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The *sum1-1* Mutation Affects Silent Mating-Type Gene Transcription in *Saccharomyces cerevisiae*

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The silent mating-type genes (*HML* and *HMR*) of *Saccharomyces cerevisiae* are kept under negative transcriptional control by the *trans*-acting products of the four *MAR/SIR* loci. *MAR/SIR* gene mutations result in the simultaneous derepression of *HML* and *HMR* gene expression. The *sum1-1* mutation was previously identified as an extragenic suppressor of mutations in *MAR1* (*SIR2*) and *MAR2* (*SIR3*). As assayed genetically, *sum1-1* is capable of restoring repression of silent mating-type information in cells containing *mar1* or *mar2* null mutations. We show here that the mating-type phenotype associated with *sum1-1* results from a dramatic reduction in the steady-state level of *HML* and *HMR* gene transcripts. At the same time, the *sum1-1* mutation has no significant effect on the level of each of the four *MAR/SIR* mRNAs.

Mating type in *Saccharomyces cerevisiae* is determined by the type of information (**a** or α) residing at the constitutively expressed *MAT* locus on chromosome III. The products of the *MAT* transcripts act to regulate cell type (for reviews, see references 22 and 28). Cryptic copies of mating-type information also exist at the *HML* and *HMR* loci (herein collectively referred to as *HM* loci or cassettes) (9, 20). The *HM* loci are capable of donating mating-type information to *MAT* via a genetic transposition event (11, 12, 16, 17, 20, 23, 30, 33, 39).

The products of four unlinked *MAR/SIR* genes (*SIR1*, *SIR2* [or MAR1], *SIR3* [or *MAR2*], and *SIR4*) act to repress transcription of the silent mating-type loci (8, 13, 18, 32, 33). A mutation in any *MAR/SIR* gene results in the simultaneous expression of both *HML* and *HMR*. *MAR/SIR*-mediated repression involves regulatory sites that flank the *HM* loci, called E and I (1, 7). Mutations at these sites result in the specific loss of repression of only the adjacent *HM* locus. *HMR* E can act in an orientation-independent manner to repress the transcription of other genes and consequently has been termed a silencer sequence (2, 35). DNA replication appears to be required for *MAR/SIR*-mediated repression since (i) cells must complete the S phase to establish *MAR/SIR* regulation (25), (ii) both *HML* E and *HMR* E contain *ARS* elements (putative origins of DNA replication) (3, 4, 38), and (iii) the *SIR1-4* gene products are necessary for high mitotic stability of a plasmid containing *HMR* E (15). Furthermore, Nasmyth (29) has shown that derepression of *HMLα* leads to a change in chromatin structure at that locus equivalent to that observed at the expressed *MATα* locus. These data suggest that *HM* gene repression is due to an interaction of the *MAR/SIR* gene products, either independently or as part of a multimeric protein complex, with the *cis*-acting control sites (13, 32). However, two factors identified as *HMR* E-binding proteins do not correspond to any of the *MAR/SIR* gene products (5, 14, 36, 37). One of these proteins (RAP1 or GRF1) is essential for cell viability, suggesting that it may be a general transcription-regulatory factor (27). Whether the MAR/SIR proteins also interact with the silencer DNA is not known.

Several new genes involved in regulating *HML* and *HMR* have been identified genetically by isolating suppressors of *MAR/SIR* mutations. These genes include *SUM1* (19), *SUM2* and *SUM3* (C.-I. P. Lin, G. P. Livi, J. M. Ivy, and A. J. S. Klar, Genetics, in press), and *SAN1*, *SAN2*, and *SAN3* (34). The *sum2-1* and *sum3-1* mutations suppress null *MAR2* (*SIR3*) mutations, whereas *san1-1*, *san2-1*, and *san3-1* suppress mutations in *SIR4*. In contrast, the *sum1-1* mutation is neither allele specific nor locus specific, suppressing null mutations in both *MAR1* (*SIR2*) and *MAR2* (*SIR3*). The *sum1-1* mutation (i) is recessive, (ii) does not correspond genetically to *MAT*, *HML*, *HMR*, or any of the *MAR/SIR* loci, (iii) has no discernible phenotype in Mar^+ strains, and (iv), on the basis of qualitative mating-type tests, affects expression of both **a** and α information at the *HM* loci (19). The role of the *SUM1* gene product remains particularly enigmatic, since the genetic data suggest that it acts as a positive regulator of the silent cassettes.

In this study, we investigated further the function of the *SUM1* gene product by asking whether the *sum1-1* mutation affects silent mating-type gene expression by restoring repression of transcription and whether, if so, this effect is due to a compensating increase in the level of expression of any of the *MAR/SIR* genes.

The *sum1-1* mutation affects *HMa* gene transcription. To address the question of whether *sum1-1* affects silent mating-type gene expression by restoring transcriptional repression of *HML* and *HMR*, we compared the levels of **a**1 and **a**2 transcripts in wild-type (*MAR/SIR*$^+$ *SUM1*$^+$) cells as well as in cells of genotypes *HML***a** *MATα* *HMR***a** *mar1-1* *SUM1*$^+$ and *HML***a** *MATα* *HMR***a** *mar1-1* *sum1-1* (see Table 1 for genotypes of all strains). Whereas *mar1-1* cells contained both **a**1 and **a**2 transcripts, the steady-state level of these transcripts was significantly reduced in *mar1-1* cells carrying *sum1-1* (Fig. 1). At the same time, *sum1-1* had no effect on the level of *MAT* gene transcripts (α1 and α2).

The *sum1-1* mutation affects *HMLα* gene transcription. Similar to the results presented above, *sum1-1* also was

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TABLE 1. Yeast strains[a]

Strain	Mating-type genotype					Other genotype	Mating phenotype
	HML	*MAT*	*HMR*	*MAR1*	*SUM1*		
DC5	α	**a**	**a**	+	+	*can1 gal2 his3 leu2 mal*	**a**
DC6	α	α	**a**	+	+	*can1 gal2 his4 leu2 mal*	α
K23	**a**	α	**a**	*mar1-1*	+	*trp1 ura1 ade6 mal*	NM
K694	**a**	α	**a**	*mar1-1*	*sum1-1*	*trp1 ura1 ade6 mal*	α
K713	**a**	α	**a**	+	*sum1-1*	*his4 leu2 MAL*$^+$	α
K165	α	**a62**	**a62**	*mar1-1*	+	*trp1-1 lys1-1 his4 leu2 thr4 met13 mal*	α
K728	α	**a62**	**a62**	*mar1-1*	*sum1-1*	*trp1-1 lys1-1 his4 leu2 thr4 met13 ade6 mal*	BM

[a] All strains are heterothallic (*ho*) and have been previously described (13; Lin et al., in press), except K728, which was constructed for this study by using standard techniques for yeast genetic crosses, sporulation, and tetrad dissection (26). All media for growth and sporulation were prepared as described previously (10). The **a62** allele was isolated at *HMR* following ethyl methane sulfonate mutagenesis and transposed to *MAT* as described by Klar et al. (20). The **a62** allele defines a mutation in **a1** that destroys sporulation function. It is not suppressed by either amber or ochre suppressors (20). NM, Nonmating (sterile); BM, bimating.

found to affect the level of α1 and α2 transcripts derived from *HML* (Fig. 2). The same results were obtained with RNA from *sum1-1* cells containing either a *mar1*::*LEU2* or *mar2*::*LEU2* gene disruption (13) (data not shown). It is unlikely that *sum1-1* specifically affects the processing or stability of all of the mating-type gene transcripts. Therefore, we favor the conclusion that the effect of this suppressor mutation on mating behavior is due to its ability to restore repression of *HM* gene transcription in *mar1* and *mar2* mutant strains.

The *sum1-1* mutation does not increase *MAR/SIR* mRNA levels. Hypothetically, one way in which repression of *HM* gene transcription may be restored is by enhancing the activity of the *MAR/SIR* gene products. To test whether *sum1-1* affects the abundance of any of the *MAR/SIR* mRNAs, the levels of *SIR1*, *SIR2* (*MAR1*), *SIR3* (*MAR2*), and *SIR4* RNA in *SUM1*$^+$ and *sum1-1* cells were compared (Fig. 3). Clearly, *sum1-1* has no significant effect on the abundance of any of these transcripts, with the possible exception of *SIR2* (*MAR1*), in which a 2.1-fold decrease was observed. We conclude that *sum1-1* does not regulate *HM* gene transcription by increasing the steady-state level of *MAR/SIR* mRNA. The results with *SIR2* (*MAR1*) and *SIR3* (*MAR2*) are consistent with the fact that *sum1-1* suppresses genetically engineered null mutations in these loci.

Proposed function of *SUM1*. These data support the genetic model which was originally proposed to account for the action of *SUM1*. In this model, the *MAR/SIR* loci negatively regulate *SUM1*, the product of which is subsequently required for *HM* gene expression, either by acting as a positive regulator or by negatively regulating another downstream repressor (19). However, the existence of such a downstream repressor has not been detected genetically, thus

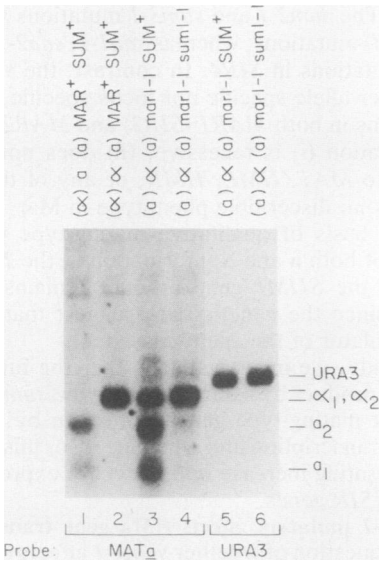

FIG. 1. Northern (RNA) blot analysis of *HMa* mating-type gene transcripts. Total RNA was isolated from various yeast strains (6) (relevant genotypes are listed at the top), and poly(A)$^+$ RNA was selected by using oligo(dT)-cellulose. Poly(A)$^+$ RNA was size fractionated on a 1.5% ME agarose gel (SeaKem) containing 2.2 M formaldehyde (24), transferred to nitrocellulose, and probed with ^{32}P-labeled (31) pMAT1 (*MAT***a**) DNA (20). pMAT1 contains sequences homologous to both **a** and α transcripts. Each lane contained 2 μg of poly(A)$^+$ RNA. Lanes: 1, DC5; 2, DC6; 3, K23; 4, K694. Lanes 5 and 6 are identical to lanes 3 and 4 but stripped and reprobed with the *URA3*-containing YIp5 plasmid. This served as an internal control for relative RNA abundance. Bands corresponding to the **a1**, **a2**, α1, α2, and *URA3* transcripts are indicated.

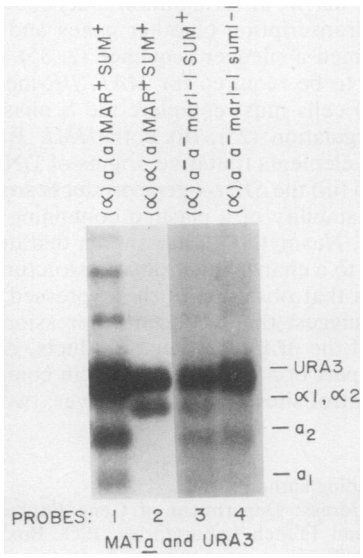

FIG. 2. Northern blot analysis of *HMLα* mating-type gene transcripts. The procedures described in the legend to Fig. 1 were followed except that 12 μg of total RNA was used and the blot was probed simultaneously with pMAT1 and YIp5. Lanes: 1, DC5; 2, DC6; 3, K165; 4, K728.

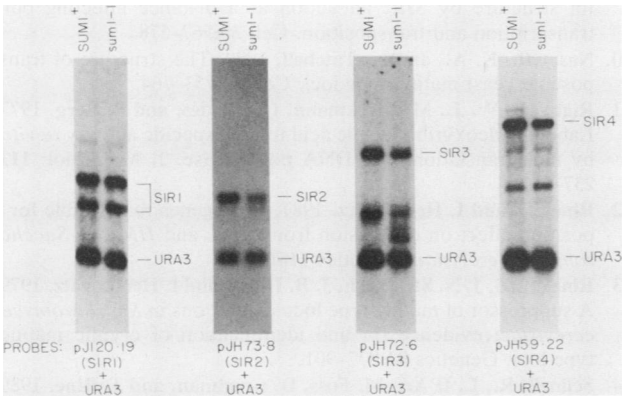

FIG. 3. Northern blot analysis of *MAR/SIR* gene transcripts. The procedures described in the legend to Fig. 1 were followed except that each blot was probed with a mixture of YIp5 (*URA3*) and a plasmid containing one of the *SIR* genes (pJI20.19, *SIR1*; pJH73.8, *SIR2* or *MAR1*; pJH72.6, *SIR3* or *MAR2*; pJH59.22, *SIR4*). In each blot, the first lane contains mRNA from strain DC6 (*SUM1*$^+$) and the second lane contains mRNA from strain K713 (*sum1-1*). Fluorographs were scanned in an LKB 2222-010 Ultroscan XL laser densitometer, and data were recorded and analyzed by using accompanying LKB software. The abundance of *MAR/SIR* mRNAs between strains relative to the abundance of *URA3* mRNA was determined.

promoting the view of *SUM1* as a positive regulator. This raises the question of why cells would evolve a mechanism for positively controlling the expression of genes not normally expressed.

As pointed out by Schnell et al. (34), *sum1-1* may not be a simple loss-of-function mutation. In contrast to other suppressors of *MAR/SIR* mutations (34; Lin et al., in press), *sum1-1* was isolated as the sole Sir$^+$ revertant from a large population of heavily mutagenized cells (19). Thus, the genetic model in which *SUM1* acts directly as a positive regulator may be incorrect. Ivy et al. (13) demonstrated that there is a lack of transcriptional regulation among the four *MAR/SIR* genes. However, allele-specific suppression of mutations in *SIR4* by increased gene dosage of *SIR3* was observed (13). Thus, one possibility is that *sum1-1* suppresses mutations in *MAR1* (*SIR2*) and *MAR2* (*SIR3*) through a compensatory increase in *MAR/SIR* gene function. Since *sum1-1* does not increase the abundance of *MAR/SIR* mRNAs, such an effect would have to occur posttranscriptionally.

Genetic data indicate that derepression of the *HM* loci in Sir$^-$ cells requires the presence of a wild-type *SUM1* allele (19). Another possibility is that the *SUM1* gene product is involved in controlling chromatin structure at the *HM* loci, thereby making them accessible to specific transcriptional control factors (36, 37). In this model, *SUM1* may be viewed as an antagonist of the normal silencer state of each *cis*-acting *HM* control sequence. This antagonism may also reflect an involvement of *SUM1* in mating-type interconversion. One feature that distinguishes *MAT* from the *HM* loci is its role in interconversion: whereas rearrangements normally occur at *MAT*, they do not normally occur at the *HM* loci, despite the presence of the same genetic information. This position effect is regulated by the *MAR/SIR* genes, since in appropriate *mar1* mutant strains the *HM* loci efficiently interconvert (21). It will be interesting to determine the effect of *sum1-1* on mating-type interconversion. Resolution of these issues will require further molecular analysis of *SUM1* as well as of the other *MAR/SIR* mutant suppressor genes.

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