

## NOTE

### *rme1* Mutation of *Saccharomyces cerevisiae*: Map Position and Bypass of Mating Type Locus Control of Sporulation

JASPER RINE,† GEORGE F. SPRAGUE, JR.,‡\* AND IRA HERSKOWITZ§

Department of Biology and Institute of Molecular Biology, University of Oregon, Eugene, Oregon 97403

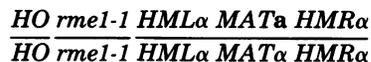
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Sporulation in *Saccharomyces cerevisiae* normally occurs only in *MATa*/*MATα* diploids. We show that mutations in *RME1* bypassed the requirement for both *a* and *α* mating type information in sporulation and therefore allowed *MATa*/*MATa* and *MATα*/*MATα* diploids to sporulate. *RME1* was located on chromosome VII, between *LEU1* and *ADE6*.

Sporulation of the yeast *Saccharomyces cerevisiae* is subject to genetic control which is exerted through alleles of the mating type locus *MAT*; *MATa*/*MATα* diploids sporulate, whereas *MATa*/*MATa* and *MATα*/*MATα* diploids do not. Each mating type locus allele codes for a function necessary for sporulation, *a1* from *MATa* and *α2* from *MATα* (reviewed in reference 3). A number of mutations have been identified that allow *MATa*/*MATa* and *MATα*/*MATα* diploids to sporulate. These mutations include *rme1-1* (6), *csp1-1* (5), *sca* (1), *sir1-1* (10), and *cmt* (2). For some of these mutations, it has been shown that they allow sporulation also of *mata1*/*MATα* and *MATa*/*mata2* strains (2, 6, 10). Analysis of *sir1-1* has revealed that this mutation does not bypass the requirement for the *a1* and *α2* products in promoting sporulation. Rather, the *sir1-1* mutation provides both of these functions by allowing expression of cryptic *α* and *a* mating type genes at *HML* and *HMR*. Does the *rme1-1* mutation allow sporulation to occur independently of the *a1* and *α2* products, or does it act in a manner similar to *sir1-1*?

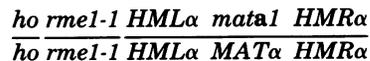
***rme1* acts independently of *HML* and *HMR*.** To determine whether expression of the cryptic *a* mating type information at *HMRa* allows *MATα*/*MATa* *rme1-1* diploids to sporulate, a *MATα*/*MATα* strain carrying only cryptic *α* information was analyzed. Such a strain was obtained by mitotic recombination from a

*MATa*/*MATα* diploid (XR156-38d) of genotype



(see reference 3 for a discussion of the origin of the *HMRα* allele). The *MATα*/*MATα* recombinant was isolated by screening colonies of XR156-38d (which are auxotrophic) for the ability to form prototrophs with a *MATa* strain containing complementary auxotrophic mutations. The *α/α* diploid (XR156-38d *α/α*) sporulated at a frequency typical of sporulation promoted by *rme1-1* (~10%; 6) and yielded 4*a*:0*α* segregants in each of 14 tetrads. *rme1-1* thus relieves the requirements for *a* information in sporulation.

To insure that sporulation of the *HO*/*HO* *MATα*/*MATα* diploid XR156-38d *α/α* was due to *rme1-1* and not to an unexpected property of diploids containing *HO*, we tested the sporulation capability of diploid XR293 of genotype



This diploid was also able to sporulate (~10%) and yielded 2*α*:2*a* segregants in each of 32 tetrads. Each of the *a* segregants, when mated to a *MATα* *RME1* strain, formed diploids incapable of sporulation, thus confirming the presence of the *mata1* mutation in XR293 (see footnote to Table 2). These results indicate that the ability of *rme1-1* to suppress the sporulation defect of *mata1* is not dependent on the presence of cryptic *a* mating type information. Diploids XR156-38d and XR293 contained the naturally occurring *rme1-1* allele described by Kassir and

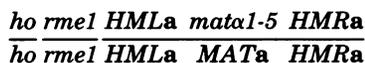
† Present address: Department of Biochemistry, Stanford University School of Medicine, Stanford, CA 94305.

‡ Present address: Department of Biology, University of Oregon, Eugene, OR 97403.

§ Present address: Department of Biochemistry and Biophysics, University of California School of Medicine, San Francisco, CA 94143.

Simchen (6). However, in experiments described below, diploids G203 and G114 contained an *rme1* allele which we discovered in strain XT1172-S245c. We designated the S245c allele as simply *rme1*, since it is not known whether this allele is identical to *rme1-1*.

To determine whether the ability of *MATa*/*MATa rme1/rme1* diploids to sporulate requires cryptic  $\alpha$  information, we isolated an ultraviolet-induced *MATa*/*MATa* mitotic recombinant from a diploid (G203) of genotype



This diploid (G203 a/a) was able to sporulate, and it yielded 4a:0 $\alpha$  segregants in each of 25 tetrads, demonstrating that *rme1* allows a/a diploids to sporulate independently of the presence of cryptic  $\alpha$  mating type information.

**Effect of *rme1* on mating efficiency.** In contrast to *MATa*, *MAT $\alpha$* , *MATa*/*MATa*, and *MAT $\alpha$* /*MAT $\alpha$*  strains, *MATa*/*MAT $\alpha$*  diploids are unable to mate. Since *rme1* imparts some properties of *MATa*/*MAT $\alpha$*  diploids to strains that are capable of mating, it was of interest to know whether *rme1* affected mating by *MATa* or *MAT $\alpha$*  cells. The ability to score mating type in all segregants from the diploids containing *rme1* mutations (XR156-38d  $\alpha/\alpha$ , XR293 a1<sup>-</sup>/ $\alpha$ , G203 a/a, and G114a/ $\alpha$ ) indicates qualitatively that *rme1* does not cause a nonmating phenotype. A further indication that *rme1* does not affect mating efficiency comes from cell-to-cell efficiency of mating assays performed with a and  $\alpha$  segregants from a *MATa*/*MAT $\alpha$*  *RME1/rme1* diploid (G114). Under the conditions tested here, the presence of an *rme1* mutation caused no reduction in mating efficiency (Table 1). Hence, *rme1* leads to induction of sporulation without a simultaneous inhibition of mating.

**Map position of *RME1*.** The map position of *RME1* was determined in a cross between a *MAT $\alpha$*  *trp5 leu1 ade6 rme1* strain and a *MATa* *TRP5 LEU1 ADE6 RME1* strain. The data are consistent with the *TRP5-LEU1-RME1-ADE6* map order (Table 2) (see also reference 11).

It is clear from the analysis presented here that *rme1* bypasses mating type control of sporulation and therefore promotes sporulation in a manner distinctly different from the action of *sir1-1*. Based upon the recessive nature of *rme1-1*, Kassir and Simchen (6) proposed that *RME1* was a regulatory locus that controls expression of the genes required for sporulation. The ability of *MATa*/*MAT $\alpha$*  diploids to sporulate may be due to negative regulation of the *RME1* gene or gene product by the action of the *MATa1* and *MATa2* gene products, as depicted in Fig. 1.

TABLE 1. Effect of *rme1* on mating efficiency<sup>a</sup>

Mating	No. of cell pairs	No. of zygotes	Efficiency of mating <sup>b</sup>
<i>MAT<math>\alpha</math></i> <i>RME1</i> × <i>MATa</i> <i>RME1</i>	57	21	0.37
<i>MATa</i> <i>rme1</i> × <i>MATa</i> <i>rme1</i>	63	53	0.84
<i>MAT<math>\alpha</math></i> <i>RME1</i> × <i>MATa</i> <i>rme1</i>	57	52	0.91
<i>MATa</i> <i>rme1</i> × <i>MATa</i> <i>RME1</i>	62	24	0.39

<sup>a</sup> Single unbudded cells of one mating type were placed in contact with single unbudded cells of the opposite mating type by micromanipulation. Strains were segregants from diploid G114 (see footnote a to Table 2).

<sup>b</sup> Efficiency of mating was calculated as the fraction of cell pairs that formed a zygote within one generation.

TABLE 2. Map position of *RME1*<sup>a</sup>

Interval	Tetrad type (no.)			Map distance (cM)
	PD	NPD	T	
<i>rme1 leu1</i>	44	0	16	13
<i>rme1 ade6</i>	39	0	19	16
<i>leu1 ade6</i>	28	0	31	26
<i>rme1 trp5</i>	25	0	35	29

<sup>a</sup> Data were obtained by analyzing the meiotic products from a diploid (G114) formed between a *MATa* *rme1 ade6 leu1 trp5 met1 LYS2* strain (XT1172-S245c) and a *MATa* *RME1 ADE6 LEU1 TRP5 met1 lys2* strain (5A). Media and standard genetic techniques were described previously by Hicks and Herkowitz (4). Genetic distances were calculated in accordance with the formula of Perkins (9). *rme1* is able to suppress the sporulation defect associated with the *mata1* (a\*) mutation and is recessive to *RME1* (6). Thus, diploids of the genotype *mata1*/*MAT $\alpha$*  *rme1/rme1* are capable of sporulation, whereas *mata1*/*MAT $\alpha$*  *RME1/rme1* and *mata1*/*MATa* *RME1/RME1* diploids are not. The presence of *rme1* in  $\alpha$  strains was determined by mating the  $\alpha$  strains to a *mata1 rme1* strain and testing the resulting diploid for the ability to sporulate. The presence of *rme1* in a strains was tested by mating the a strain to a *mata1 HML $\alpha$  HMRa sir1-1 rme1* strain, which mates as  $\alpha$ , since the  $\alpha$  information at *HML $\alpha$*  and *HMRa* is expressed because of the *sir1-1* mutation (10). When the *mata1 HML $\alpha$  HMRa sir1-1 rme1* strain was mated to a *MATa* strain, the recessive *sir1-1* mutation no longer allowed expression of *HML $\alpha$*  and *HMRa*. Therefore, the ability of the diploid to sporulate was dependent upon the presence of *rme1* in the *MATa* strain. Sporulation promoted by *rme1* typically requires 5 days on sporulation medium at 30°C rather than 2 to 3 days for sporulation of a *MATa*/*MAT $\alpha$*  diploid. The efficiency of sporulation promoted by *rme1* is typically 10% that of a *MATa*/*MAT $\alpha$*  diploid. cM, Centimorgan.

Such a role for the a1 and a2 gene products has been proposed to account for regulation of *MAT $\alpha$* 1 and *HO*, since production of transcripts corresponding to *MAT $\alpha$* 1 (7, 8) and to *HO* (R. Jensen and G. Sprague, unpublished data) is inhibited in a/ $\alpha$  cells.

The efficiency of sporulation induced by

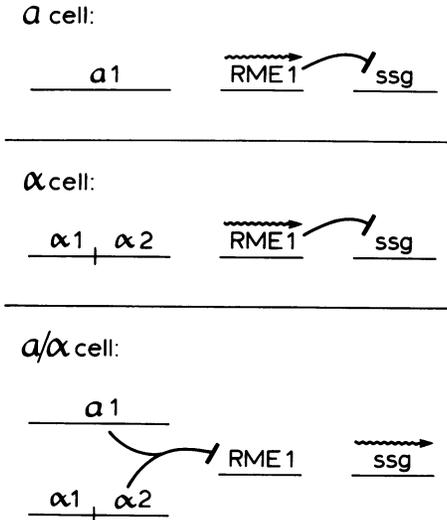


FIG. 1. A complex of *a1* and  $\alpha 2$  products is proposed to act as a negative regulator of *RME1*, which codes for a negative regulator of genes necessary for sporulation. Expression of *RME1* and sporulation-specific genes (*ssg*) is shown for *a*,  $\alpha$ , and *a/α* cells. The wavy line indicates expression of *RME1* or *ssg*; the line with a terminal bar indicates inhibition of expression of those genes.

*rme1-1* is typically 10% of that induced by a *MATa/MATα* diploid. The low level of sporulation associated with *rme1-1* may be due to leakiness of the *rme1-1* allele. On the other hand, the low level of sporulation may indicate that other genes (e.g., a hypothetical *RME2* locus) also have roles in the control of sporulation. The isolation of nonsense or deletion alleles of *RME1* should resolve this issue. Do the other sporulation bypass mutations, *csp1-1* and *sca*, define additional *RME* genes? The relationship among *rme1-1*, *csp1-1*, and *sca* is not known, although it is possible they are allelic or identical to one another. This possibility is supported by two observations. First, *csp1-1* was isolated in a diploid subsequently shown to be *rme1-1/RME1* (A. Hopper, personal communication). Second, although most laboratory strains contain the *RME1* allele, some common strains believed to be unrelated to the original *rme1-1* isolate con-

tain *rme1* mutations (e.g., strain XT1172-S245c; see above). Since *sca* was isolated from an unmutagenized strain, it may be an allele of *RME1* also. Knowledge of the map position of *RME1* should make it possible to determine the relationship among these mutations.

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