in agreement with genetic observations at *HML* (*24*). Some other mechanism must determine the boundary of the silenced domain at *HML* and *HMR*. The silencers may possibly nucleate the assembly of a domain whose size is limited by the quantity of some other component, such as a SIR protein (*37*).

Genetic assays indicate that *HMR*-E is capable of repressing transcription in the absence of *HMR*-I (*16*). The restriction endonuclease–based assay revealed that the extent of the protected domain created by *HMR*-E alone was indistinguishable from that created by both silencers acting in concert. However, in the absence of *HMR*-E, no restriction sites were protected. Thus, *HMR*-I may have no role in governing the linear extent of the protected structure. Instead, *HMR*-I may support a role of E that is not reflected in the linear extent of the protected structure, perhaps enhancing its nucleation rather than its spreading.

Several genetic observations point to a distinction between the initial establishment of the repressed state and its subsequent stable propagation and maintenance (*19, 38*); the silencers have been implicated in establishment (*39, 40*). However, it is not known whether the silencers are also required for the subsequent maintenance of repression, as required by some models (*41*). Our study addressed the requirement for an intact DNA duplex as well as for the silencers to be on the same DNA duplex in maintenance of a *SIR*-dependent structure at *HMR*. Two sensitive Dra I sites allowed the selective excision of a 2-kb DNA fragment bearing *HMR* without the associated silencer sequences. This fragment retained the specialized chromatin structure at *HMR* as judged by retention of *SIR*-dependent protection from restriction endonuclease cleavage. These observations are consistent with silencers not being required for the maintenance of repression. We emphasize that cleavage of the DNA duplex in this manner does not necessarily disrupt higher order structures that might constrain *HMR* in isolated nuclei. Recovery of the Dra I–Dra I fragment for more detailed analysis will shed more light on the maintenance requirements at *HMR* as well as the protein components physically associated with silenced DNA.

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42. Nuclei were prepared by a modification of two methods [B. B. Amati and S. M. Gasser, *Cell* **54**, 967 (1988); J. P. Aris and G. Blobel, *Methods Enzymol.* **194**, 735 (1991)]. Cells (1 liter) were grown at 30°C in YPD medium and harvested at an optical density at 600 nm of 1.5. The cells were washed in water and suspended in 25 ml of a spheroplast medium (1 M sorbitol, 1% glucose, 0.2% Bacto yeast nitrogen base, 0.2% Bacto casamino acids, 25 mM Hepes, 50 mM tris base). After addition of 60 μl of 1 M dithiothreitol and 10,000 U of lyticase (Sigma), the suspension was incubated uncovered at 30°C for up to 2 hours. Unless noted, all subsequent steps were performed at 4°C. Spheroplasts were collected, washed twice in glycerol SS+SS buffer [1× SS (see below), 1% (v/v) thiodiglycol, 10% (v/v) glycerol, 0.25 mM phenylmethylsulfonyl fluoride (PMSF), 2 mM spermidine, 0.8 mM spermine], suspended in 20 ml of lysis buffer (1× SS, 18% Ficoll 400, 0.25 mM PMSF), and homogenized with a Dounce homogenizer. Cell particles, including nuclei, were separated by centrifugation and washed twice in glycerol SS+SS buffer. The final pellet was suspended in 10 ml of glycerol SS+SS buffer and 15 ml of Percoll solution [1× SS, 60% (v/v) Percoll, 10% (v/v) glycerol, 1% (v/v) thiodiglycol, 0.25 PMSF, 2mM spermidine, 0.8 mM spermine] with a Dounce homogenizer, and centrifuged at 35,000 *g* for 40 min. The band containing nuclei was collected by centrifugation after dilution with glycerol SS buffer [1× SS, 1% (v/v) thiodiglycol, 10% (v/v) glycerol, 0.25 mM PMSF]. The pellet was washed twice with 20 ml of 1 × SS buffer [10 mM tris-Cl (pH 7.5), 2 mM EDTA (K^+ salt), 0.125 mM spermidine, 0.05 mM spermine, 20 mM KCl] and suspended in 1× SS buffer such that the optical density at 600 nm of the suspension was between 2 and 6. For each assay, 200 μl of nuclei were incubated for 1 hour at 25°C in the presence of 10 mM $MgCl_2$, 0 to 100 mM NaCl (as appropriate for the specific endonuclease), and 20 to 100 units of restriction endonuclease or partially purified HO endonuclease. The reactions were stopped by adding 2 volumes of a detergent solution (2% Triton X-100, 1% SDS, 100 mM NaCl) and heating to 65°C for 20 min before organic extractions and ethanol precipitation. HO endonuclease was purified by standard protein purification procedures and a modification of protocols of R. Kanaar and R. Kostriken (available upon request). In all assays, some *HMR* molecules remained uncut by the endonuclease used to detect *SIR*-dependent protection. The amount of uncut *HMR* was not attributable to residual silencing in *sir* strains because a similar amount of uncut *MAT* fragment was detected. The amount of DNA that escaped digestion was insensitive to the amount of endonuclease added and may have resulted from some nuclei being impermeable. Alternatively, sites may have been masked by random nucleosome placement unrelated to silencing.
43. Strains used were as follows: W303-1A (*MAT***a** *ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 can1-100*) (R. Rothstein), YAB62 (*MATα ade2-1 his3-11,15 leu2-3,112 trp1-1 ura3-1 hmr***a**Δe358–303 *can1-100*) (*27*), RS862 (W303-1A; *sir3::TRP1*) [E. M. Stone, M. J. Swanson, A. M. Romeo, J. B. Hicks, R. Sternglanz, *Mol. Cell. Biol.* 11, 2253 (1991)], JRY3009 (W303-1A; *MATα*), JRY3411 (W303-1A; *lys2 sir4::HIS3*), JRY3433 (W303-1A; *sir2::HIS3*), JRY3436 (JRY3009; *sir3::TRP1*), JRY3525 (JRY3009; *hmr***a**Δpromoter *sir3::TRP1*), JRY3528 (JRY3009; *hmr***a**Δpromoter), JRY3842 (JRY3009; *hmr***a**ΔI), JRY3843 (JRY3009; *hmr***a**ΔI *sir2::HIS3*), JRY3846 (YAB62; *sir2::HIS3*), JRY4006 (JRY3009; *hmr***a**Δpromoter and silencers-inverted), JRY4009 (JRY3009; *hmr***a**Δpromoter and silencers-inverted *sir3::TRP1*).

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