MOLECULAR AND CELLULAR BIOLOGY, Oct. 1981, p. 958-960
0270-7306/81/100958-03$02.00/0

Vol. 1, No. 10

NOTE

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

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Received 13 April 1981/Accepted 6 July 1981

Sporulation in *Saccharomyces cerevisiae* normally occurs only in *MAT***a**/*MAT*α diploids. We show that mutations in *RME1* bypassed the requirement for both **a** and α mating type information in sporulation and therefore allowed *MAT***a**/*MAT***a** and *MAT*α/*MAT*α 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*; *MAT***a**/*MAT*α diploids sporulate, whereas *MAT***a**/*MAT***a** and *MAT*α/*MAT*α diploids do not. Each mating type locus allele codes for a function necessary for sporulation, **a**1 from *MAT***a** and α2 from *MAT*α (reviewed in reference 3). A number of mutations have been identified that allow *MAT***a**/*MAT***a** and *MAT*α/*MAT*α 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 *mat***a**1/*MAT*α and *MAT***a**/*mat*α2 strains (2, 6, 10). Analysis of *sir1-1* has revealed that this mutation does not bypass the requirement for the **a**1 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 **a**1 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 *MAT*α/*MAT*α *rme1-1* diploids to sporulate, a *MAT*α/*MAT*α strain carrying only cryptic α information was analyzed. Such a strain was obtained by mitotic recombination from a *MAT***a**/*MAT*α diploid (XR156-38d) of genotype

$$\frac{HO\ rme1\text{-}1\ HML\alpha\ MAT\mathbf{a}\ HMR\alpha}{HO\ rme1\text{-}1\ HML\alpha\ MAT\alpha\ HMR\alpha}$$

(see reference 3 for a discussion of the origin of the *HMR*α allele). The *MAT*α/*MAT*α recombinant was isolated by screening colonies of XR156-38d (which are auxotrophic) for the ability to form prototrophs with a *MAT***a** 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 4α:0**a** 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* *MAT*α/*MAT*α 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

$$\frac{ho\ rme1\text{-}1\ HML\alpha\ mat\mathbf{a}1\ HMR\alpha}{ho\ rme1\text{-}1\ HML\alpha\ MAT\alpha\ HMR\alpha}$$

This diploid was also able to sporulate (~10%) and yielded 2α:2**a** segregants in each of 32 tetrads. Each of the **a** segregants, when mated to a *MAT*α *RME1* strain, formed diploids incapable of sporulation, thus confirming the presence of the *mat***a**1 mutation in XR293 (see footnote to Table 2). These results indicate that the ability of *rme1-1* to suppress the sporulation defect of *mat***a**1 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

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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 *MAT***a**/*MAT***a** *rme1*/*rme1* diploids to sporulate requires cryptic α information, we isolated an ultraviolet-induced *MAT***a**/*MAT***a** mitotic recombinant from a diploid (G203) of genotype

$$\frac{ho\ rme1\ HML\mathbf{a}\ mat\alpha1\text{-}5\ HMR\mathbf{a}}{ho\ rme1\ HML\mathbf{a}\ MAT\mathbf{a}\ HMR\mathbf{a}}$$

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

Effect of *rme1* on mating efficiency. In contrast to *MAT***a**, *MAT*α, *MAT***a**/*MAT***a**, and *MAT*α/*MAT*α strains, *MAT***a**/*MAT*α diploids are unable to mate. Since *rme1* imparts some properties of *MAT***a**/*MAT*α diploids to strains that are capable of mating, it was of interest to know whether *rme1* affected mating by *MAT***a** or *MAT*α cells. The ability to score mating type in all segregants from the diploids containing *rme1* mutations (XR156-38d α/α, XR293 **a**1$^-$/α, G203 **a**/**a**, and G114**a**/α) 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 α segregants from a *MAT***a**/*MAT*α *RME*/*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*α *trp5 leu1 ade6 rme1* strain and a *MAT***a** *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 *MAT***a**/*MAT*α diploids to sporulate may be due to negative regulation of the *RME1* gene or gene product by the action of the *MAT***a***1* and *MAT*α*2* gene products, as depicted in Fig. 1.

TABLE 1. *Effect of rme1 on mating efficiency*[a]

Mating	No. of cell pairs	No. of zygotes	Efficiency of mating[b]
MATα *RME1* × *MAT***a** *RME1*	57	21	0.37
MATα *rme1* × *MAT***a** *rme1*	63	53	0.84
MATα *RME1* × *MAT***a** *rme1*	57	52	0.91
MATα *rme1* × *MAT***a** *RME1*	62	24	0.39

[a] 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).

[b] Efficiency of mating was calculated as the fraction of cell pairs that formed a zygote within one generation.

TABLE 2. *Map position of RME1*[a]

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

[a] Data were obtained by analyzing the meiotic products from a diploid (G114) formed between a *MAT*α *rme1 ade6 leu1 trp5 met1 LYS2* strain (XT1172-S245c) and a *MAT***a** *RME1 ADE6 LEU1 TRP5 met1 lys2* strain (5A). Media and standard genetic techniques were described previously by Hicks and Herskowitz (4). Genetic distances were calculated in accordance with the formula of Perkins (9). *rme1* is able to suppress the sporulation defect associated with the *mat***a***1* (**a***) mutation and is recessive to *RME1* (6). Thus, diploids of the genotype *mat***a***1*/*MAT*α *rme1*/*rme1* are capable of sporulation, whereas *mat***a***1*/*MAT*α *RME1*/*rme1* and *mat***a***1*/*MAT*α *RME1*/*RME1* diploids are not. The presence of *rme1* in α strains was determined by mating the α strains to a *mat***a***1 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 *mat***a***1 HML*α *HMR*α *sir1-1 rme1* strain, which mates as α, since the α information at *HML*α and *HMR*α is expressed because of the *sir1-1* mutation (10). When the *mat***a***1 HML*α *HMR*α *sir1-1 rme1* strain was mated to a *MAT***a** strain, the recessive *sir1-1* mutation no longer allowed expression of *HML*α and *HMR*α. Therefore, the ability of the diploid to sporulate was dependent upon the presence of *rme1* in the *MAT***a** 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 *MAT***a**/*MAT*α diploid. The efficiency of sporulation promoted by *rme1* is typically 10% that of a *MAT***a**/*MAT*α diploid. cM, Centimorgan.

Such a role for the **a**1 and α2 gene products has been proposed to account for regulation of *MAT*α*1* and *HO*, since production of transcripts corresponding to *MAT*α*1* (7, 8) and to *HO* (R. Jensen and G. Sprague, unpublished data) is inhibited in **a**/α cells.

The efficiency of sporulation induced by

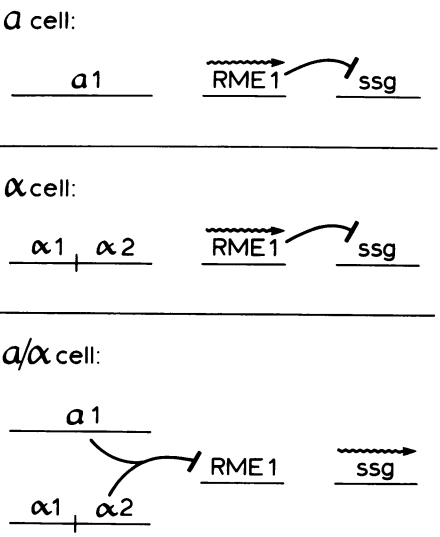

FIG. 1. *A complex of* **a***1 and* α*2 products is proposed to act as a negative regulator of RME1, which codes for a negative regulator of genes necessary for sporulation. Expression of RME1 and sporulation-specific genes (ssg) is shown for* **a**, α, *and* **a**/α *cells. The wavy line indicates expression 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 *MAT***a**/*MAT*α 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 contain *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.

We thank Drena Larson and Diane Morton for experimental contributions in the early stages of this work and Julie Dunn for preparation of the manuscript.

This work was supported by a Public Health Service Research Career Development Award (AI-00163), Research Grant (AI-13452), and Molecular Biology Training Grant (GM-00715), and by an American Cancer Society postdoctoral fellowship to G.F.S. (PF-1282).

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