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 HMLa MATa HMRa 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 (suppressor of mar). 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 HMLa and HMRa 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 (α 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 Klär, 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; Klär et al. 1980). Although these loci contain complete structural and promoter sequences for the expression of α 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; Klär and Fogel 1979; Kushner, Blair and Herskowitz 1979; Hicks, Strathern and Klär 1979; Nasmyth and Tatchell 1980; Klär 1980).

This position effect on expression is controlled by two cis-acting "silencer" sequences, called E (essential) and I (important), 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 (Klär, Fogel and MacLeod 1979; Haber and George 1979; Rine et al. 1979; Ivy, Klär 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,
it has been shown that the \textit{SIR2}, \textit{SIR3} and \textit{SIR4} gene products act to control the replication of plasmids containing \textit{HMR E} (Kimmerly and Rine 1987). Another feature that distinguishes \textit{MAT} from the \textit{HM} loci is its role in mating-type interconversion. Whereas interconversion can occur at \textit{MAT}, it does not normally do so at the \textit{HM} loci despite the presence of the same genetic information. This position-effect control of gene rearrangement is also regulated by the \textit{MAR/SIR} genes, since in appropriate \textit{marl} mutant strains the \textit{HM} loci efficiently interconvert (Klar, Stratman and Hicks 1981).

Several models have been proposed to account for the involvement of the four \textit{MAR/SIR} loci in regulating \textit{HML} and \textit{HMR} (Ivy, Klar and Hicks 1986; Rine and Herskowitz 1987; Kimmerly and Rine 1987). Based solely on genetic data, the repression of \textit{HM} gene transcription has been ascribed to the interaction of the \textit{MAR/SIR} gene products, either independently or as part of a multimeric protein, with the \textit{cis}-acting control sites (see Figure 1). However, no direct evidence for DNA binding activity has been reported for any \textit{MAR/SIR} gene product (Shore \textit{et al}. 1987). In fact, at least two \textit{HML E}- and \textit{HMR E}-specific DNA binding proteins, which do not correspond to any of the \textit{MAR/SIR} gene products, have recently been identified (Shore \textit{et al}. 1987; Shore and Nasmyth 1987; Nasmyth and Shore 1987; Buchman \textit{et al}. 1988). The silencer region of \textit{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 \textit{HMR B} region (as well as to \textit{ARS1}) but not to \textit{HML E}. Another protein, ABFI (ARS-binding factor I), recognizes controlling ele-
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The sum2-I and sum3-1 mutations are recessive: Among a total of 35,000 colonies screened, we obtained two mutants that mate as a (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 a mating phenotype could be due to a variety of mutational events: (1) simultaneous mutation of both HML and HMRa, (2) reversion of mar2-1 to MAR2, (3) certain mutations in MATa2 (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 (suppressor of mar after Klar et al. 1985), which are involved in regulating 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 a (K566) tester strain. "Restrictive" mating-type tests utilized leu2 tester strains (DC5a and DC6a) 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 Carlsson 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). 32P-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 (HMLa MATa HMRa mar2-1), which exhibits a sterile (nonmating) phenotype due to the simultaneous expression of both a and a 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., HMLa MATa HMRa will simply read a.a.a.) Cells of strain PL1 were mutagenized and colonies were screened for those exhibiting an a mating type. Among a total of 35,000 colonies screened, we obtained two mutants that mate as a (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 a mating phenotype could be due to a variety of mutational events: (1) simultaneous mutation of both HMLa and HMRa, (2) reversion of mar2-1 to MAR2, (3) certain mutations in MATa2 (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 (suppressor 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 (a.a. mar2-1 sum2-1) and PL13 (a.a.
mar2-1 sum3-1) to strain J562 (a^-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 a 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 (aaa); upon sporulation, such diploids generated the expected relative frequency of a and nonmating segregants, rather than 2a:2a 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 MATa allele were phenotypically a, indicating that both sum2-1 and sum3-1 identify single loci which segregate independently of MATa.

**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 a a a mar2-1 sum3-1 + × PL60 a^-a^- Δ mar2::LEU2 + sum2-1
2. K724 a a a mar2::LEU2 sum1-1 + × PL60 a^-a^- Δ mar2::LEU2 + sum2-1
3. K724 a a a mar2::LEU2 sum1-1 + × PL66 a^-a^- Δ mar2-1 + sum3-1

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-
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Master Plate

<table>
<thead>
<tr>
<th></th>
<th>DC5^a</th>
<th>PL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>PL1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL11</td>
<td>DC6^X</td>
<td>PL1</td>
</tr>
<tr>
<td>PL13</td>
<td>K122</td>
<td>PL1</td>
</tr>
<tr>
<td>PL136</td>
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<td>PL1</td>
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<tr>
<td>PL78</td>
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<td>PL1</td>
</tr>
</tbody>
</table>

**Figure 2.** Mating-type tests indicating suppression of the mar2-1 mutation in strains PL11 (aα mar2-1 sum2-1) and PL13 (aα mar2-1 sum3-1) as compared to the nonmating parental strain PL1 (aα 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 MATa (strain K567; left) or MATα (strain K566; right) mating-type tester cells. Growth (scored after 48 hr) on the bottom left plate (Xa) indicates an α mating type; growth on the bottom right plate (Xα) indicates an α mating type.

Diploids from each cross were found to be sterile and exhibited wild-type levels of sporulation, indicating the expression of functional α 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. K780 × PL78 aα mar2-1 sum2-1 aα mar2-1 sum2-1

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. K782 × PL13 aα mar2-1 sum3-1 aα mar2-1 sum3-1

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 mar2-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 (aα 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 (MATa 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

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

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"a" 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"a" 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"a" 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 tetrad analysis was recovered (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 (aaa sum3-1), PL48 (aaa mar2-1 sum2-1) and K775 (aaa 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, a mating-type Leu"a" 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 aaa sum3-1 were generated from a cross between
strains PL134 (aaa sum3-1) and PL81 (aαa
mar2::LEU2 sum3-1). All ascis contained 2a and 2α
segregants (Table 2, line 12), which included a signif-
icant number of Leu-a 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 2a 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 a1 and a2 transcripts in wild-type vs. aαa
mar2-1 sum2-1 and aαa 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 MAKα that recognizes both a and α
transcripts (KLAR et al. 1981). Figure 3 shows that,
whereas cells carrying the mar2-1 mutation contain
both a1 and a2 transcripts, the level of these tran-
scripts 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 (a1 and a2). 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 tran-
scripts could be due to an effect on mRNA processing
and/or stability, given the fact that the a1 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) PL46 × PL75
    aaa mar2-1 sum2-1
    aαa mar2::LEU2 sum2-1
(2) PL42 × PL58
    aaa mar2::LEU2 sum3-1
    aαa mar2-1 sum3-1

In both cases, selected diploids were found to be
nonmating and sporulation proficient (>80%), indicat-
ing expression and correct processing of the a1
transcript derived from MAKα.

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 ge-
etic evidence to suggest that, like sum1-1 (KLAR et al.
1985), sum2-1 also partially represses (albeit very
weakly) the expression of a information at HMR.
Strain PL46 (aaa mar2-1 sum2-1) was mated with
strain K388 [aαa α mar2-1 (α phenotype)]. If sum2-1
was capable of repressing the expression of HMRα,
we expected a number of ascis to contain 3a and 4a
mating-type segregants, and to exhibit an overall seg-
regation pattern of 12a:1α:4α. In fact, tetrads con-
taining both 3a and 4a mating-type segregants were
observed (Table 2, line 13). Furthermore, the pres-
ence of a unique class of bimating segregants suggests
that the sum2-1 mutation is leaky. Expression of
HMRα will result in an α phenotype when both HML
and MAT contain a^- information. In some cells, ap-
parently enough α information is expressed to allow
them to mate as α, whereas in other cells the HMRα
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 func-
tional a information, leaky expression of HMRα would
produce a mixture of a (no α expression) and non-
mating (α expression) cell types. A colony with this
mixture would appear to be an a mater. Because sum2-
1 restores repression to HMLa, 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: HMLa (or a^-) MAKα^- HMRα 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. How-
ever, 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 com-
plementation 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 dele-
tion mutations of MAR2, indicating that they are not	ranslational 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 (MATa) (shown below; KLAR et al. 1981) which contains a fragment of MATa homologous to both α and ω 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 α1, α2, ω1, ω2, 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 HMLα MATα HMRα mar2-1 sum2-1 cells vs. the bimating behavior of HMLα (or ω) mata- HMRα 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 HMLα is expressed to produce an α mating phenotype in only a fraction of the cells. (Expression of both α and ω in a single cell results in an α mating phenotype; e.g., MATα/mata- diploids mate with a cells). The fraction of cells expressing insufficient levels of HMLα will exhibit an α mating behavior (mata- cells mate with MATα tester strains). This interpretation is not inconsistent with the α mating behavior of HMLα MATα HMRα mar2-1 sum2-1 (or sum3-1) cells. Complete repression of both HMLα loci will give an α mating behavior, and expression of some HMLα 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 α1 transcript from MATα 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 sum-1 mutation exhibits no effect on transcription of any of the four MAR/SIR genes (Livi, Hicks and Klar 1990), and that sum-3-1 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 sum-1-1 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 SUM 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 sum-2-1 and sum-3-1 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 (GRFl) (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 (Whitehead 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 sum-1-1 mutation (Klar et al. 1985). Clearly, understanding the function of the SUM genes will require further genetic and molecular dissection.

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