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Extragenic Suppressors of *mar2*(*sir3*) Mutations in *Saccharomyces cerevisiae*

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ABSTRACT

The silent mating-type genes (*HML* and *HMR*) of *Saccharomyces cerevisiae* are kept under negative transcriptional control by four *trans*-acting *MAR* (or *SIR*) loci. We have isolated extragenic suppressors of the *mar2-1* mutation which, based on genetic complementation tests, define two additional loci involved in regulating the expression of *HML* and *HMR*. A strain with the genotype *HML***a** *MAT*α *HMR***a** *mar2-1* is sterile due to the simultaneous expression of **a** and α information. Two mutants exhibiting an α phenotype (which may result from the restoration of *MAR/SIR* repression) were isolated and genetically characterized. The mutations in these strains: (1) are recessive, (2) are capable of suppressing a *mar2*-deletion mutation, (3) are unlinked to *MAT*, (4) complement one another as well as the previously identified *sum1-1* mutation, and (5) are not new alleles of the known *MAR/SIR* loci. We designate these new regulatory loci *SUM2* and *SUM3* (*su*pressor of *m*ar). Unlike the *sum1-1* mutation, suppression by *sum2-1* and *sum3-1* is *mar2*-locus specific. Both *sum2-1* and *sum3-1* affect the expression of **a** information at the *HM* loci. Transcript analysis shows a significant reduction in *HML***a** and *HMR***a** gene transcription in *mar2-1 sum2-1* and *mar2-1 sum3-1* cells. Furthermore, we have found genetic evidence to suggest that *mar2-1 sum2-1* cells exhibit only partial expression of silent α information. We conclude that the *SUM2* and *SUM3* gene products are required for expression of the *HM* loci and act downstream of the *MAR2* (*SIR3*) gene function. Possible mechanisms for the action of the *SUM* gene products are discussed.

MATING specificity in the yeast *Saccharomyces cerevisiae* is determined by the type of information (**a** or α) present at the constitutively expressed mating-type (*MAT*) locus on chromosome *III*. Each allele at *MAT* encodes two transcripts, the products of which act to regulate cell type (STRATHERN, HICKS and HERSKOWITZ 1980; for reviews see KLAR, STRATHERN and HICKS 1984; NASMYTH 1982a). Additional copies of mating-type information reside at the *HML* and *HMR* loci, also on chromosome *III* (Figure 1) (HARASHIMA and OSHIMA 1976; KLAR *et al.* 1980). Although these loci contain complete structural and promoter sequences for the expression of **a** or α information, they are not normally transcribed. Both *HML* and *HMR* (collectively referred to as *HM*) serve as donor loci for mating-type interconversion, an event that involves a genetic rearrangement in which copies of these silent mating-type genes (or "cassettes") are transposed to and expressed at *MAT* (TAKANO, KUSUMI and OSHIMA 1973; HICKS, STRATHERN and HERSKOWITZ 1977; KLAR and FOGEL 1979; KUSHNER, BLAIR and HERSKOWITZ 1979; HICKS, STRATHERN and KLAR 1979; NASMYTH and TATCHELL 1980; KLAR 1980).

This position effect on expression is controlled by two *cis*-acting "silencer" sequences, called *E* (*e*ssential) and *I* (*i*mportant), which flank each locus (ABRAHAM *et al.* 1984; FELDMAN, HICKS and BROACH 1984). The *HMR E* sequence has been shown to affect the transcription of other genes, including those transcribed by polymerase III, and is capable of acting in an orientation-independent manner up to 2.5 kb from a targeted promoter (BRAND *et al.* 1985; SCHNELL and RINE 1986). *HM* gene repression also requires the action of four unlinked *MAR* (or *SIR*) loci, such that a mutation in any *MAR/SIR* gene results in the simultaneous expression of both silent cassettes (KLAR, FOGEL and MACLEOD 1979; HABER and GEORGE 1979; RINE *et al.* 1979; IVY, KLAR and HICKS 1986; RINE and HERSKOWITZ 1987). *SIR*-mediated repression appears to involve DNA replication since both *HML E* and *HMR E* contain *ARS* elements (putative origins of DNA replication) (STINCHCOMB, STRUHL and DAVIS 1979; BROACH *et al.* 1982). In this regard, MILLER and NASMYTH (1984) have demonstrated that cells must complete the S phase to establish *MAR/SIR* regulation of the silent cassettes, and more recently,

This manuscript is dedicated to the memory of Laurie Lowman.

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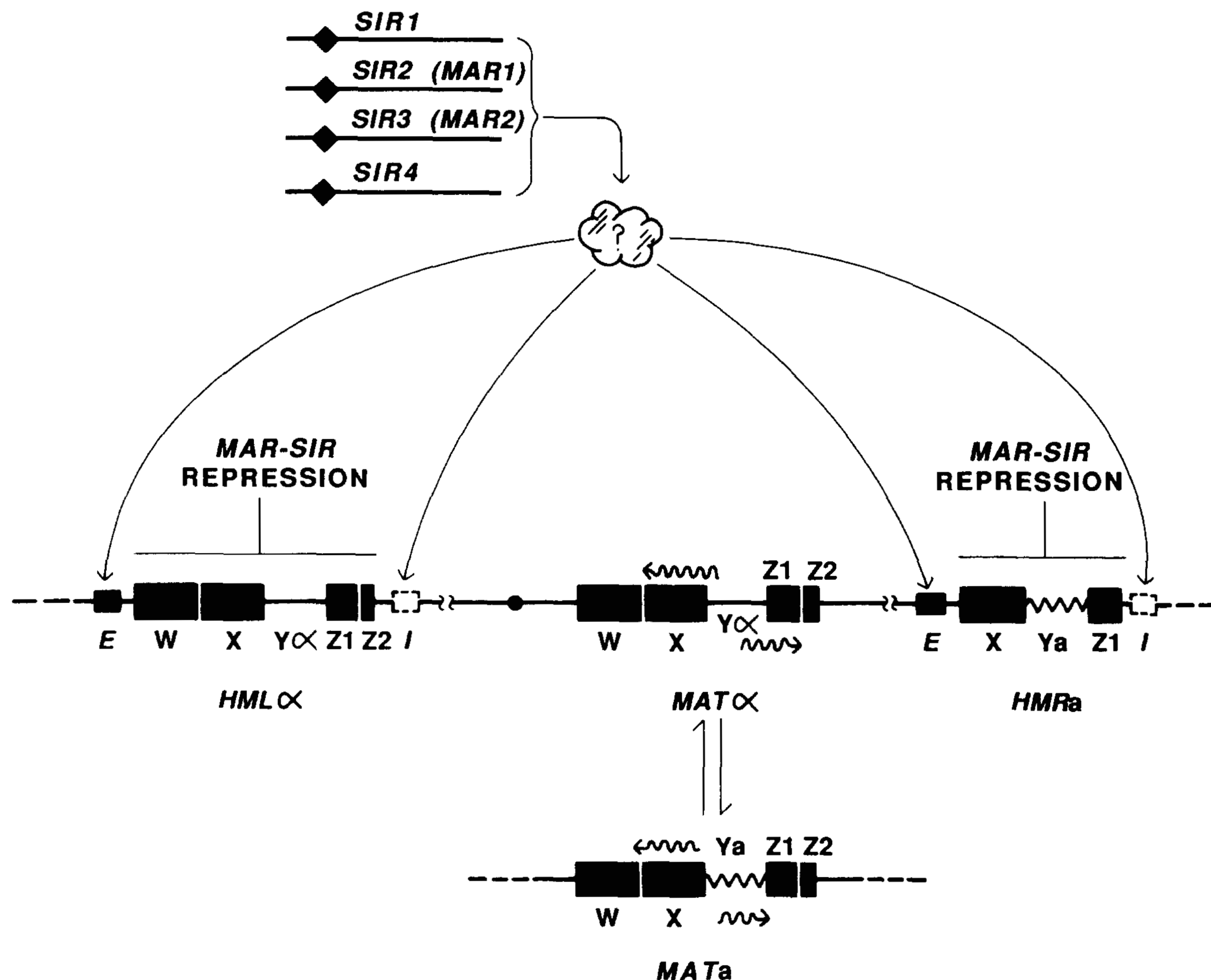


FIGURE 1.—Diagram of the mating-type cassettes on chromosome *III* of *S. cerevisiae*. The W (732-bp), X (704-bp), Z1 (239-bp) and Z2 (88-bp) boxes represent regions of homology shared by the mating-type loci *HML*, *MAT* and *HMR*. The *MAT* locus contains either **a** or α information; each allele encodes two divergently transcribed mRNAs indicated by the wavy line with arrow (KLAR *et al.* 1981; NASMYTH *et al.* 1981). Homothallic switching results in the replacement of the Y sequence at *MAT* with either **Ya** (642-bp, normally at *HMR*) or Yα (747-bp, normally at *HML*) (ASTELL *et al.* 1981). Transcriptional repression of *HML* and *HMR* requires the *cis*-acting elements E and I (ABRAHAM *et al.* 1984; FELDMAN, HICKS and BROACH 1984) and the products of four *trans*-acting *MAR* (or *SIR* genes) (RINE and HERSKOWITZ 1987). Our lack of understanding of how the *MAR/SIR* gene products act to control the silent mating-type cassettes is indicated by the question mark.

it has been shown that the *SIR2*, *SIR3* and *SIR4* gene products act to control the replication of plasmids containing *HMR E* (KIMMERLY and RINE 1987). Another feature that distinguishes *MAT* from the *HM* loci is its role in mating-type interconversion. Whereas interconversion can occur at *MAT*, it does not normally do so at the *HM* loci despite the presence of the same genetic information. This position-effect control of gene rearrangement is also regulated by the *MAR/SIR* genes, since in appropriate *mar1* mutant strains the *HM* loci efficiently interconvert (KLAR, STRATHERN and HICKS 1981).

Several models have been proposed to account for the involvement of the four *MAR/SIR* loci in regulating *HML* and *HMR* (IVY, KLAR and HICKS 1986; RINE and HERSKOWITZ 1987; KIMMERLY and RINE 1987). Based solely on genetic data, the repression of *HM* gene transcription has been ascribed to the interaction of the *MAR/SIR* gene products, either independently or as part of a multimeric protein, with the *cis*-acting control sites (see Figure 1). However, no direct evidence for DNA binding activity has been reported for any *MAR/SIR* gene product (SHORE *et al.* 1987). In fact, at least two *HML E*- and *HMR E*-specific DNA binding proteins, which do not correspond to any of the *MAR/SIR* gene products, have recently been identified (SHORE *et al.* 1987; SHORE and NASMYTH 1987; NASMYTH and SHORE 1987; BUCHMAN *et al.* 1988). The silencer region of *HMR E* consists of three different regulatory elements (called A, E and B) all contained within a 120-bp region (NASMYTH and SHORE 1987; BRAND, MICKLEM and NASMYTH 1987). One silencer binding protein (SBF-B) binds specifically to the ARS element at the *HMR* B region (as well as to *ARS1*) but not to *HML E*. Another protein, ABFI (ARS-binding factor I), recognizes controlling ele-

ments with *HMR E*, *HMR I*, and *HML I*, in addition to sequences adjacent to *ARS1*, *ARS2* and the 2-μm plasmid *ARS* (BUCHMAN *et al.* 1988). Yet another protein(s) (SBF-E or RAP1, and GRFI) binds to sequences present at both *HM* loci, to UAS sequences at *MATα*, and apparently recognizes UAS sequences present at a number of other loci (SHORE *et al.* 1987; SHORE and NASMYTH 1987; NASMYTH and SHORE 1987; BUCHMAN *et al.* 1988). The *RAP1* gene is essential for growth, indicating that its gene product may be a general regulatory factor for transcription (NASMYTH and SHORE 1987).

NASMYTH (1982b) has shown that the *MAR/SIR* genes also function in regulating silent mating-type gene chromatin structure as assayed by nuclease hypersensitivity. Specifically, derepression of *HMLα* leads to a change in chromatin structure at that locus equivalent to that observed at the expressed *MAT* locus. Consistent with this observation is the fact that N-terminal deletions of histone H4 can lead to derepression of the silent mating-type cassettes (KAYNE *et al.* 1988). Recently, two new genes (*ARD1* and *NAT1*), which code for N-terminal acetyltransferases, have been shown to be specifically required for repression of *HML*. Derepression of *HML* (as well as alleles of *HMR* containing specific silencer deletions) occurs in *ard1* and *nat1* strains, possibly due to a lack of acetylation of histone H2B (WHITEWAY *et al.* 1987; MULLEN *et al.* 1989). The specific molecular role that the silencer DNA binding proteins and histone acetyltransferases proteins play in regulating silent mating-type gene expression remains to be determined.

To understand more fully the nature of this type of control, we have tried to identify new regulatory genes involved in the repression of *HML* and *HMR*. In a previous report, KLAR *et al.* (1985) identified a recessive mutation (designated *sum1-1*) which acts to suppress mutations in *MAR1* (*SIR2*) and *MAR2* (*SIR3*). The gene defined by this extragenic suppressor (*SUM1*) acts to regulate the expression of both *HM* loci, and its product appears to function downstream of certain *MAR/SIR* control elements. More recently, SCHNELL *et al.* (1989) isolated suppressors of *SIR4* mutations which identify three new genes (*SAN1*, *SAN2* and *SAN3*). In this report, we demonstrate the existence of two new suppressor loci (*SUM2* and *SUM3*) which are involved in controlling silent mating-type gene transcription.

MATERIALS AND METHODS

Strains, genetic methods and media: Yeast strains are listed in Table 1. Mating-type tests were performed by replica-plating patches of cells grown on rich medium (YEPD) onto synthetic minimal medium (SD) pre-seeded with cells of a mating-type **a** (K567) or α (K566) tester strain. "Restrictive" mating-type tests utilized *leu2* tester strains (DC5a and DC6α) in order to assay the mating of cells that had retained plasmids carrying the *LEU2* gene. Successful mating was scored as confluent growth of prototrophic diploids. Genetic crosses, sporulation and tetrad dissection techniques were performed according to MORTIMER and HAWTHORNE (1969). All media for growth and sporulation were prepared as described previously (HICKS and HERSKOWITZ 1976).

Mutagenesis: Strain PL1 was mutagenized by exposure to ethyl methanesulfonate (EMS; Eastman Kodak Co.) as described previously (KLAR, FOGEL and RADIN 1979). The frequency of survivors was 3%.

Transformation: Yeast transformants were generated and selected according to the method of BEGGS (1978), involving the formation of spheroplasts generated by Glusulase (du Pont Pharmaceuticals) treatment.

Biochemical techniques: Total RNA from yeast was isolated by the method of CARLSON and BOTSTEIN (1982). Poly(A^+) RNA was selected following passage over oligo(dT)-cellulose (AVIV and LEDER 1972), using an ISCO UA5 absorbance detector to monitor RNA fractions. Poly(A^+)-selected RNA was size fractionated by electrophoresis through 1.5% ME agarose (SeaKem) in the presence of 2.2 M formaldehyde (LEHRACH *et al.* 1977). Samples were transferred to nitrocellulose according to SOUTHERN (1975). ^{32}P-labeled probes were prepared by nick translation (RIGBY *et al.* 1977), and hybridization conditions were as described previously (IVY, KLAR and HICKS 1986).

RESULTS

Isolation of *mar2-1* suppressors: To obtain suppressors of the *mar2-1* mutation, we constructed strain PL1 (*HML***a** *MATα HMR***a** *mar2-1*), which exhibits a sterile (nonmating) phenotype due to the simultaneous expression of both **a** and α information (IVY, KLAR and HICKS 1986; RINE and HERSKOWITZ 1987). (For brevity, genotypes will be abbreviated according to the mating-type information at *HML*, *MAT* and *HMR* in the order that they map on chromosome *III*; *e.g.*, *HML***a** *MATα HMR***a** will simply read **aαa**.) Cells of strain PL1 were mutagenized and colonies were screened for those exhibiting an α mating type. Among a total of 35,000 colonies screened, we obtained two mutants that mate as α (strains PL11 and PL13) (Figure 2); we presumed that this phenotype resulted from the restoration of *MAR/SIR* control of *HM* gene expression. However, acquisition of an α mating phenotype could be due to a variety of mutational events: (1) simultaneous mutation of both *HML***a** and *HMR***a**, (2) reversion of *mar2-1* to *MAR2*, (3) certain mutations in *MATα2* (STRATHERN *et al.* 1988), (4) translational suppression of *mar2-1*, or (5) extragenic suppression of *mar2-1*. The following genetic experiments establish that the *mar2-1* suppressor mutations in these strains identify two new loci, designated *SUM2* and *SUM3* (*su*pressor of *mar* after KLAR *et al.* 1985), which are involved in regulating the expression of *HML* and *HMR*.

The *sum2-1* and *sum3-1* mutations are recessive: In order to assess the dominance or recessiveness of each mutation, diploids were constructed by mating strains PL11 (**aαa** *mar2-1 sum2-1*) and PL13 (**aαa**

TABLE 1

Strain list

Strain	Mating-type genotype					Other Markers	Mating Phenotype
	HML	*MAT*	*HMR*	*MAR/SIR*	*SUM*		
PL1	**a**	α	**a**	*mar2-1*	+	leu2 lys2	nm
PL11	**a**	α	**a**	*mar2-1*	*sum2-1*	leu2 lys2	α
PL13	**a**	α	**a**	*mar2-1*	*sum3-1*	leu2 lys2	α
PL42	**a**	**a**	**a**	*mar2::LEU2*	*sum3-1*	cry1 leu2 his4	**a**
PL46	**a**	**a**	**a**	*mar2-1*	*sum2-1*	leu2 lys2 mal	**a**
PL48	**a**	α	**a**	*mar2-1*	*sum2-1*	leu2 lys2 his1	α
PL58	**a**	α	**a**	*mar2-1*	*sum3-1*	leu2 his1	α
PL60	**a62**	**a62**	Δ	*mar2::LEU2*	*sum2-1*	cry1 leu2 his4	**a**
PL66	**a62**	**a62**	**a**	*mar2-1*	*sum3-1*	leu2 trp1	**a**
PL75	**a**	α	**a**	*mar2::LEU2*	*sum2-1*	leu2 thr4 metx mal	α
PL78	**a62**	**a62**	α	*mar2-1*	*sum2-1*	leu2 lys1-1 lys2	bm
PL81	**a**	α	**a**	*mar2::LEU2*	*sum3-1*	leu2 lys2 his4 thr4	α
PL134	**a**	**a**	**a**	+	*sum3-1*	ade6 his4 leu2 ura1 mal	**a**
PL136	**a**	α	**a**	+	*sum3-1*	ade6 his4 leu2 lys2 mal	α
DC5	α	**a**	**a**	+	+	can1 gal2 his3 leu2 mal	**a**
DC6	α	α	**a**	+	+	can1 gal2 his4 leu2	α
JI562	**a62**	**a62**	**a62**	*mar2-1*	+	leu2 trp1	**a**
K122	**a**	α	**a**	+	+	leu2 his4 mal	α
K388	**a61**	**a62**	α	*mar2-1*	+	leu2 lys1-1 lys2 mal	α
K566	**a**	α	**a**	+	+	ilv5 mal	α
K567	α	**a**	**a**	+	+	ilv5 mal	**a**
K596	**a**	**a**	**a**	*mar2-1*	+	leu2 his1 mal	**a**
K700	**a**	**a**	**a**	*mar1::LEU2*	+	cry1 his4 leu2 thr4	**a**
K712	**a**	**a**	**a**	*mar2::LEU2*	+	cry1 his4 leu2 thr4	**a**
K724	**a**	α	**a**	*mar2::LEU2*	*sum1-1*	cry1 leu2 his4	α
K733	**a**	**a**	**a**	*sir4::LEU2*	+	can1 cry1 ade2 his4 leu2 tyr1	**a**
K775	**a**	α	**a**	+	*sum2-1*	leu2 metx	α
K780	**a**	α	**a**	*mar2-1*	*sum2-1*	leu2 thr4 metx	α
K782	α	**a62**	**a62**	*mar2-1*	*sum3-1*	leu2 ura1 trp1	bm

All strains are heterothallic (*ho*). The *mar1::LEU2, mar2::LEU2* and *sir4::LEU2* deletion mutations were constructed as previously described (IVY, KLAR and HICKS 1986). All strains were constructed for this study, except K700 and K712 (KLAR *et al.* 1985), K566 and K567 (IVY, KLAR and HICKS 1986). The **a61** allele is an amber mutation, whereas the **a62** allele is not suppressed by either amber or ochre suppressors (KLAR *et al.* 1980); both defective alleles are abbreviated $\mathbf{a}^-$ in the text. The symbol Δ indicates deletion of a particular cassette [their construction was described earlier (KLAR, HICKS and STRATHERN 1982)]. nm = nonmating (sterile); bm = bimating.

mar2-1 sum3-1) to strain JI562 ($\mathbf{a}^-\mathbf{a}^-\mathbf{a}^-$ *mar2-1*), and assayed for their mating and sporulation capabilities. Such diploids were found to be sterile and sporulation proficient; since both phenotypes require functional **a** and α information (ROMAN and SANDS 1953), we conclude that *HML* and/or *HMR* are expressed in heterozygous *SUM/sum* diploids, and that both *sum2-1* and *sum3-1* are recessive to their wild-type alleles. This result also suggests that *sum2-1* and *sum3-1* are not reversion mutations of *mar2-1*. This was confirmed by crossing each original mutant strain to strain DC5 (α**aa**); upon sporulation, such diploids generated the expected relative frequency of α and nonmating segregants, rather than 2**a**:2α ratio that would result if each suppressor mutation were a reversion of *mar2-1*.

***sum2-1* and *sum3-1* identify single genes:** Diploids constructed between each original mutant strain and strain K596 (**aaa** *mar2-1*) were subjected to tetrad analysis in order to determine whether the suppression of *mar2-1* is due to single gene mutations. As shown in Table 2 (lines 1 and 2), approximately half of the segregants from each cross which inherited the *MAT*α allele were phenotypically α, indicating that both *sum2-1* and *sum3-1* identify single loci which segregate independently of *MAT*α.

***sum1-1, sum2-1* and *sum3-1* complement one another:** We performed complementation tests by assaying mating and sporulation in heterozygous *SUM* diploids. The *sum1-1* mutation was included in this analysis because it is also capable of suppressing mutations in *MAR2* (*SIR3*) (KLAR *et al.* 1985). The following diploids were constructed:

(1) PL58 × PL60 $\dfrac{\mathbf{a}\ \alpha\ \mathbf{a}}{\mathbf{a}^-\mathbf{a}^-\Delta}\ \dfrac{mar2\text{-}1}{mar2::LEU2}\ \dfrac{sum3\text{-}1}{+}\ \dfrac{+}{sum2\text{-}1}$

(2) K724 × PL60 $\dfrac{\mathbf{a}\ \alpha\ \mathbf{a}}{\mathbf{a}^-\mathbf{a}^-\Delta}\ \dfrac{mar2::LEU2}{mar2::LEU2}\ \dfrac{sum1\text{-}1}{+}\ \dfrac{+}{sum2\text{-}1}$

(3) K724 × PL66 $\dfrac{\mathbf{a}\ \alpha\ \mathbf{a}}{\mathbf{a}^-\mathbf{a}^-\mathbf{a}^-}\ \dfrac{mar2::LEU2}{mar2\text{-}1}\ \dfrac{sum1\text{-}1}{+}\ \dfrac{+}{sum3\text{-}1}.$

The *mar2::LEU2* allele is a deletion/insertion mutation constructed *in vitro* using the *LEU2* gene of *S. cerevisiae* (IVY, KLAR and HICKS 1986). Selected dip-

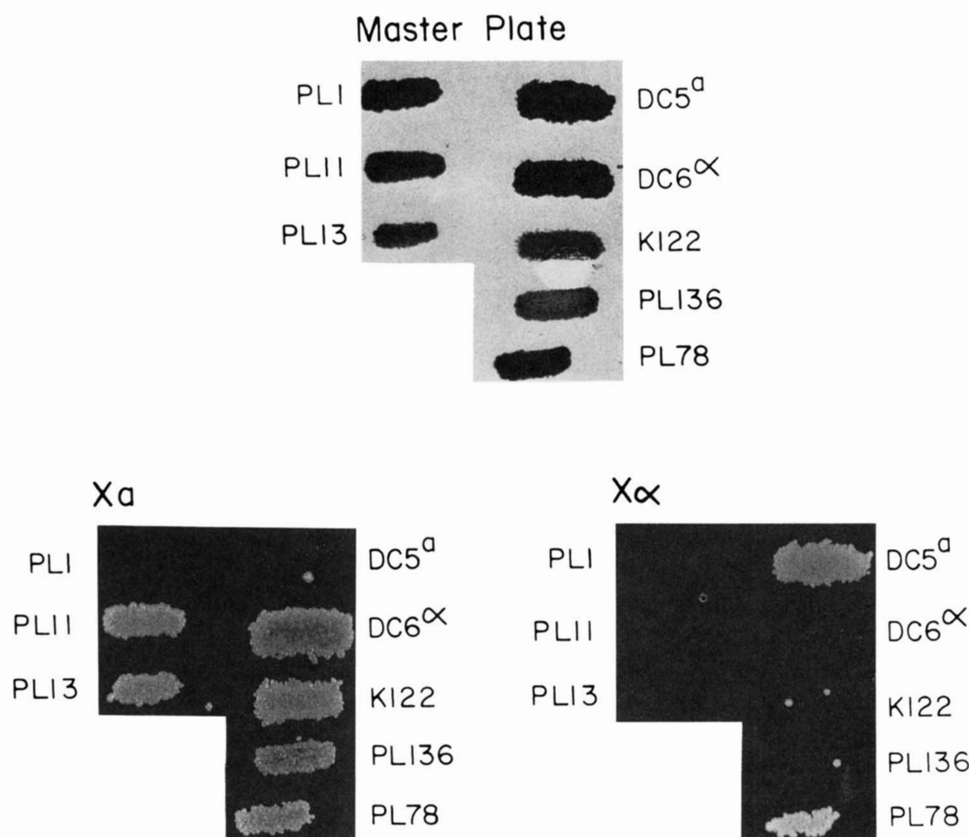
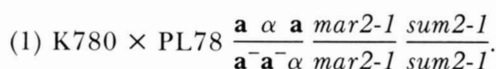


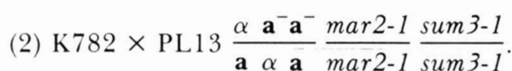
FIGURE 2.—Mating-type tests indicating suppression of the *mar2-1* mutation in strains PL11 (**a**α**a** *mar2-1 sum2-1*) and PL13 (**a**α**a** *mar2-1 sum3-1*) as compared to the nommating parental strain PL1 (**a**α**a** *mar2-1*). Refer to Table 1 for the genotype of other strains. Cells grown on a YEPD master plate (top) were replicated to SD medium containing either *MAT***a** (strain K567; left) or *MAT*α (strain K566; right) mating-type tester cells. Growth (scored after 48 hr) on the bottom left plate (X**a**) indicates an α mating type; growth on the bottom right plate (Xα) indicates an **a** mating type.

loids from each cross were found to be sterile and exhibited wild-type levels of sporulation, indicating the expression of functional **a** information at the silent mating-type loci. As controls for each complementation test, we isolated diploids from the following two crosses and assayed their ability to mate and sporulate:



$$(1)\ \text{K780} \times \text{PL78}\ \frac{\mathbf{a}\ \alpha\ \mathbf{a}}{\mathbf{a}^-\mathbf{a}^-\alpha}\ \frac{mar2\text{-}1}{mar2\text{-}1}\ \frac{sum2\text{-}1}{sum2\text{-}1}.$$

Diploids from this cross mated as α (as well as the parent strain K780) and were sporulation-deficient (0% sporulation in 48 hr, 5% sporulation in 96 hr).



$$(2)\ \text{K782} \times \text{PL13}\ \frac{\alpha\ \mathbf{a}^-\mathbf{a}^-}{\mathbf{a}\ \alpha\ \mathbf{a}}\ \frac{mar2\text{-}1}{mar2\text{-}1}\ \frac{sum3\text{-}1}{sum3\text{-}1}.$$

Diploids from this cross also mated as α and were sporulation-deficient (0% sporulation in 96 hr). We conclude that the *sum1-1*, *sum2-1* and *sum3-1* mutations complement one another and therefore are likely to define separate genes.

***sum2-1* and *sum3-1* do not correspond to any of the *MAR/SIR* loci:** Cloned sequences containing each of the wild-type *MAR/SIR* genes, carried on the high copy number yeast plasmid YEp13 (pJH570-*SIR1*, pJH16-*SIR2*, pKAN63-*SIR3*, pKAN59-*SIR4*) (IVY, KLAR and HICKS 1986), failed to complement *sum2-1* and *sum3-1* when introduced into strains PL11 and PL13 by transformation (data not shown). We conclude that these mutations do not correspond to any of the *MAR/SIR* loci.

Suppression by *sum2-1* and *sum3-1* is *mar-2*-allele nonspecific: To determine whether suppression by *sum2-1* and *sum3-1* is *mar2*-allele specific, strains PL11 and PL13 were each crossed to strain K712 (**aaa** *mar2::LEU2 leu2*), and the resulting diploids were subjected to tetrad analysis (Table 2, lines 3 and 4). Since PL11 and PL13 are both *leu2*, the Leu$^+$ phenotype may be used to identify those meiotic segregants containing the *mar2::LEU2* deletion mutation. Both crosses generated mating-type α segregants that were also Leu$^+$, indicating suppression of the *mar2::LEU2* deletion mutation by *sum2-1* and *sum3-1*. These data rule out the possibility that *sum2-1* and *sum3-1* are translational suppressors. Further analysis of the data from these crosses leads to the conclusion that *sum2-1* and *sum3-1* are not linked to *MAT* or to *MAR2*. First, the observation that nonmating α segregants (*MAT*α *SUM2* or *SUM3*) arise at high frequency indicates that the *SUM* genes are not linked to *MAT* (see Table 2, lines 1 and 2, as well). Secondly, the observation that Leu$^+$ and Leu$^-$ phenotypes are equally frequent among segregants that mate as α implies that the *SUM* loci are not linked to *MAR2*.

Suppression by *sum2-1* and *sum3-1* is *mar2*-locus specific: The *sum1-1* mutation is capable of suppressing mutations in both *MAR1* (*SIR2*) and *MAR2* (*SIR3*) (KLAR *et al.* 1985). To determine whether *sum2-1* and

TABLE 2

Genetic analysis of the *sum2-1* and *sum3-1* mutations

	Cross	Genotype		Mating type of segregants
(1)	K596 × PL11	**aaa** / a**α**a	*mar2-1* + / *mar2-1 sum2-1*	30**a**:16α:16nm
(2)	K596 × PL13	**aaa** / a**α**a	*mar2-1* + / *mar2-1 sum3-1*	31**a**:13α:15nm
(3)	K712 × PL11	**aaa** / a**α**a	*mar2::LEU2* + / *mar2-1 sum2-1*	47**a**:21α (8 Leu$^+$, 13 Leu$^-$):29nm
(4)	K712 × PL13	**aaa** / a**α**a	*mar2::LEU2* + / *mar2-1 sum3-1*	47**a**:21α (14 Leu$^+$, 7 Leu$^-$):31nm
(5)	K700 × PL11	**aaa** / a**α**a	*mar1::LEU2* + + / + *mar2-1 sum2-1*	39**a**:12α (all Leu$^-$):25nm
(6)	K700 × PL13	**aaa** / a**α**a	*mar1::LEU2* + + / + *mar2-1 sum3-1*	32**a**:7α (all Leu$^-$):25nm
(7)	K700 × K775	**aaa** / a**α**a	*mar1::LEU2* + / + *sum2-1*	96**a**:49α (all Leu$^-$):35nm
(8)	K700 × PL136	**aaa** / a**α**a	*mar1::LEU2* + / + *sum3-1*	62**a**:30α (all Leu$^-$):33nm
(9)	K733 × PL136	**aaa** / a**α**a	*sir4::LEU2* + / + *sum3-1*	83**a**:32α (all Leu$^-$):27nm
(10)	K733 × PL48	**aaa** / a**α**a	*sir4::LEU2* + + / + *mar2-1 sum2-1*	133**a**:33α (all Leu$^-$):101nm
(11)	K733 × K775	**aaa** / a**α**a	*sir4::LEU2* + / + *sum2-1*	81**a**:44α (all Leu$^-$):26nm
(12)	PL134 × PL81	**aaa** / a**α**a	+ *sum3-1* / *mar2::LEU2 sum3-1*	20**a**:20α:0nm (all asci contained 2**a** and 2α spores)
(13)	K388 × PL46	**a**$^-$**a**$^-$α / **a a a**	*mar2-1* + / *mar2-1 sum2-1*	30**a**:2α:9nm:3bm (of 11 total asci, 6 contained either 4**a** or 3**a** spores)

nm, nonmating, ; bm, bimating segregants.

sum3-1 are capable of suppressing mutations in other *MAR/SIR* genes, we performed the following crosses. First, strains PL11 and PL13 were each crossed to strain K700 (**aaa** *mar1::LEU2*) and subjected to tetrad analysis (Table 2, lines 5 and 6). If *sum2-1* or *sum3-1* were able to suppress the *mar1::LEU2* mutation, then Leu$^+$ α mating-type meiotic segregants would be expected to be recovered. Assuming random segregation of *mar1*, *mar2*, *sum2* and *sum3*, crosses 5 and 6 (Table 2) should have produced a total of nine and eight such segregants, respectively. The absence of any Leu$^+$ α mating type segregants from either cross indicates the inability of *sum2-1* and *sum3-1* to suppress this *MAR1* deletion mutation. A chi square analysis of the data from crosses 5 and 6 support this model (d.f. = 3, $P = 0.91$ and 0.48, respectively). The probability that the absence of Leu$^+$ α mating-type segregants is due to chance is very low ($P = 0.07$ and 0.15, respectively). To demonstrate this further, strains containing *sum2-1* (K775) or *sum3-1* (PL136) alone were crossed to strain K700, and the resulting tetrads were analyzed (Table 2, lines 7 and 8). Again, the data show that *sum2-1* and *sum3-1* do not suppress *mar1::LEU2*.

Likewise, results of crosses between strain K733 (**aaa** *sir4::LEU2*) and PL136 (a**α**a *sum3-1*), PL48 (a**α**a *mar2-1 sum2-1*) and K775 (a**α**a *sum2-1*) showed a lack of suppression of this *sir4* deletion mutation by *sum2-1* and *sum3-1* (Table 2, lines 9–11). If *sum2-1* and *sum3-1* were able to suppress *sir4::LEU2*, α mating-type Leu$^+$ segregants would be expected to be recovered; none were observed. Thus, unlike the *sum1-1* mutation, *sum2-1* and *sum3-1* appear to be able to suppress mutations only in *MAR2* (*SIR3*). Suppression of *SIR1*-deletion mutations was not assayed due to their leaky phenotype (IVY, KLAR and HICKS 1986; RINE and HERSKOWITZ 1987).

***sum2-1* and *sum3-1* exhibit no discernible phenotype in a wild-type background:** Cells of genotype a**α**a *sum3-1* were generated from a cross between

strains PL134 (**aaa** *sum3-1*) and PL81 (**a**α**a** *mar2::LEU2 sum3-1*). All asci contained 2**a** and 2α segregants (Table 2, line 12), which included a significant number of Leu⁻ α mating type segregants. Had *sum3-1* itself affected mating behavior, a fraction of the Leu⁻ segregants should have displayed an altered mating type. The recovery of 2**a** and 2α spores per tetrad indicates that the *sum3-1* mutation alone has no obvious effect on mating behavior. An analogous cross involving the *sum2-1* mutation yielded the same result (data not shown).

sum2-1* and *sum3-1* affect silent mating-type gene transcription:** To determine whether *sum2-1* and *sum3-1* affect *HM* gene transcription, we compared the level of **a**1 and **a**2 transcripts in wild-type *vs.* **a**α**a** *mar2-1 sum2-1* and **a**α**a** *mar2-1 sum3-1* mutant strains. Poly(A^+) RNA was prepared, size fractionated on an agarose gel, blotted to nitrocellulose, and probed with a subclone of *MATa** that recognizes both **a** and α transcripts (KLAR *et al.* 1981). Figure 3 shows that, whereas cells carrying the *mar2-1* mutation contain both **a**1 and **a**2 transcripts, the level of these transcripts is significantly reduced in *mar2-1* cells carrying either *sum2-1* or *sum3-1*. At the same time, *sum2-1* and *sum3-1* do not appear to affect the level of *MAT* transcripts (α1 and α2). Similarly, the *sum1-1* mutation in combination with *mar1-1* has also been found to affect transcription of both **a** and α cassettes from the silent mating-type loci (LIVI, HICKS and KLAR 1990). These data indicate that the suppressor mutations affect mating behavior by restoring repression of *HM* gene transcription in *mar* mutant strains.

Alternatively, the drastic reduction in **a** gene transcripts could be due to an effect on mRNA processing and/or stability, given the fact that the **a**1 primary transcript is known to contain introns (MILLER 1984). To test this hypothesis, we assayed the mating and sporulation ability of diploids from two crosses:

$$(1)\ \text{PL46} \times \text{PL75}\ \frac{\mathbf{aaa}}{\mathbf{a}\alpha\mathbf{a}}\ \frac{mar2\text{-}1}{mar2{::}LEU2}\ \frac{sum2\text{-}1}{sum2\text{-}1}$$

$$(2)\ \text{PL42} \times \text{PL58}\ \frac{\mathbf{aaa}}{\mathbf{a}\alpha\mathbf{a}}\ \frac{mar2{::}LEU2}{mar2\text{-}1}\ \frac{sum3\text{-}1}{sum3\text{-}1}.$$

In both cases, selected diploids were found to be nonmating and sporulation proficient (>80%), indicating expression and correct processing of the **a**1 transcript derived from *MAT***a**.

We predict from these data that our suppressor mutations should be capable of suppressing *HM* gene expression regardless of the information carried at these loci. In fact, we have obtained preliminary genetic evidence to suggest that, like *sum1-1* (KLAR *et al.* 1985), *sum2-1* also partially represses (albeit very weakly) the expression of α information at *HMR*. Strain PL46 (**aaa** *mar2-1 sum2-1*) was mated with strain K388 [**a**⁻**a**⁻α *mar2-1* (α phenotype)]. If *sum2-1* was capable of repressing the expression of *HMR*α, we expected a number of asci to contain 3**a** and 4**a** mating-type segregants, and to exhibit an overall segregation pattern of 12**a**:1α:4nm. In fact, tetrads containing both 3**a** and 4**a** mating-type segregants were observed (Table 2, line 13). Furthermore, the presence of a unique class of bimating segregants suggests that the *sum2-1* mutation is leaky. Expression of *HMR*α will result in an α phenotype when both *HML* and *MAT* contain **a**⁻ information. In some cells, apparently enough α information is expressed to allow them to mate as α, whereas in other cells the *HMR*α product is insufficient resulting in an **a** mating type. The frequency of bimating segregants is consistent with the expected frequency of the genotype **a**⁻**a**⁻α *mar2-1 sum2-1* from this cross, assuming independent assortment of these genes. If *MAT* contained functional **a** information, leaky expression of *HMR*α would produce a mixture of **a** (no α expression) and nonmating (α expression) cell types. A colony with this mixture would appear to be an **a** mater. Because *sum2-1* restores repression to *HML***a**, it is difficult to predict the **a** allele present at *HML* in the bimating colonies. The bimating phenotype of one segregant of this cross [strain PL78: *HML***a** (or **a**⁻) *MAT***a**⁻ *HMR*α *mar2-1 sum2-1*] is shown in Figure 2. These data suggest that *sum2-1* assorts independently of *MAT* and *HMR*, but segregation relative to *HML* cannot be assessed. However, since *SUM2* and *SUM3* each coregulate the expression of both *HML* and *HMR*, it is extremely unlikely that our suppressor mutations correspond to lesions within these genes.

DISCUSSION

We have identified two extragenic suppressors of mutations in the *MAR2* (*SIR3*) gene as defined by the mutations *sum2-1* and *sum3-1*. Based on genetic complementation tests these suppressors define two genes which act to regulate the silent mating-type loci in that *mar2-1 sum2-1* and *mar2-1 sum3-1* cells fail to express both *HML* and *HMR* as determined by mating-type tests and direct transcript analysis. Mutations in *SUM2* and *SUM3* are also capable of suppressing deletion mutations of *MAR2*, indicating that they are not translational suppressors. The suppression exhibited by *sum2-1* and *sum3-1* differs from that exhibited by the *sum1-1* mutation in that it is *mar2*-locus specific. In contrast, *sum1-1* suppresses deletion mutations in both *MAR1* (*SIR2*) and *MAR2* (*SIR3*). [Although *sum1-1* was previously reported to weakly suppress an ochre mutation in *SIR4* (KLAR *et al.* 1985), we have found that it fails to suppress a *sir4::LEU2* deletion mutation. Perhaps segregation of some other weak translational suppressor of the *sir4*-ochre mutation misled us into making the previous interpretation.] Based on our genetic observations we conclude that

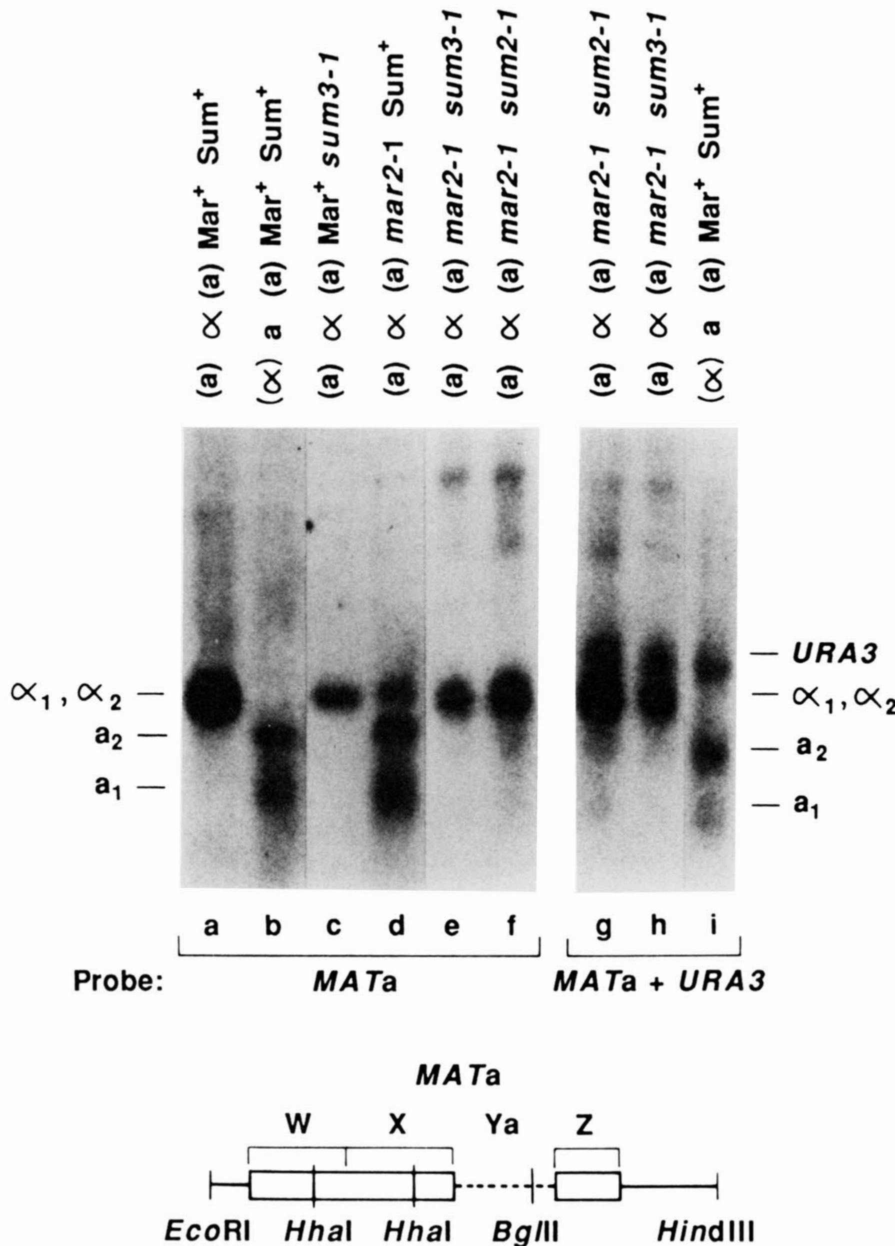


FIGURE 3.—Northern blot analysis of mating-type transcripts. Poly(A^+) RNA was isolated from various yeast strains (relevant genotypes are listed at the top), size fractionated on a 1.5% agarose gel, blotted to nitrocellulose and probed with pMAT1 (*MAT***a**) (shown below; KLAR *et al.* 1981) which contains a fragment of *MAT***a** homologous to both **a** and α transcripts. Each lane contained 3 μg poly(A^+) RNA. Lane a = strain K122; b and i = strain DC5; c = strain PL136; d = strain PL1; e and h = strain PL13; f and g = strain PL11. The right panel was probed with a mixture of pMAT1 and the *URA3*-containing YIp5 plasmid which served as an internal control for relative RNA abundance. Bands corresponding to the **a**1, **a**2, α1, α2, and *URA3* transcripts are indicated.

the *SUM2* and *SUM3* gene products act downstream of the *MAR2* (*SIR3*) gene product.

The level of suppression of *mar2* mutations by *sum2-1* and *sum3-1*, as assayed by mating-type tests, appears to depend on the type of information at the silent cassettes. This is exemplified by the α mating behavior of *HML***a** *MAT*α *HMR***a** *mar2-1 sum2-1* cells *vs.* the bimating behavior of *HML***a** (or **a**$^-$) *mat***a**$^-$ *HMR*α *mar2-1 sum2-1* cells. The mating-type test measures mating ability of a population of cells. We interpret bimating ability of the latter genotype to indicate that sufficient *HML*α is expressed to produce an α mating phenotype in only a fraction of the cells. (Expression of both α and **a**$^-$ in a single cell results in an α mating phenotype; *e.g.*, *MAT*α/*mat***a**$^-$ diploids mate with **a** cells). The fraction of cells expressing insufficient levels of *HML*α will exhibit an **a** mating behavior (*mat***a**$^-$ cells mate with *MAT*α tester strains). This interpretation is not inconsistent with the α mating behavior of *HML***a** *MAT*α *HMR***a** *mar2-1 sum2-1* (or *sum3-1*) cells. Complete repression of both *HM***a** loci will give an α mating behavior, and expression of some *HM***a** in a fraction of the cells will produce a nonmater; a colony comprised of a mixture of these two cell types would mate as α. On the basis of our genetic analysis of *mar sum* strains, it appears that **a** information at the *HM* loci is repressed more efficiently than α information. The significance of this difference is not clear, although it is unlikely that it is due to post-transcriptional processing or mRNA stability since the **a**1 transcript from *MAT***a** provides wild-type **a** function(s).

IVY, KLAR and HICKS (1986) have demonstrated a lack of transcriptional control among the four *MAR*/

SIR genes, and it has also been proposed that regulation of *HML* and *HMR* requires specific interaction of certain *MAR/SIR* gene products (IVY, KLAR and HICKS 1986; MARSHALL *et al.* 1987; KIMMERLY *et al.* 1988). We have found that the *sum1-1* mutation exhibits no effect on transcription of any of the four *MAR/SIR* genes (LIVI, HICKS and KLAR 1990), and that *sum3-1* has no effect on either *MAR1* (*SIR2*) or *SIR4* gene transcription (C. P. LIN, unpublished results). Whether a specific mode of protein-protein interaction exists between the *MAR/SIR* and *SUM* gene products awaits further biochemical investigations.

The identity of *SUM2* and *SUM3* is based primarily on complementation tests which included the previously identified *sum1-1* mutation (KLAR *et al.* 1985). More complicated allelism tests have not yet been performed. Since our pilot screens have revealed only a single "allele" per locus, it will be important to rescreen more thoroughly for additional suppressor mutations. It is clear, however, that none of our suppressor mutations suppress defects in *SIR4*, suggesting that *SIR4* may be the most distal gene in the pathway leading to control of the *HM* loci. Suppressors of *SIR4* mutations have now been isolated independently (SCHNELL *et al.* 1989). These suppressors identify three additional genes (*SAN1*, *SAN2* and *SAN3*) involved in controlling *SIR4* function. Analogous to the effect of *sum2-1* and *sum3-1* on *mar2::LEU2*, *san1* mutations are locus-specific. However, because *san1* is unable to suppress a particular *sir4* allele (*sir4-351*, an ochre mutation), *SAN1* may act to regulate *SIR4* protein activity.

How are the *SUM* genes involved in controlling *HM* gene expression? Our present data fit the simple genetic model which was proposed to account for the reaction of *SUM1* (KLAR *et al.* 1985): in this model the *MAR/SIR* loci negatively regulate the *SUM* loci, whose gene products are subsequently required for *HM* gene expression either by acting as a positive regulator, or by negatively regulating another downstream repressor. It seems unlikely that yeast cells would have evolved a mechanism to positively regulate genes that are not normally expressed. What remains inconsistent with the alternative hypothesis, however, is the inability to detect genetically the "last gene" in the pathway leading to *HM* gene repression, since mutations in such a gene would be expected to exhibit the same phenotype as *MAR/SIR* gene mutations. Alternatively, repression of transcription might involve an essential gene product, not directly under *MAR/SIR* control, which exhibits multiple functions (such as RAP1) (SHORE and NASMYTH 1987) or (GRFI) (BUCHMAN *et al.* 1988). As pointed out by SHORE and NASMYTH (1987), the proposed dual repressor/activator function of RAP1 may indicate its involvement in effecting a structural change in silencer (as well as activator) DNA sequences which is a prerequisite to transcriptional regulation. Thus, certain silencer DNA-binding proteins could control chromatin structure in such a way as to make the *HM* loci accessible to specific transcriptional control factors (*MAR/SIR* proteins?). In the absence of these transcriptional control factors not only is repression abolished, but in addition, *HM* gene chromatin becomes hypersensitive to nuclease digestion (NASMYTH 1982b) suggesting that the *MAR/SIR* gene products do in fact play some role in determining chromatin structure. Additionally, it is known that the *HO*-encoded endonuclease (KOSTRIKEN *et al.* 1983; KOSTRIKEN and HEFFRON 1984) catalyzes a site-specific cleavage at *MAT* that is required to initiate *MAT* interconversion (STRATHERN *et al.* 1982), yet it fails to cleave the same site at the *HM* loci. In *mar* cells, however, the sites at *HML* and *HMR* are accessible to cleavage, thus again implicating the *MAR/SIR* gene functions in regulating chromatin structure (KLAR, STRATHERN and HICKS 1981).

In the context of all of this, the function of the *SUM* gene products remains puzzling since they appear to act as antagonists of the normal silencer state of each *cis*-acting sequence. For example, derepression of *HML* and *HMR* in a *sir2* or *sir3* mutant strain requires the presence of a wild-type *SUM1* allele (in fact, *HM* gene expression could only occur when the *SUM* genes are active if their expression is controlled by the *MAR/SIR* genes). Perhaps the *SUM* gene products act to promote some kind of site-specific change in nucleosome structure which permits active transcription. In this regard, the *SUM* gene products may be involved in regulating some aspect of histone biochemistry, but in a manner distinct from the recently identified *NAT1* and *ARD1* acetyltransferases [mutations in these loci result in silent mating-type gene derepression (WHITEWAY *et al.* 1987; MULLEN *et al.* 1989)]. The function of the *SUM1* gene product remains particularly enigmatic because of the locus-nonspecific nature of the *sum1-1* mutation (KLAR *et al.* 1985). Clearly, understanding the function of the *SUM* genes will require further genetic and molecular dissection.

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LITERATURE CITED

ABRAHAM, J., K. A. NASMYTH, J. N. STRATHERN, A. J. S. KLAR and J. B. HICKS, 1984 Regulation of mating-type information in yeast: negative control requiring sequences both 5′ and 3′ to the regulated region. J. Mol. Biol. **176:** 307–331.

ASTELL, C., L. AHLSTROM-JONASSON, M. SMITH, K. TATCHELL, K.

NASMYTH and B. D. HALL, 1981 The sequence of the DNAs coding for the mating-type loci of *Saccharomyces cerevisiae*. Cell **27:** 15–23.

AVIV, H., and P. LEDER, 1972 Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA **69:** 1408–1412.

BEGGS, J. D., 1978 Transformation of yeast by a replicating hybrid plasmid. Nature **275:** 104–109.

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

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

BROACH, J. R., Y.-Y. LI, J. FELDMAN, M. JAYARAM, J. ABRAHAM, K. A. NASMYTH and J. B. HICKS, 1982 Localization and sequence analysis of yeast origins of DNA replication. Cold Spring Harbor Symp. Quant. Biol. **47:** 1165–1173.

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

CARLSON, M., and D. BOTSTEIN, 1982 Two differentially regulated mRNAs with different 5′ ends encode secreted and intracellular forms of invertase. Cell **28:** 145–154.

FELDMAN, J. B., J. B. HICKS and J. R. BROACH, 1984 Identification of sites required for repression of a silent mating type locus in yeast. J. Mol. Biol. **178:** 815–834.

HABER, J. E., and J. P. GEORGE, 1979 A mutation that permits the expression of normally silent copies of mating-type information in *Saccharomyces cerevisiae*. Genetics **93:** 13–35.

HARASHIMA, S., and Y. OSHIMA, 1976 Mapping of the homothallic genes, *HMα* and *HM***a**, in *Saccharomyces* yeasts. Genetics **84:** 437–451.

HICKS, J. B., and I. HERSKOWITZ, 1976 Interconversion of yeast mating types. I. Direct observations of the action of the homothallism (*HO*) gene. Genetics **83:** 245–258.

HICKS, J. B., J. N. STRATHERN and I. HERSKOWITZ, 1977 The cassette model of mating-type interconversion, p. 457 in *DNA Insertion Elements, Plasmids, and Episomes*, edited by A. I. BUKHARI *et al.* Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

HICKS, J. B., J. N. STRATHERN and A. J. S. KLAR, 1979 Transposable mating-type genes in *Saccharomyces cerevisiae*. Nature **282:** 478–483.

IVY, J. M., A. J. S. KLAR and J. B. HICKS, 1986 Cloning and characterization of four *SIR* genes of *Saccharomyces cerevisiae*. Mol. Cell. Biol. **6:** 688–702.

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

KIMMERLY, W. J., and J. RINE, 1987 Replication and segregation of plasmids containing *cis*-acting regulatory sites of silent mating-type genes in *Saccharomyces cerevisiae* are controlled by the *SIR* genes. Mol. Cell. Biol. **7:** 4225–4237.

KIMMERLY, W., A. BUCHMAN, R. KORNBERG and J. RINE, 1988 Roles of two DNA-binding factors in replication, segregation and transcriptional repression mediated by a yeast silencer. EMBO J **7:** 2241–2253.

KLAR, A. J. S., 1980 Interconversion of yeast cell types by transposable genes. Genetics **95:** 631–648.

KLAR, A. J. S., and S. FOGEL, 1979 Activation of mating-type genes by transposition in *Saccharomyces cerevisiae*. Proc. Natl. Acad. Sci. USA **76:** 4539–4543.

KLAR, A. J. S., S. FOGEL and K. MACLEOD, 1979 *MAR1*—a regulator of *HM***a** and *HMα* loci in *Saccharomyces cerevisiae*. Genetics **93:** 37–50.

KLAR, A. J. S., S. FOGEL and D. N. RADIN, 1979 Switching of a mating-type **a** mutant allele in budding yeast *Saccharomyces cerevisiae*. Genetics **92:** 759–776.

KLAR, A. J. S., J. B. HICKS and J. N. STRATHERN, 1982 Directionality of a yeast mating-type interconversion. Cell **28:** 551–561.

KLAR, A. J. S., J. N. STRATHERN and J. B. HICKS, 1984 Developmental pathways in yeast, pp. 151–195 in *Microbial Development*, edited by R. LOSICK and L. SHAPIRO. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

KLAR, A. J. S., J. N. STRATHERN and J. B. HICKS, 1981 A position-effect control for gene transposition: state of expression of yeast mating-type genes affects their ability to switch. Cell **25:** 517–524.

KLAR, A. J. S., J. MCINDOO, J. B. HICKS and J. N. STRATHERN, 1980 Precise mapping of the homothallism genes, *HML* and *HMR*, in *Saccharomyces cerevisiae*. Genetics **96:** 315–320.

KLAR, A. J. S., J. N. STRATHERN, J. R. BROACH and J. B. HICKS, 1981 Regulation of transcription in expressed and unexpressed mating-type cassettes of yeast. Nature **289:** 239–244.

KLAR, A. J. S., S. N. KAKAR, J. M. IVY, J. B. HICKS, G. P. LIVI and L. M. MIGLIO, 1985 *SUM1*, an apparent positive regulator of the cryptic mating-type loci in *Saccharomyces cerevisiae*. Genetics **111:** 745–758.

KOSTRIKEN, R., and F. HEFFRON, 1984 The product of the *HO* gene is a nuclease: purification and characterization of the enzyme. Cold Spring Harbor Symp. Quant. Biol. **49:** 89–96.

KOSTRIKEN, R., J. N. STRATHERN, A. J. S. KLAR, J. B. HICKS and F. HEFFRON, 1983 A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. Cell **35:** 167–174.

KUSHNER, P., L. C. BLAIR and I. HERSKOWITZ, 1979 Control of yeast cell type by mobile genes: a test. Proc. Natl. Acad. Sci. USA **76:** 5264–5268.

LEHRACH, H., D. DIAMOND, J. M. WOZNEY and H. BOEDTKER, 1977 RNA molecular weight determinations by gel electrophoresis under denaturing conditions, a critical reexamination. Biochemistry **16:** 4743–4751.

LIVI, G. P., J. B. HICKS and A. J. S. KLAR, 1990 The *sum1-1* mutation affects silent mating-type gene transcription in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **10:** 409–412.

MARSHALL, M., D. MAHONEY, A. ROSE, J. B. HICKS and J. R. BROACH, 1987 Functional domains of *SIR4*, a gene required for position effect regulation in *Saccharomyces cerevisiae*. Mol. Cell. Biol. **7:** 4441–4452.

MILLER, A. M., 1984 The yeast *MAT***a**1 gene contains two introns. EMBO J. **3:** 1061–1065.

MILLER, A. M., and K. A. NASMYTH, 1984 Role of DNA replication in the repression of silent mating-type loci in yeast. Nature **312:** 247–251.

MORTIMER, R. K., and D. C. HAWTHORNE, 1969 Yeast genetics, pp. 385–460 in *The Yeasts*, Vol. 1 edited by A. H. ROSE and J. S. HARRISON. Academic Press, New York.

MULLEN, J. R., P. S. KAYNE, R. P. MOERSCHELL, S. TSUNASAWA, M. GRIBSKOV, M. COLAVITO-SHEPANSKI, M. GRUNSTEIN, F. SHERMAN and R. STERNGLANZ, 1989 Identification and characterization of genes and mutants for a N-terminal acetyltransferase from yeast. EMBO J. **8:** 2067–2075.

NASMYTH, K. A., 1982a Molecular genetics of yeast mating type. Annu. Rev. Genet. **16:** 439–500.

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

NASMYTH, K., and D. SHORE, 1987 Transcriptional regulation in the yeast life cycle. Science **237:** 1162–1170.

NASMYTH, K. A., and K. TATCHELL, 1980 The structure of trans-

posable yeast mating type loci. Cell **19:** 753–764.

NASMYTH, K. A., K. TATCHELL, B. D. HALL, C. ASTELL and M. SMITH, 1981 A position effect in the control of transcription at yeast mating-type loci. Nature **289:** 244–250.

RIGBY, P. W. J., M. DIECKMANN, C. RHODES and P. BERG, 1977 Labelling deoxyribonucleic acid to high specific activity *in vitro* by nick translation with DNA polymerase. J. Mol. Biol. **113:** 237–251.

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

RINE, J. D., J. N. STRATHERN, J. B. HICKS and I. HERSKOWITZ, 1979 A suppressor of mating type locus mutations in *Saccharomyces cerevisiae*: evidence for and identification of cryptic mating-type loci. Genetics **93:** 877–901.

ROMAN, H., and S. SANDS, 1953 Heterogeneity of clones of *Saccharomyces* derived from haploid ascospores. Proc. Natl. Acad. Sci. USA **39:** 171–179.

SCHNELL, R., and J. RINE, 1986 A position effect on the expression of a tRNA gene mediated by the *SIR* genes in *Saccharomyces cerevisiae.* Mol. Cell. Biol. **6:** 494–501.

SCHNELL, R., L. D'ARI, M. FOSS, D. GOODMAN and J. RINE, 1989 Genetic and molecular characterization of suppressors of *SIR4* mutations in *Saccharomyces cerevisiae.* Genetics **122:** 29–46.

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

SOUTHERN, E. M., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:** 503–517.

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

STINCHCOMB, D. C., K. STRUHL and R. W. DAVIS, 1979 Isolation and characterization of a yeast chromosomal replicator. Nature **282:** 39–43.

STRATHERN, J. N., J. B. HICKS and I. HERSKOWITZ, 1980 Control of cell type in yeast by the mating-type locus: the $\alpha 1$-$\alpha 2$ hypothesis. J. Mol. Biol. **147:** 357–372.

STRATHERN, J. N., A. J. S. KLAR, J. B. HICKS, J. A. ABRAHAM, J. M. IVY, K. A. NASMYTH and C. MCGILL, 1982 Homothallic switching of yeast mating-type cassettes is initiated by a double-stranded cut in the *MAT* locus. Cell **31:** 183–192.

STRATHERN, J., B. SHAFER, J. HICKS and C. MCGILL, 1988 **a**/α-Specific repression by *MAT*α2. Genetics **120:** 75–81.

TAKANO, I., T. KUSUMI and Y. OSHIMA, 1973 An α mating-type allele insensitive to the mutagenic action of the homothallic gene system in *Saccharomyces diastaticus.* Mol. Gen. Genet. **126:** 19–28.

WHITEWAY, M., R. FREEDMAN, S. VAN ARSDELL, J. W. SZOSTAK and J. THORNER, 1987 The yeast *ARD1* gene product is required for repression of cryptic mating-type information at the *HML* locus. Mol. Cell. Biol. **7:** 3713–3722.

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