A SUPPRESSOR OF MATING-TYPE LOCUS MUTATIONS IN *SACCHAROMYCES CEREVISIAE*: EVIDENCE FOR AND IDENTIFICATION OF CRYPTIC MATING-TYPE LOCI

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ABSTRACT

A mutation has been identified that suppresses the mating and sporulation defects of all mutations in the mating-type loci of *S. cerevisiae*. This suppressor, *sir1–1*, restores mating ability to *matα1* and *matα2* mutants and restores sporulation ability to *matα2* and *mat***a***1* mutants. *MAT***a** *sir1–1* strains exhibit a polar budding pattern and have reduced sensitivity to α-factor, both properties of **a**/α diploids. Furthermore, *sir1–1* allows *MAT***a**/*MAT***a**, *matα1*/*matα1*, and *MATα*/*MATα* strains to sporulate efficiently. All actions of *sir1–1* are recessive to *SIR1*. The ability of *sir1–1* to supply all functions necessary for mating and sporulation and its effects in **a** cells are explained by proposing that *sir1–1* allows expression of mating type loci which are ordinarily not expressed. The ability of *sir1–1* to suppress the *matα1–5* mutation is dependent on the *HM***a** gene, previously identified as required for switching of mating types from **a** to α. Thus, as predicted by the cassette model, *HM***a** is functionally equivalent to *MATα* since it supplies functions of *MATα*. We propose that *sir1–1* is defective in a function, Sir ("*S*ilent-*i*nformation *r*egulator"), whose role may be to regulate expression of *HM***a** and *HMα*.

THE mating-type locus is of central importance in determining cell type in the yeast *Saccharomyces cerevisiae*. Cells with the *MAT***a** allele have **a** mating type; cells with the *MATα* allele have α mating type. *MAT***a**/*MATα* strains are a third cell type, which, unlike *MAT***a** and *MATα* (or *MAT***a**/*MAT***a** and *MATα*/*MATα*) strains, are able to sporulate, but not to mate. Genes necessary for mating have been identified by isolation of mating-deficient mutants and are located both at the mating-type locus and at several other loci unlinked to the mating-type locus (MACKAY and MANNEY 1974a,b; MANNEY and WOODS 1976; J. RINE, unpublished observations; L. HARTWELL, personal communication). MACKAY and MANNEY (1974b) have proposed that the mating-type loci control expression of the unlinked genes necessary for mating (and for sporulation). Mutations in the *MATα* locus define at least two complementation groups necessary for mating, *MATα*1 and *MATα*2 (HICKS 1975; STRATHERN 1977; STRATHERN, HICKS and HERSKOWITZ, in preparation). The mating-type loci also code for functions neces-

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sary for sporulation in *MAT***a**/*MAT*α cells; the sporulation requirements appear to include the $\alpha 2$ function of *MAT*α (MACKAY and MANNEY 1974a) and the **a**1 function of *MAT***a** (KASSIR and SIMCHEN 1976).

Studies of mating-type interconversion have led to the proposal that yeast cells have unexpressed copies of *MAT***a** and *MAT*α information at the *HM*α and *HM***a** loci, respectively (HICKS, STRATHERN and HERSKOWITZ 1977b) (Figure 1). Cells of one mating type switch to the other mating type, either at low frequency in strains with the *ho* allele (heterothallic strains) or at high frequency in strains with the *HO* allele (homothallic strains) (HAWTHORNE 1963a,b; HARASHIMA, NOGI and OSHIMA 1974; HICKS and HERSKOWITZ 1976). The *HM***a** and *HM*α loci have been identified by their requirement for switching from *MAT***a** to *MAT*α and from *MAT*α to *MAT***a**, respectively, in strains carrying the *HO* gene (NAUMOV and TOLSTORUKOV 1973; HARASHIMA, NOGI and OSHIMA 1974). Based on these results, HARASHIMA, NOGI and OSHIMA (1974) proposed that *HM***a** and *HM*α are controlling elements analogous to those of maize, and that association of the *HM***a** element or *HM*α element with the mating-type locus leads to an α or **a** strain, respectively (see also OSHIMA and TAKANO 1971). Based on the finding that mutations in the α and **a** mating-type loci are efficiently "healed" by the interconversion process (HICKS and HERSKOWITZ 1977; D. HAWTHORNE, personal communication; STRATHERN 1977; STRATHERN, BLAIR and HERSKOWITZ 1979; KLAR, FOGEL and RADIN 1979), it has been proposed that mating-type interconversion occurs by transposition of a copy of *HM*α *or HM***a** (a *MAT***a** or *MAT*α "cassette") into a site at the mating-type locus (HICKS, STHATHERN and HERSKOWITZ 1977b).

According to this hypothesis, a strain of genotype *ho HM***a** *MAT*α *HM*α is equivalent to *ho* [*MAT*α] *MAT*α [*MAT***a**]. Three lines of evidence indicate that the additional *MAT* loci must be unexpressed: (1) an *ho HM***a** *MAT*α *HM*α strain has the phenotype of an α cell and not of an **a**/α diploid; (2) mating type is determined by a single Mendelian gene that maps at the mating-type locus; (3) recessive mutations have been isolated in both *MAT***a** and *MAT*α. We have thus proposed that the position of a *MAT* cassette determines whether or not it is expressed. For example, the cassette at the mating-type locus might be adjacent to an active promoter, whereas the cassettes at *HM***a** and *HM*α are not.

Circumstantial evidence supporting the hypothesis that *HM***a** and *HM*α are silent *MAT* cassettes comes from two kinds of chromosomal rearrangements. Heterothallic strains can switch from α to **a** by a deletion on the right arm of chromosome *III* (HAWTHORNE 1963a), which may result in fusion of the *HM*α locus to the active controlling site at the mating-type locus (HICKS, STRATHERN and HERSKOWITZ 1977b; STRATHERN 1977). Another rearrangement, associated with switches from **a** to α, has been interpreted as a fusion of the *HM***a** locus to the mating-type-locus controlling site (STRATHERN 1977).

In this paper we describe the isolation and characterization of the mutation *sir1–1* (formerly called *ssp515* [HICKS 1975; STRATHERN 1977; HERSKOWITZ *et al.* 1977]), which suppresses the defects of all mutations tested in *MAT*α and *MAT***a**. The ability of *sir1–1* to provide all functions associated with *MAT*α and

*MAT***a** leads to the proposal that *sir1–1* allows expresseion of cryptic copies of *MAT*α and *MAT***a** located in the yeast genome. We report, further, that the ability of *sir1–1* to suppress the mating defect of strains with a mutation in *MAT*α is dependent on the *HM***a** locus. *HM***a**, identified by its role in mating-type interconversion as required for switching from **a** to α, is thus shown to be functionally equivalent to the α mating-type locus. These studies provide independent support for the hypothesis that *HM***a** is a silent *MAT*α locus and, in addition, provide information on the mechanism by which the silent *MAT* loci are kept silent.

MATERIALS AND METHODS

Media: YEPD (complete medium), SD (minimal medium), and supplements have been described previously (HICKS and HERSKOWITZ 1976).

Strains: Strains are described in Table 1. Standard laboratory strains contain the *HM***a** and *HM*α alleles. The genotypes of *ho hm***a** or *hm*α strains were confirmed by appropriate backcrosses (see HARASHIMA, NOGI and OSHIMA 1974).

Construction of strains: Crosses are described in Table 2. A *MAT***a**/*mat*α*1–5 SIR1/sir1–1* strain homozygous for *hm***a** (XR213–20) was constructed by UV-induced mitotic recombination from XR213, which has the genotype *HM***a** *HIS4 LEU2 mat*α*1–5/hm***a** *his4 leu2 MAT***a** *sir1–1/SIR1* (see Figure 1). XR213 was lightly UV irradiated and screened for colonies that became *His*$^-$*Leu*$^-$. Assuming that the frequency of double mitotic recombinants is low, the strain obtained (XR213–20) is expected to be homozygous for all markers distal to *leu2*, thus homozygous for *hm***a**, which is on the left end of chromosome *III* (HARASHIMA and OSHIMA 1976). Upon sporulation, XR213–20 yielded 2 **a** : 2 nonmating segregation in all 79 tetrads. All four segregants from a single tetrad of XR213–20 were shown to be *hm***a** by backcrosses to homothallic strains (data not shown). To determine whether XR213–20 is still heterozygous for *sir1–1*, segregants were mated (by prototroph selection) to *mat***a***–1 sir1–1* (strain XJ116–6a). Half of these diploids sporulated, and half did not, indicating that XR213–20 is heterozygous for *sir1–1* (see RESULTS).

In the course of this work, it has often been necessary to sporulate diploids that are normally incapable of sporulating. In order to induce such strains to sporulate, we have used the *rme* (KASSIR and SIMCHEN 1976) and *csp1* (HOPPER and HALL 1975a) mutations, which allow a low level of sporulation (typically 1 to 5% that of an **a**/α strain) in *MAT***a**/*MAT***a**, *MAT*α/*MAT*α and *mat***a***–1*/*MAT*α strains. We have found that some common laboratory strains contain *rme* (*e.g.*, XT1172–S245c). The relationship between *rme* and *csp1* is unclear, though they may be allelic. *rme* was identified in α strains by determining whether the α strain, when crossed to *mat***a***–1* (17–15), formed a diploid capable of sporulating. The ability to sporulate defined the α parent as being *rme*. *csp1* in α strains was identified by selecting rare matings between an α strain and an α *csp1* strain from HOPPER and HALL (1975a) or XT1172–S245c. The ability to sporulate and produce asci containing spores all with α mating type defined the *csp1* genotype. The ability of both α *rme* and α *csp1* strains to sporulate when crossed to XT1172–S245c suggests either that *csp1* and *rme* are allelic or that XT1172–S245c contains mutations in both genes. The presence of *rme* and *csp1* in **a** strains was determined by testing the α segregants from an outcross of the **a** strain in question to an α *RME CSP1* strain. The *rme-csp1* phenotype was designated *csp1* if the mutation came from strains described in HOPPER and HALL (1975a) or *rme* if the mutation came from strains described in KASSIR and SIMCHEN (1976).

Isolation of sir1–1: *sir1–1* was isolated in a screen for suppressors of the mating defect of *mat*α*1–5* (originally called *ste1–5*; MACKAY and MANNEY, 1974a). Strain VN33 (*mat*α*1–5*) was grown overnight at 30° on YEPD agar, suspended at 5×10^5 cells per ml, and irradiated with a germicidal UV lamp (600 ergs/mm^2) to approximately 22% survival. Cells were grown on YEPD plates at 30° at a density of 400 to 600 colonies per plate. These colonies were replica-plated onto a lawn of **a** mating-type tester strain (73) on minimal medium, where mating-proficient cells formed colonies due to prototrophic complementation.

TABLE 1

*Strain list**

Strain	Genotype	Source/Reference
XT1172–S245c	*MATα ade6 his6 leu1 met1 trp5–1 gal2 can1 rme*	T. MANNEY/MACKAY and MANNEY (1974a)
VN33	*matα1–5* derivative of XT1172–S245c	T. MANNEY/MACKAY and MANNEY (1974a)
VC2	*matα1–2* derivative of XT1172–S245c	T. MANNEY/MACKAY and MANNEY (1974a)
VP1	*matα2–4* derivative of XT1172–S245c	T. MANNEY/MACKAY and MANNEY (1974a)
VC73	*matα2–1* derivative of XT1172–S245c	T. MANNEY/MACKAY and MANNEY (1974a)
R515	*sir1–1* derivative of VN33	This paper
17–15	*mat***a***–1 ade2 ura3 leu1 can1–11 cyh2–21 rme*	Y. KASSIR/KASSIR and SIMCHEN (1975)
70	*MATα thr3–10*	F. SHERMAN
73	*MAT***a** *ilv3*	F. SHERMAN
227	*MAT***a** *cry1–3 lys1*	This work
247	*MATα cry1–3 his4 leu2–1 ade6 lys2*	This work
349	*MAT***a** *hm***a** *HO HMα his5 ade5 ade1 ura4 met⁻*	This work
406	*MATα* isolated as an α-factor resistant derivative of XMB4–12b	J. MCCULLOUGH
D609–28c	*MAT***a** *arg4–17 his5–2 lys1–1 trp1–1 tyr7–1*	G. FINK
G58–25c	*MATα sir1–1 arg4 can1*	G. SPRAGUE
G58–28d	*MATα sir1–1 arg4 leu1 trp5*	G. SPRAGUE
X10–1b	*MAT***a***/MATα HO/HO HM***a***/HMα HMα/HMα his5/his5 ade5/ade5 ura4/ura4 met4/met4 met13/met13*	HICKS and HERSKOWITZ (1976)
X50–2b	*MATα cry1–3 ade6 leu2 his4 lys2 RME*	This work
X57–1b	*MAT***a** *cry1–3 his4 leu2–1 ade6 lys2*	This work
X77–40c	*MAT***a** *sir1–1*	This work
X77–68d	*matα1–5 cry1–3 sir1–1 leu2–1 ade6 his4 RME*	This work
XHB21–1c	*MATα ade6*	L. BLAIR
XJ24–25d	*MAT***a** *arg4–17 trp1 tyr7*	This work
XJ89–7a	*matα1–5 sir1–1 cry1–3 ade6 tyr7–1 csp1*	This work
XJ89–13a	*matα1–5 cry1–3 sir1–1 leu2–1 ade6 arg4–17 lys2 csp1*	This work
XJ104–25a	*matα2–1 sir1–1 his6 ade6 lys2 arg4–17 csp1*	This work
XJ104–27a	*matα2–1 sir1–1 ade6 arg4–17 csp1*	This work

TABLE 1—Continued

Strain	Genotype	Source/Reference
XJ109-7a	*MAT***a** *HM***a** *hmα cry1-3 ade6 his⁻ leu⁻*	This work
XJ116-6a	*mat***a**-*1 sir1-1 cry1-3 leu⁻ arg4 lys2 tyr7 rme*	This work
XJ116-22c	*mat***a**-*1 sir1-1 ade6 ura3 rme*	This work
XJ116-27b	*mat***a**-*1 sir1-1 ade6 arg4 tyr7 leu⁻ rme*	This work
XMB3-7b	*MAT***a** *ura1 arg9 ilv3*	L. BLAIR
XMB4-12b	*MAT***a** *arg9 ilv3 ura1 sst*	L. BLAIR
XR28-1c	*MAT***a** *cry1-3 his4 leu2-1 arg4-17 lys2*	This work
XR28-9c	*MAT***a** *cry1-3 his4 leu2-1 trp1*	This work
XR29-10c	*MAT***a** *cry1-3 sir1-1 ade6 arg4-17 leu2 RME*	This work
XR29-10d	*MAT***a** *sir1-1 lys2*	This work
XR29-23a	*MAT***a** *sir1-1 ade6 arg4-17 RME*	This work
XR30-23d	*MAT***a** *his4 leu2 lys2 trp1 tyr7*	This work
XR59-11a	*MATα sir1-1 ade6 arg4 lys2 trp⁻ leu⁻*	This work
XR59-11d	*MAT***a** *SIR1 cry1-3 ade6 lys2 arg4 trp⁻ leu⁻ his⁻*	This work
XR101-12d	*MAT***a** *hm***a** *ho ade6 ade1 ura4 met⁻ RME*	This work
XR107-15b	*matα1-5 sir1-1 ade6 ura4 met⁻*	This work
XR107-30c	*matα1-5 HM***a** *cry1-3 sir1-1 ade6 lys2*	This work
XR107-38d	*MAT***a** *hm***a** *ho his4 leu2 ade6 ura4*	This work

* Unless otherwise indicated, all strains have the standard *ho HM***a** *HMα* alleles.

TABLE 2

*Cross list**

Diploids	Parents	Diploids	Parents
X77	R515 × D609–28c	XR107	X77–68d × XR101–12d
XJ104	VC73 × XJ89–13a	XR128E	VP1 × XR29–10c
XJ110	VC73 × XJ89–13a	XR152	XR167–15b × XJ109–7a
XJ111	VP1 × XJ89–13a	XR202	VC2 × XR29–10c
XJ115	XJ104–25a × XR29–10c	XR213	XR107–38d × XR107–30c
XJ116	XJ89–13a × 17–15	XR242	G58–25c × XR29–10c
XR29	X77–68d × XJ24–25d	XR243	G58–28d × XR29–10c
XR57	X77–68d × XR29–23a	XS5F	VP1 × XJ89–7a
XR58	X77–68d × XR29–10c	XS8E	VC2 × XJ89–7a
XR81	XJ116–22C × XR59–11a		

* Another series of crosses is described in Table 10.

Efficiency of mating determinations: Mating-type tester strains, **a** (XMB4–12b) and α (406), were suspended in YEPD at 2×10^8 cells per ml and kept on ice. Known numbers of cells from the strains being tested and 0.15 ml of each tester were spread on minimal agar plates, and 2×10^7 cells of each strain were also spread onto SD without a tester strain to test for reversion of auxotrophic markers: Zero or one auxotroph was observed for all strains. Efficiency of mating was calculated as the number of colonies on the mating-type tester lawns divided by the number of cells plated (assayed on YEPD agar). The mating efficiencies of wild-type **a** and α strains were normalized to 1.0, and the mating efficiency of other strains was expressed as a fraction of that of wild-type strains.

α-factor confrontation assays: These assays were performed as described in HICKS and HERSKOWITZ (1976), with strain 70 as the source of α factor.

Rare matings: Diploid strains were formed between mating-defective and mating-proficient cells by selection for prototrophs. Parents were mixed and either plated directly onto selective medium or first grown on YEPD plates and then replica-plated onto selective medium. Diploid strains were formed from mating-proficient haploid strains by cell-to-cell matings or by isolation of prototrophs from mating mixtures.

Mitotic recombination: In order to isolate diploid strains that had undergone mitotic recombination, colonies on YEPD agar were screened for the desired genotype by replica-plating. In some cases, cells on the YEPD plate were lightly UV irradiated at a low dose (approximately one-fifth the dose used for mutagenesis) before incubation.

Mating-type determination: Mating-type segregation was followed by replica-plating patches of the auxotrophic strain being tested onto SD agar plates previously spread with either **a** or α tester lawns containing complementary auxotrophic mutations. **a** mating type was indicated by the formation of prototrophs after mating with the α lawn, and α mating type by the formation of prototrophs after mating with the **a** lawn.

Summary of symbols used: The nomenclature used in this paper corresponds to previous symbols as indicated (previous symbols in parentheses): *MAT***a**(**a**), *MAT*α(α), *mat*α*1–5* (*ste1–5*), *mat*α*1–2* (*ste1–2*), *mat*α*2–1* (*ste73*), *mat*α*2–4* (*ste1–4*), *sir1–1* (*ssp515*), *mat***a***-1* (**a***) (MACKAY and MANNEY 1974a,b; HERSKOWITZ *et al.* 1977; KASSIR and SIMCHEN 1976).

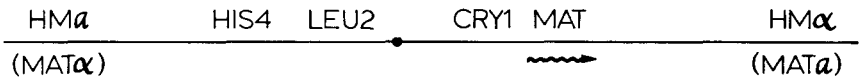

FIGURE 1.—Chromosome *III* of *Saccharomyces cerevisiae* with relevant markers drawn roughly to genetic scale.

RESULTS

From approximately 10,000 colonies of strain VN33 (*matα1–5*), seven colonies were identified as having regained the ability to mate as α (HICKS 1975). In five of these revertants, the determinant responsible for mating ability was unlinked to the mating-type locus. One of these revertants (R515) was studied in detail.

Suppression of matα1–5 *by an unlinked mutation,* sir1–1

The diploid formed between R515 and an **a** strain yielded 11 PD tetrads (2 α : 2 **a**), nine NPD tetrads (2 nm : 2 **a**), and 60 T tetrads (1 α : 1 nm : 2 **a**) (Table 3, Cross 1). Pooling these data with those from an analogous cross involving strain X77–68d (Table 3, Cross 2), a PD:NPD:T ratio of 19:25:95 is observed, which approximates a 1:1:4 ratio. These results indicate that α mating ability is determined by two unlinked loci, the *matα 1–5* mutation and an unlinked suppressor, which we call *sir1–1*.

Since two segregants with **a** mating ability were obtained in all tetrads, *sir1–1* does not affect the scoring of the **a** phenotype. The presence of *sir1–1* in two **a** segregants was confirmed by backcrosses of two **a** segregants from different NPD tetrads to *matα1–5 sir1–1*, which yielded no mating-defective segregants (Table 2, Crosses 3 and 4). More sensitive analysis of **a** sir1–1 strains described below indicates that *sir1–1* does exhibit a phenotype in *MAT***a** cells.

The *sir1–1* mutation restores mating ability of *matα1–5* strains to near normal efficiency (Table 4). *matα1–5/matα1–5* diploids heterozygous for *sir1–1* show low mating efficiency, indicating that *sir1–1* is recessive (Table 4). *sir1–1* not only allows *matα1–5* to mate, but also to produce α factor (as assayed by the confrontation assay; see MATERIALS AND METHODS).

Suppression by sir1–1 *is not allele specific*

Strain VC2 is a mating-defective mutant, isolated independently of VN33 (*matα1–5*), which carries the mutation *matα1–2* (MACKAY and MANNEY 1974a). We examined the ability of *sir1–1* to suppress the mating defect of *matα1–2* in two ways:

(1) As in many crosses described in this work, the mating-type loci have been marked with alleles of the *CRY1* locus, which is approximately 4 cM from the

TABLE 3

Suppression of the mating defect of matα1–5 *by* sir1–1

Cross	Genotype	Tetrad Types* PD	NPD	T
(1) X77	*MAT***a** *SIR1* × *matα1–5 sir1–1*	11	9	60
(2) XR29	*CRY1 MAT***a** *SIR1* × *cry1–3 matα1–5 sir1–1*	8	16	35
	Total (1) + (2)	19	25	95
(3) XR58	*MAT***a** *sir1–1* × *matα1–5 sir1–1*	15	0	0
(4) XR57	*MAT***a** *sir1–1* × *matα1–5 sir1–1*	10	0	0

* PD = 2 α : 2 **a**; NPD = 2 nm : 2 **a**; T = 1 α : 1 nm : 2 **a**.

TABLE 4

*Efficiency of mating determinations for selected strains**

Strain	Genotype	Allele	Efficiency of Mating × **a** Strain	Efficiency of Mating × α Strain
XT1172-S245c	*MATα*	*SIR1*	1	1.2×10^{-4}
VN33	*matα1-5*	*SIR1*	2.1×10^{-4}	5.5×10^{-4}
VC2	*matα1-2*	*SIR1*	3.7×10^{-5}	4.3×10^{-4}
VP1	*matα2-4*	*SIR1*	1.1×10^{-5}	4.1×10^{-4}
VC73	*matα2-1*	*SIR1*	1.2×10^{-5}	3.2×10^{-3}
227	*MAT***a**	*SIR1*	9.5×10^{-6}	1
G58-25a	*MATα*	*sir1-1*	0.16	2.1×10^{-6}
G58-28b	*MATα*	*sir1-1*	0.85	1.2×10^{-4}
XR29-10c	*MAT***a**	*sir1-1*	5.7×10^{-5}	0.63
XR29-10d	*MAT***a**	*sir1-1*	1.2×10^{-6}	0.092
XJ89-13a	*matα1-5*	*sir1-1*	0.4	NT
X77-68d	*matα1-5*	*sir1-1*	0.6	NT
XS10B	*matα1-5/matα1-5*	*sir1-1/SIR1*	1.1×10^{-3}	NT
XR202-3a	*matα1-2*	*sir1-1*	0.96	1.5×10^{-5}
XJ104-25a	*matα2-1*	*sir1-1*	0.1	NT
XR128-3b	*matα2-4*	*sir1-1*	0.28	2.7×10^{-4}
XR128-68b	*matα2-4*	*sir1-1*	0.98	1.0×10^{-4}
17-15	*mat***a***-1*	*SIR1*	2.9×10^{-5}	0.88
XR197-2b	*mat***a***-1*	*sir1-1*	5.5×10^{-3}	0.81
XR197-11b	*mat***a***-1*	*sir1-1*	4.1×10^{-3}	0.78
XR197-3b	*mat***a***-1 hmα*	*sir1-1*	7.6×10^{-2}	1.5×10^{-6}
XR197-3d	*mat***a***-1 hmα*	*sir1-1*	1.34	4.0×10^{-4}

* All mating-efficiency determinations were conducted on the same day except for XJ89-13a, X77-68d and XS10B, which were assayed on a different day with the same *MATα* control, and XJ104-25, which was determined by quantitative cell-to-cell matings. Unless otherwise indicated, all strains contain the standard *ho HM***a** *HMα* alleles.

mating-type locus (SKOGERSON, MCLAUGHLIN and WAKATAMA 1973; GRANT, SANCHEZ and JIMENEZ 1974). In the diploid XR202, *cry1-3 MAT***a**/*CRY1 matα1-2 sir1-1/SIR1*, formed between VC2 and XR29-10c (*cry1-3 MAT***a** *sir1-1*), *MAT***a** is coupled to *cry1-3* (resistance to cryptopleurine, CryR) and *matα1-2* is coupled to *CRY1* (sensitivity to cryptopleurine, CryS). XR202 produced 13 PD tetrads (2 nm : 2**a**), 14 NPD tetrads (2α : 2 **a**), and 51 T tetrads (1 nm : 1 α : 2 **a**), in which the **a** segregants were predominantly CryR, and the nonmating α segregants were predominantly CryS (Table 5, line 1). This segregation pattern indicates that a single mutation unlinked to the mating-type locus, presumably *sir1-1*, suppresses the mating defect of *matα1-2* and does not affect scoring of the **a** phenotype. To determine whether the suppressor is indeed *sir1-1*, or whether it is some new suppressor selected in the mating between VC2 and XR29-10c, we selected diploids from a mating mixture of *mat***a***-1 sir1-1* and individual segregants from XR202 and without the suppressor. The prototrophs formed by segregants containing the suppressor were capable of sporulating, whereas the prototrophs formed by segregants lacking the suppressor were not.

TABLE 5

Suppression of the mating defect of matα1–5, matα2–4, *and* matα2–1 *by* sir1–1

Diploid	Genotype	PD	Tetrad Types* NPD	T
XR202	*CRY1 matα1–2 / cry1–3 MAT***a** *SIR1 / sir1–1*	13	14	51
XS8E	*CRY1 matα1–2 / cry1–3 matα1–5* *SIR1 / sir1–1* *rme / csp1*	1	2	3
XR128E	*CRY1 matα2–4 / cry1–3 MAT***a** *SIR1 / sir1–1*	13	21	52
XS5F	*CRY1 matα2–4 / cry1–3 matα1–5* *SIR1 / sir1–1* *rme / csp1*	2	2	4
XJ111	*CRY1 matα2–4 / cry1–3 matα1–5* *SIR1 / sir1–1* *rme / csp1*	1	5	8
XJ104	*CRY1 matα2–1 / cry1–3 matα1–5* *SIR1 / sir1–1* *rme / csp1*	1	3	5
XJ110	*CRY1 matα2–1 / cry1–3 matα1–5* *SIR1 / sir1–1* *rme / csp1*	4	1	10

* For XR202 and XR128E, PD = 2 nm : 2 **a**; NPD = 2 α : 2 **a**; T = 1 α : 1 nm : 2 **a**. For other crosses, PD = 2 CryR α : 2 CryS nm; NPD = 2 CryR nm : 2 CryS α; T = 1 CryR α : 1 CryS α : 1 CryR nm : 1 CryS nm.

Since diploids containing *matα-1* sporulate efficiently only when homozygous for *sir1–1* (discussed below), the suppressor in XR202 is indeed *sir1–1*.

(2) A diploid XS8E was isolated between *cry1–3 matα1–5 sir1–1* (XJ89–7a) and *CRY1 matα1–2 SIR1* (VC2) by prototroph selection and was able to sporulate at a low level due to the presence of *csp1* and *rme* mutations in these strains (see MATERIALS AND METHODS). Two α and two nonmating segregants were observed in the six tetrads analyzed (Table 5, line 2). We presume that XS8E is *matα1–5/matα1–2* and not *matα1–5/matα1–5*, because this strain is heterozygous for *cry*. Of the CryR segregants (which should be *matα1–5*), five mated as α and are presumed to carry *sir1–1*, whereas seven were nonmaters. More importantly, seven of the 12 CryS (*matα1–2*) segregants mated as α, whereas five did not. The CryS segregants with α mating ability are presumed to be *matα1–2 sir1–1*.

In summary, α mating segregants of the genotype *matα1–2 sir1–1* can be recovered at high frequency from two different diploids heterozygous for both *matα1–2* and *sir1–1*.

sir1–1 *suppresses the mating defect of the* matα2–4 *mutation*

The α mating type locus contains at least two complementation groups, *MATα1* and *MATα2*, as indicated by complementation of *matα1–5* and *matα2–1*

(STRATHERN 1977; STRATHERN, HICKS and HERSKOWITZ, in preparation). Strain VPI has a mutation of the α mating-type locus (originally called *ste1–4*; MACKAY and MANNEY 1974), which complements *matα1–5*, but not *matα2–1*, for mating (STRATHERN 1977; RINE, unpublished observations). Because of its inability to complement *matα2–1*, we have tentatively assigned *ste1–4* to the *MATα2* complementation group and denote it as *matα2–4*.

In order to determine if *sir1–1* is able to suppress *matα2–4*, five independent diploid strains were formed between *MAT***a** *sir1–1* and VPI by prototroph selection. Four of the diploid strains yielded 2 **a** : 2 α in all tetrads. These diploids appear to be due to matings between the **a** strain and *MATα2* revertants of VPI and were not studied further. Analysis of the fifth diploid (XR128E) demonstrated the segregation of an unlinked suppressor of *matα2–4* (Table 5, line 3). To determine whether the suppressor in this cross is *sir1–1*, rather than a newly selected suppressor, *matα2–4* segregants carrying the suppressor were mated to *sir1–1 mat***a***-1* strains. Since *mat***a***-1/MATα* strains sporulate efficiently if homozygous for *sir1–1*, but not if *sir1–1/SIR1*, the efficient sporulation observed in each case indicates that these segregants contain *sir1–1*. The efficient sporulation seen in these diploids is not due to *rme*, since approximately half of the α segregants were *RME*.

An independent demonstration that *sir1–1* suppresses *matα2–4* comes from analysis of rare diploids selected between *cry¹–3 matα1–5 sir1–1* and *CRY1 matα2–4* that were able to sporulate due to the *csp1* mutation (XS5F and XJ111; Table 5, lines 4 and 5). As before, the mating-type locus of each parent is distinguishable by its coupling to different *CRY1* alleles. All 22 tetrads from XS5F and XJ111 exhibited 2 α : 2 nm segregation, in which the α mating phenotype is not correlated with either CryR or CryS. We conclude that both mating-type loci in these diploids are defective and that both are suppressible by *sir1–1*.

sir1–1 *suppresses both the mating and sporulation defects of* matα2–1

In the preceding sections, we have described the ability of *sir1–1* to suppress the mating defect associated with mutations of *MATα*. In this section, we describe the ability of *sir1–1* to suppress other defects caused by mutations of *MATα*, in particular, by the mutation *matα2–1*, which leads to both defective mating and inability to promote sporulation in *MAT***a***/matα2–1* diploids (MACKAY and MANNEY 1974a; STRATHERN 1977) (see Table 6).

Suppression of mating defect: To determine whether *sir1–1* can suppress the mating defect due to the *matα2–1* mutation, diploids were selected between *CRY1 matα2–1* (VC73) and *cry1–3 matα1–5 sir1–1* (XJ89–13a). Two independent diploids (XJ104 and XJ110) were formed, each of which sporulated by virtue of *csp1/rme* and segregated 2 α : 2 nm per tetrad. Once again, the segregation of mating ability and sensitivity to cryptopleurine in the 24 tetrads from XJ104 and XJ110 demonstrates that both mating-type loci are suppressible by *sir1–1* (Table 5, lines 6 and 7). (We note that both diploids have α mating phenotypes, demonstrating that both *matα1–5* and *matα2–1* are recessive and that these mutations complement.)

TABLE 6

Regulation of sporulation by the mating-type locus

Diploid genotype	Sporulation ability
*MAT***a**/*MATα*	++++
*MAT***a**/*matα1–5*	++++
*MAT***a**/*matα2–4**	++
*MAT***a**/*matα2–1*	—
*mat***a**-*1*/*MATα*	—

* Reduced sporulation proficiency of *matα2–4* strains was noted by GEORGE SPRAGUE, JR. (personal communication).

In order to confirm that *matα2–1* was still segregating in XJ104 and XJ110, α mating-proficient segregants were tested for their ability to support sporulation when mated to an **a** strain (227). None of nine CryS segregants with α mating ability from XJ104 formed sporulating diploids, whereas six of eight CryR segregants with α mating ability did form sporulating diploids. Similar results were obtained with XJ110: one of 17 CryS segregants and ten of 17 CryR segregants with α phenotype formed sporulating diploids. These results indicate that diploids XJ104 and XJ110 are indeed *CRY1 matα2–1/cry1–3 matα1–5*.

Suppression of sporulation defect: As noted above, *sir1–1* is recessive to *SIR1* (Table 4). In order to determine the ability of *sir1–1* to suppress the sporulation defect of *matα2–1*, it was thus necessary to construct a diploid homozygous for *sir1–1*. *MAT***a**/*matα2–1 sir1–1/sir1–1* diploid XJ115 was constructed by cell-to-cell mating between *matα2–1 sir1–1* (XJ104–25a) and *MAT***a** *sir1–1* (XR29–10c) and sporulated efficiently (greater than 50% asci), producing spores with high viability. The sporulation proficiency of XJ115 is not due to *rme*- or *csp1*-mediated sporulation, since neither *rme* nor *csp1* is homozygous in this diploid. As expected, 2 **a** : 2 α segregation was observed in all nine tetrads. No segregant with α mating ability promoted sporulation when mated with **a** *SIR1* strain XMB3–7b, which is fully capable of supporting sporulation when mated with *MATα SIR1* and *matα1–5 sir1–1* strains (247 and XJ89–13a). These results indicate that (1) the *matα2–1* mutation is present in the segregants with α mating ability, (2) the *sir1–1* mutation is able to suppress the sporulation defect of *matα2–1*, and (3) this action is recessive. In summary, *MAT***a**/*matα2–1 sir1–1/sir1–1* sporulates efficiently, whereas *MAT***a**/*matα2–1 sir1–1/SIR1* and *MAT***a**/*matα2–1 SIR1/SIR1* do not.

Action of sir1–1 *in vegetative* **a** *cells*

Although *sir1–1* does not have an obvious effect on the mating efficiency of **a** cells when assayed by a "patch test" for mating type (see MATERIALS AND METHODS), more sensitive assays show that *MAT***a** *sir1–1* cells differ significantly from *MAT***a** *SIR1* cells.

Response to α-factor: **a** cells respond to α factor by arresting in the G1 phase of the cell cycle and undergoing a characteristic morphological alteration to form

a pear-shaped or elongated cell (DUNTZE, MACKAY and MANNEY 1970; BÜCKING-THROM *et al* 1973). To determine the response of *MAT***a** *sir1–1* cells to α factor, single unbudded cells of both *MAT***a** *sir1–1* and *MAT***a** *SIR1* (XR29–10c and 227) were placed in front of a streak of α cells (strain 70) on YEPD dissection agar and observed over an 18-hour period. All 20 *MAT***a** *SIR1* cells exhibited the characteristic response to α factor. Of the 64 *MAT***a** *sir1–1* cells, 13 responded to α factor, 44 continued to bud, and six failed to divide and were apparently dead. The *MAT***a** *sir1–1* cells that responded were removed from the presence of α factor, allowed to undergo several rounds of cell division, and then these cells were again exposed to α factor. Twelve responded, 47 continued to bud, and two failed to divide. Based on several similar experiments with different *MAT***a** *sir1–1* strains, we find that approximately 20% or fewer *MAT***a** *sir1–1* cells respond to α factor under conditions in which 90 to 100% of the *MAT***a** *SIR1* cells respond. We conclude that *sir1–1* interferes with the ability of **a** cells to respond to α factor.

Budding pattern: Haploid cells, as well as **a**/**a** and α/α diploids, have a medial budding pattern; **a**/α diploids have a polar budding pattern (CRANDALL, EGEL and MACKAY 1977; HICKS, STRATHERN and HERKSOWITZ 1977a). In the confrontation assay described in the previous paragraph, five of the 44 *MAT***a** *sir1–1* cells that did not respond to α factor exhibited a medial budding pattern; the other 39 exhibited a polar budding pattern. Of the *MAT***a** *sir1–1* cells reintroduced to the presence of α factor, seven had a medial budding pattern and 40 had a polar budding pattern. The predominantly polar budding pattern of *MAT***a** *sir1–1* cells is also observed in the absence of α factor. *MAT*α *sir1–1* cells exhibit a medial budding pattern. The *sir1–1* mutation, therefore, affects the budding pattern of **a** cells such that a fraction of *MAT***a** *sir1–1* cells exhibit the budding pattern of **a**/α diploids.

Efficiency of mating: In order to determine more accurately whether *sir1–1* affects the mating proficiency of **a** cells, quantitative efficiency of mating determinations were performed (see MATERIALS AND METHODS). For one *MAT***a** *sir1–1* strain that was used above, the mating efficiency is slightly reduced, 60% of wild-type efficiency; for another *MAT***a** *sir1–1* strain, the efficiency is reduced to 9% (Table 4).

In summary, *MAT***a** *sir1–1* cells differ strikingly from *MAT*a *SIR1* cells in at least two respects, showing reduced response to α factor and exhibiting a polar budding pattern. In both cases, a large fraction of the cells in a *MAT***a** *sir1–1* culture have the phenotype of an **a**/α cell.

sir1–1 *allows* **a**/**a** *diploids to sporulate*

The observations that *sir1–1* suppresses both the mating and sporulation defects of *matα*⁻ mutations and makes **a** cells exhibit phenotypes similar to those of **a**/α cells can be explained by proposing that *sir1–1* allows expression of a cryptic copy of the *MAT*α locus. If this is true, then **a**/**a** diploids homozygous for *sir1–1* should be able to sporulate efficiently. In order to construct such a diploid, **a**/**a** mitotic recombinants were derived from *MAT***a**/*matα1–5* *sir1–1*/

sir1–1 RME/RME diploid (XR57) after UV irradiation (see MATERIALS AND METHODS). XR57 upon sporulation yielded 2**a** : 2 α in each of ten tetrads, confirming that XR57 is indeed homozygous for *sir1–1*. Lightly irradiated cells of XR57 from three independent clones, A, B and C, were incubated at 30° and colonies tested for mating proficiency (approximately 1500 colonies in each case). Culture A yielded no colonies with **a** or α mating ability. Culture B had one colony (B16) with a sectored mating phenotype, half mating as **a** and half as α. Culture C had two colonies that exhibited mating, one (C12) mating exclusively like an **a** and another (C8) like B16. Single colonies purified from opposite sides of the B16 colony had the mating type of **a** cells or α cells. Although originally a sectored colony, single colonies purified from C8 yielded colonies only with **a** mating type. C12 was not studied further.

Consistent with our predictions, all colonies with **a** mating type from B16 and C8 sporulated efficiently (more than 90% of the cells formed asci, Table 7), and each produced four **a** segregants per tetrad in a total of 30 tetrads. Both *leu2* and *cry1*, heterozygous in the parent diploid XR57, segregated 2:2 in all tetrads, indicating that the mitotic recombination event that formed the **a**/**a** diploids occurred between the *cry1* locus and the mating-type locus. (Given the relative distances between the centromere and *cry1* and between *cry1* and the mating-type locus, the apparent position of the mitotic recombination events in B16 and C8 is somewhat surprising.)

All of the colonies derived from B16 with the mating phenotype of α cells also sporulated very efficiently. In nine complete tetrads and ten tetrads with three viable spores, all segregants had the α mating phenotype (Table 7). As before, *cry1* and *leu2* segregated 2:2. In order to determine whether these α mating diploids capable of sporulation were in fact *matα1/5 matα1/5 sir1–1/sir1–1*, all four segregants from a single tetrad were backcrossed to a *MAT***a** *SIR1* strain (XR28–9c or XR30–23d). These diploids yielded both mating (*matα1–5 sir1–1*) and nonmating (*matα1–5 SIR1*) segregants, thereby confirming the proposed genotype of the B16 α mating clone (data not shown).

Additional *MAT***a**/*MAT***a** diploids homozygous for *sir1–1* have been obtained from two *CRY1 MATα/cry1–3 MAT***a** *sir1–1/sir1–1* diploids (XR242 and XR243) by selecting for CryR mitotic recombinants with **a** mating type (XR242–1 and XR243–1). These diploids sporulated efficiently and yielded only

TABLE 7

Sporulation of MAT**a**/MAT**a** *and* matα1–5/matα1–5 *strains homozygous for* sir1–1

Clone	Mating type	Tetrad types	
		4 **a** : 0 α	4 α : 0 **a**
XR57-B16	**a**	13	0
XR57-C8	**a**	17	0
XR57–B16	α	0	9
XR242–1 CryR	**a**	20	0
XR243–1 CryR	**a**	13	0

4 **a** : 0 α tetrads in 20 tetrads from XR242–1 and in 13 tetrads from XR242–1 (Table 7).

The behavior of XR58-B16 indicates that a *mat*α*1–5/mat*α*1–5 sir1–1/sir1–1* diploid is able to sporulate. We also wished to determine whether or not *MAT*α*/MAT*α *sir1–1/sir1–1* strains are able to sporulate. Colonies grown from two independent *cry1–3 MAT***a***/CRY1 MAT*α *sir1–1/sir1–1* diploid clones (XR242), which were not UV irradiated, were screened for those with α mating ability. One colony with α mating ability was tested from each clone, and both sporulated efficiently. Surprisingly, germination was very poor: after two days, no viable spore clones were visible; after six days, 12 spore clones of very heterogeneous size were observed. All of these were of α mating type. We do not yet know whether the poor spore viability is a reflection of a property of *sir1–1* in *MAT*α*/MAT*α diploids, or whether it is a strain-specific peculiarity unrelated to the presence of *sir1–1*. Nevertheless, the efficient sporulation of two independent α mating derivatives from XR242 suggests that *sir1–1* allows strains homozygous (or possibly hemizygous) for *MAT*α to sporulate.

The experiments described in this section demonstrate that *sir1–1* in **a**/**a** diploids provides all functions associated with *MAT*α that are necessary for sporulation. *sir1–1* also appears to obviate the requirement for *MAT***a** in sporulation since *sir1–1/sir1–1* strains homozygous for *mat*α*1–5* (and presumably for *MAT*α) sporulate efficiently.

sir1–1 *suppresses a mutation of* MAT**a** (mat**a**-1)

Since *sir1–1* provides functions associated with *MAT***a** in allowing sporulation of *mat*α*1–5/mat*α*1–5* diploids, we have asked whether *sir1–1* was also able to suppress mutations in the **a** mating-type locus. The *mat***a***-1* mutation (KASSIR and SIMCHEN 1976) inactivates a function of the **a** mating-type locus, **a***1*, which is necessary to promote sporulation in **a**/α diploids (*mat***a***-1/MAT*α strains do not sporulate). *mat***a***-1* has little if any effect on mating. In order to determine whether *sir1–1* allows sporulation of a *mat***a***-1/MAT*α diploid, it was necessary to construct *mat***a***-1/MAT*α homozygous for *sir1–1*, since *sir1–1* is recessive in its other phenotypes and *mat***a***-1/MAT*α *sir1–1/SIR1* does not sporulate. A *mat***a***-1 sir1–1* recombinant was constructed by a cross between *mat*α*1–5 sir1–1* (XJ89–13a) and *mat***a***-1* (17–15) (XJ116). This diploid was sporulated *via rme*-promoted sporulation and yielded segregants of the following types (Table 8): those deficient in mating (*mat*α*1–5 SIR1*), those with α phenotype (*mat*α*1–5 sir1–1*), those with **a** mating phenotype (*mat***a***-1 SIR1*), and those with a novel bimating phenotype (mating with both **a** and α tester cells as described in MATERIALS AND METHODS). The pattern of segregation indicates that these cells are genotypically *mat***a***-1 sir1–1*.

Several *mat***a***-1 sir1–1* segregants were mated to α cells (X50–2d) to produce *mat***a***-1/MAT*α *sir1–1/SIR1* diploids, which had an α phenotype and did not sporulate. Similarly, *mat***a***-1 sir1–1* segregants mated to *MAT***a***SIR1* cells (XR59–11d) produced diploids with an **a** phenotype, which did not sporulate. In contrast, *mat***a***-1/MAT*α *sir1–1/sir1–1* diploids (XR81), which exhibited an α

TABLE 8

Construction of mata-1 sir1–1 *from* $\frac{\text{cry1–3 mat}\alpha\text{1–5}}{\text{CRY1 mata–1}}$ $\frac{\text{sir1–1}}{\text{SIR1}}$

Tetrad type	Number observed	Mate with **a**	Mate with α	*cry*	Inferred genotype
I	5	+	—	*cry1–3*	*matα1–5 sir1–1*
		+	—	*cry1–3*	*matα1–5 sir1–1*
		—	+	*CRY1*	*mat**a**-1 SIR1*
		—	+	*CRY1*	*mat**a**-1 SIR1*
II	6	+	—	*cry1–3*	*matα1–5 sir1–1*
		—	—	*cry1–3*	*matα1–5 SIR1*
		+	+	*CRY1*	*mat**a**-1 sir1–1*
		—	+	*CRY1*	*mat**a**-1 SIR1*
III	2	—	—	*cry1–3*	*matα1–5 SIR1*
		—	—	*cry1–3*	*matα1–5 SIR1*
		+	+	*CRY1*	*mat**a**-1 sir1–1*
		+	+	*CRY1*	*mat**a**-1 sir1–1*
IV	1	+	—	*cry1–3*	*matα1–5 sir1–1*
		—	—	*CRY1*	*matα1–5 SIR1*
		+	+	*cry1–3*	*mat**a**-1 sir1–1*
		—	+	*CRY1*	*mat**a**-1 SIR1*
V	1	—	—	*cry1–3*	*matα1–5 SIR1*
		—	—	*cry1–3*	*matα1–5 SIR1*
		+	—	*CRY1*	*matα1–5 sir1–1*
		—	+	*CRY1*	*mat**a**-1 SIR1*

mating phenotype, sporulated efficiently. We thus conclude that *sir1–1* suppresses the sporulation defect of a mutation in the **a** mating-type locus and that its ability to do so is recessive to *SIR1*.

Suppression of matα1–5 *by* sir1–1 *requires* HM**a**.

As noted earlier, the ability of *sir1–1* to suppress the mating and sporulation defects of *matα* mutations and to allow efficient sporulation of *MAT**a**/MAT**a*** strains can be explained by proposing that *sir1–1* leads to the expression of a copy or copies of *MATα* information that are ordinarily not expressed. HICKS, STRATHERN and HERSKOWITZ (1977b) have proposed on other grounds that the *HM**a*** locus is a silent *MATα* locus. We have thus determined whether action of *sir1–1*, in particular its ability to suppress the mating defect of *matα1–5*, is dependent on *HM**a*** (and *HMα*).

In order to examine the role of *HM**a*** and *HMα* alleles in suppression of *matα1–5* by *sir1–1*, we have monitored the production of segregants with α mating ability from *MAT**a**/matα1–5 sir1–1/SIR1* diploids with various combinations of alleles at *HM**a*** and *HMα* (Table 9).

(1) HM**a**/HM**a** HMα/HMα: In diploids homozygous for *HM**a*** and *HMα* (X77 and XR29), segregation of α maters is dependent on only two genes,

TABLE 9

Influence of HM**a** *and* HMα *on suppression of* matα1–5 *by* sir1–1

Diploid	Genotype	Tetrad types		
		2 α : 2 **a**	2 nm : 2 **a**	1 α : 1 nm : 2 **a**
X77* and XR29*	*HM**a** cry1–3 matα1–5 / HM**a** CRY1 MAT**a*** ; *sir1–1 / SIR1*	19	25	95
XR107	*HM**a** cry1–3 matα1–5 / hm**a** CRY1 MAT**a*** ; *sir1–1 / SIR1*	3	35	37
XR213	*HMα + + cry1–3 matα1–5 / hm**a** his4 leu2 CRY1 MAT**a*** ; *sir1–1 / SIR1*	0	26	17
XR213-20	*hm**a** his4 leu2 cry1–3 matα1–5 / hm**a** his4 leu2 CRY1 MAT**a*** ; *sir1–1 / SIR1*	0	79	0
XR152	*HM**a** CRY1 matα1–5 HMα / HM**a** cry1–3 MAT**a** hmα* ; *sir1–1 / SIR1*	7	7	21

* Data repeated from Table 3.

matα1–5 and *sir1–1*, as noted earlier; a PD:NPD:T ratio of 19:25:95 is observed; and 24% of the segregants have α mating ability.

(2) hm**a**/hm**a** HMα/HMα: Upon sporulation of diploid XR213–20 (whose construction is described in MATERIALS AND METHODS), *MAT**a**/matα1–5 sir1–1/ SIR1/ hm**a**/hm**a** HMα/HMα*, no α mating segregants were observed in 79 tetrads. We presume that the failure to produce α mating segregants is due to homozygosity of *hm**a***. The possibility that the loss of α mating segregants is due to homozygosity of some other marker on the left arm of chromosme *III* cannot be rigorously excluded by this experiment. Analysis of *HM**a**/hm**a*** diploids (below) confirms that *HM**a*** is required for *sir1–1* suppression of *matα1–5*.

(3) HM**a**/hm**a**: Sporulation of *MAT**a**/matα1–5 sir1–1/SIR1 HM**a**/hm**a** HMα/HMα* diploids (XR107 and XR213) yields a PD:NPD:T ratio of 3:61:54; only one-eighth of the segregants have α mating ability. Since the presence of *hm**a*** in these crosses greatly reduces the number of α segregants, *sir1–1* suppression of *matα1–5* apparently requires *HM**a***. We have analyzed segregants with α mating ability further to determine whether they indeed carry both *sir1–1* and *HM**a***.

Presence of sir1–1: The presence of *sir1–1* was determined by mating the α segregants from XR107 with *mat**a**-1 sir1–1* (XJ116–27b) strains and assaying the ability of these diploids to sporulate. Thirty-eight of 40 α segregants formed diploids capable of sporulation, indicating that they carry *sir1–1*. The inability of the other two segregants tested to form sporulating diploids is not understood, but may be due to defects in sporulation *per se*.

Presence of HM**a**: To determine whether the α mating segregants from XR107 and XR213 are *HM**a*** or *hm**a***, we crossed 12 α segregants from XR107 and XR213

to a strain of the genotype *hm***a** *MAT***a** *HM*α *HO*. If an α mater is *hm***a**, then the resulting diploid, being homozygous for *hm***a**, will not produce any *MAT***a** spores capable of switching mating type and thus diploidizing. Each tetrad will have at least two **a** maters since *MAT***a** *hm***a** cells have **a** mating type regardless of whether they are *HO* or *ho*. (More than two **a** mating colonies would be produced in tetrads with an *HO hm***a** *mat*α*1–5 SIR1* spore, which would switch to *MAT***a** and appear to be homogeneous **a** colony.) If, however, an α mater is *HM***a**, then the resulting diploid will produce *MAT***a** spores capable of diploidizing and will have tetrads with fewer than two **a** maters. Two observations indicate that the diploids are heterozygous for *HM***a**. (1) These diploids produce CryS spores, most of which should be *MAT***a** because of its coupling to *CRY1*, which are able to switch mating types to form nonmating, sporulation-proficient cells (Table 10). For a diploid heterozygous for *HO*, *SIR1*, *MAT* and *HM***a**, the number of CryS segregants able to diploidize is expected to be approximately the same as the number of CryR segregants able to diploidize, and the fraction of diploidizing CryR or CryS segregants is expected to be approximately one-eighth of the total segregants. Both expectations are observed. (2) The twelve diploids each gave rise to a large fraction of tetrads with less than two **a** mating segregants (Table 10). The frequency of such tetrads observed (39%) is comparable to that expected if HO, *HM***a**, *MAT* and *SIR1* segregate independently of each other.

The heterozygosity of *HM***a** in the diploids formed between segregants from XR107 and XR213 with *HO hm***a** *MAT***a** *HM*α shows that the α mating segregants from XR107 and XR213 are all *HM***a** and thus confirms that *HM***a** is required for suppression of *mat*α*1–5* by *sir1–1*.

TABLE 10

Analysis of α mating segregants from XR107 and XR213

Diploid*	Segregants able to diploidize†		Tetrads with < 2a maters
	CryS	CryR	tetrads scored
XR220	10	10	7/19
XR222	‡	‡	11/27
XR223	14	15	9/23
XR225	5	3	8/19
XR226	15	16	10/30
XR244	10	8	9/23
XR245	8	4	8/18
XR246	11	18	12/29
XR247	14	11	11/25
XR248	12	17	9/28
XR249	10	12	10/27
XR250	13	11	11/23
Total:	122	125	115/291

* Diploids XR220–XR226 are crosses between α segregants from XR107 and strain 349. Diploids XR244–XR250 are crosses between α segregants from XR213 and strain 349.

† Includes data from tetrads with less than four viable spores.

‡ Diploid XR222 is homozygous *CRY1*.

(4) HMα/hmα: To determine whether *sir1–1* requires both *HM***a** and *HM*α to suppress *mat*α*1–5*, or whether *HM***a** is sufficient, we have analyzed a strain heterozygous for *hm*α. A diploid of genotype *HM***a**/*HM***a** *cry1–3 mat*α*1–5*/*CRY1 MAT***a** *hm*α/*HM*α *sir1–1*/*SIR1* (XR152) yielded a PD:NPD:T ratio of 7:7:21, with 25% of the segregants having α mating type. These results indicate that *hm*α has no effect on the pattern of suppression of *mat*α*1–5* by *sir1–1* and that *HM*α is not necessary for suppression.

DISCUSSION

Studies of physiological suppressor mutations have led to both the identification of functional interactions and the uncovering of cryptic genes. For example, bacterial mutants (*hfl*$^-$) have been identified in which the phage lambda positive regulator *cIII* is no longer required for efficient lysogenization (BELFORT and WULFF 1971). These studies suggest that *cIII* promotes lysogenization by inhibiting Hfl protein, which is itself an inhibitor of lysogenization (GAUTSCH and WULFF 1974). A suppressor that appears to lead to expression of cryptic genes is the *sbcA* mutation of *E. coli* (BARBOUR *et al.* 1970). In this case, *RecB*$^+$ pseudorevertants appear to have activated ordinarily silent genes of a defective prophage that are able to compensate for the *recB*$^-$ defect (GOTTESMAN *et al.* 1974). Our studies of the suppressor mutation *sir1–1* indicate that it is more analogous to suppressors of the second kind. We believe that suppression by the *sir1–1* mutation occurs by allowing expression of genes that are silent in heterothallic yeast strains under ordinary physiological conditions. These studies indicate that suppression by *sir1–1* acts *via* the *HM***a** (and *HM*α) genes previously identified by virtue of their action in mating-type interconversion in homothallic strains. We discuss below the properties of *sir1–1* and the implications of these findings for mating-type interconversion.

Phenotypic consequences of sir1–1

Locus-specific, allele-nonspecific suppression by sir1–1*: sir1–1*, isolated as a suppressor of the mating defect due to the *mat*α*1–5* mutation, is able to suppress all mutations tested in *MAT*α (*mat*α*1–5*, *mat*α*1–2*, *mat*α*2–4*, and *mat*α*2–1*); it thus suppresses mutations in both of the two unknown complementation groups of *MAT*α. More specifically, *sir1–1* suppresses the mating deficiency, the α-factor deficiency and the sporulation deficiency associated with the different *mat*α mutations.

sir1–1 is also able to suppress the defects caused by mutations in the **a** mating-type locus. *mat***a***–1*/*MAT*α diploids are unable to sporulate, but *mat***a***-1*/*MAT*α *sir1–1*/*sir1–1* do sporulate, indicating that *sir1–1* is able to suppress *MAT***a** mutations. *sir1–1* is recessive to *SIR1* in its suppression of both *MAT***a** and *MAT*α mutations.

sir1–1 is unable to suppress mating defects of mutations affecting α mating ability that are not in the α mating-type locus. Two independent mutations have been tested: *ste3–1* (MACKAY and MANNEY 1974a,b) and *ste13–1* (RINE, unpublished; G. SPRAGUE, JR., personal communication). In addition, *sir1–1* does not

suppress nonsense mutations (*ochre* mutations *arg4–17*, *his5–2*, *lys1–1* and *leu2–1*; *amber* mutations *trp1–1* and *tyr7–1*). *sir1–1* thus is a locus-specific suppressor, acting on mutant alleles of the mating-type loci.

Sporulation by **a**/**a** *and* α/α *diploids: sir1–1* allows efficient sporulation by *MAT***a**/*MAT***a**, *MAT*α/*MAT*α, and *mat*α*1–5*/*mat*α*1–5* diploids. In this regard, *sir1–1* behaves as a mutation leading to a bypass of normal control of sporulation by the mating-type locus, which ordinarily requires both *MAT***a** and *MAT*α. As discussed below, we believe that the sporulation proficiency of these strains is due not to a bypass of the requirement for both *MAT***a** and *MAT*α loci for sporulation, but rather is due to the production of the functions of the mating-type loci in a novel manner.

Action in **a** *cells:* In addition to the ability of *sir1–1* to promote sporulation in **a**/**a** diploids, it also exhibits three phenotypes in haploid **a** cells: reducing response to α-factor, causing cells to exhibit a polar budding pattern and slightly lowering the efficiency of mating with α cells. In all of these behaviors, **a** *sir1–1* strains have acquired properties of **a**/α cells.

sir1–1 *and* HM**a**

A simple view of *sir1–1* is that it leads to the expression of all functions normally controlled by the **a** and α mating-type loci, independent of the particular allele at the mating-type locus. We propose that *sir1–1* acts by allowing expression of cryptic copies of *MAT***a** and *MAT*α. Candidates for these silent *MAT* loci are the genes *HM***a** and *HM*α, which have been proposed to be silent *MAT*α and *MAT***a**, respectively (HICKS, STRATHERN and HERSKOWITZ 1977b; see also HARASHIMA, NOGI and OSHIMA 1974). Our work shows that the ability of *sir1–1* to suppress the mating defect of *mat*α*1–5* is dependent on *HM***a** (the proposed silent *MAT*α) and not on *HM*α. Although not yet tested, we expect that *sir1–1* suppression of *mat***a***-1* requires *HM*α. We stress that the requirement of *HM***a** in suppression of *mat*α*1–5* does not occur by mating-type interconversion—the *mat*α*1–5* mutation remains at the mating-type locus in strains suppressed by *sir1–1*.

We propose that suppression by *sir1–1* results from expression of *HM***a** *in situ*, that is, without movement of the information at this locus. Since *sir1–1* is recessive to *SIR1*, we propose that *SIR1* codes for a negative regulator of expression of *HM***a** (and *HM*α). We discuss the implications of *SIR1* for the mechanism of mating-type interconversion after consideration of additional properties of *sir1–1*.

sir1–1 *may be a "leaky" mutation in the* SIR1 *gene*

If SIR1 is involved in keeping *HM***a** and *HM*α silent, mutants completely lacking *SIR1* function might be expected to have the phenotype of **a**/α diploids. In other words, haploid **a** or α *sir1* mutants might have been expected to be completely defective in mating. Indeed, *MAT***a** *sir1–1* strains do exhibit several properties of **a**/α cells, *e.g.*, lower efficiency of mating. KLAR, FOGEL and RADIN (1979) have recently identified a mutation, *mar1–1*, which is similar to *sir1–1*

in that it appears to allow expression of both *HM***a** and *HMα* in heterothallic strains. In contrast to *sir1–1* strains, *MAT***a** and *MATα* strains carrying *mar1–1* exhibit a severe defect in mating. Recent results suggest that the *cmt* mutation (HOPPER and HALL 1975b) may also be similar to *mar1–1* (HABER and GEORGE 1979). *mar1–1* and *sir1–1* appear to be in different genes, since *mar1–1* is linked to *trp1* (KLAR, FOGEL and MACLEOD 1979), whereas *sir1–1* is not (RINE, unpublished).

Why do *sir1–1* and *mar1–1* have different phenotypes? Since *sir1–1* was selected for its ability to suppress the mating defect of *matα1–5*, *sir1–1* strains might maintain partial *SIR1* activity. For example, the *sir1–1* mutation itself might lower, but not abolish, *SIR1* activity, as observed for the *c1857* mutation of the phage lambda repressor gene (see KLECKNER and SIGNER 1977). It is also possible that the apparent partial expression of *HM***a** and *HMα* by *sir1–1* mutants reflects the phenotype of a *sir1* null mutation.

Given that *sir1–1* allows expression of both *HM***a** and *HMα* at some level, the ability of *sir1–1* to promote sporulation in **a**/**a** and *α*/*α* diploids without severely inhibiting mating and the ability of *sir1–1* to allow mating by *matα1* and *matα2* mutants implies that the level of mating-type locus functions necessary to promote sporulation (**a***1* and *α2*) is lower than the level necessary to turn off mating. In *matα1* or *matα2 sir1–1 HM***a***HMα* strains, the level of *α1* and *α2* functions from *HM***a** must be sufficient to complement the defect at the mating type locus, but the level of **a***1* function from *HMα* must be inadequate for inhibition of mating. The inability of *matα1 sir1–1 hm***a** *HMα* to mate may be caused by a lack of *α1* function *per se* or because the strain has adequate **a***1* function from *hm***a** and *HMα* and adequate *α2* function from the mating-type locus to have an **a**/*α* phenotype. Further understanding of the interactions among the functions coded by *HM***a**, *HMα* and the mating-type locus itself will require studies of mutations within *HM***a** and *HMα* and the isolation of known null alleles of *SIR1*.

The behavior of mat**a**-1 sir1–1 *and* MAT**a** sir1–1 *strains*

Why do *mat***a***-1 sir1–1* strains mate rather well as **a** and, to a measurable extent, as *α*, whereas *MAT***a** *sir1–1* strains mate only as **a** (Table 4)? Although we do not know whether a given *mat***a***-1 sir1–1* can mate with both **a** and *α* cells, our working hypothesis is that the mating behavior reflects differences within the population of *mat***a***-1 sir1–1* cells. If *sir1–1* allows a low-level expression of *HM***a** and *HMα*, then some cells may have sufficient activities of *MATα* functions from *HM***a** to allow mating as *α*; other cells may not have sufficient activities. Since expression of functions necessary for **a** mating ability is believed to be constitutive in the absence of *MATα* (STRATHERN, HICKS and HERSKOWITZ, in preparation), the latter class of *mat***a***-1 sir1–1* cells will have the mating phenotype of **a** cells. Strains of genotype *HM***a** *mat***a***-1 hmα sir1–1* (XR197–3b, –3d) have an *α* mating phenotype instead of a bimating phenotype (Table 4). This behavior can be explained by the proposal of NAUMOV and TOLSTORUKOV (1973) that *hmα* is functionally equivalent to *HM***a**. These cells

would then be expressing two normally silent *MAT*α genes, and we would therefore expect these cells to have fewer phenotypes of an **a** cell.

*MAT***a** *sir1–1* strains may be similar to *mat***a**-*1 sir1–1* strains in that expression of *MAT*α functions from *HM***a** may be low in some cells and essentially absent in others. However, the ***a1*** function produced in high level by the **a** mating-type locus may interact with *α2* product from the *HM***a** locus to give some cells an **a**/α phenotype. Thus, cells with a low level of *HM***a** expression would mate as **a**; cells with a higher level of *HM***a** functions would be phenotypically **a**/α and would exhibit the various phenotypes seen in some *MAT***a** *sir1–1* cells.

Although one might have expected individual cells in a *MAT***a** *sir1–1 HM***a** *HM*α population to respond slowly to α-factor, we observe quite a different result—a given cell either responds to α-factor or it does not. Since α-factor-resistant cells give rise to α-factor-sensitive cells and *vice versa*, *MAT***a** *sir1–1 HM***a** *HM*α cells exhibit a physiological switch between resistance and sensitivity to α-factor. This behavior is rationalized, as described above, on the hypothesis that the probability per generation of a cell producing *MAT*α functions from *HM***a** is low. Cells that produce adequate *α2* function behave for at least one cell-division cycle as an **a**/α cell and are resistant to α-factor; cells that fail to produce adequate *α2* function behave as **a** cells and are sensitive to α-factor.

Implications for mating-type interconversion

HM**a** *is functionally equivalent to* MATα: The *HM***a** locus was originally identified by its role in mating-type interconversion as required for switching from **a** to α in homothallic cells. Our studies of *sir1–1* indicate that the *HM***a** locus can exhibit a phenotype in heterothallic cells—that of supplying *MAT*α functions. The role of *HM***a** in cells containing *sir1–1* thus provides independent support for the proposal that *HM***a** and *HM*α are cryptic copies of *MAT*α and *MAT***a**. These studies also allow us to distinguish between two models for *HM***a** and *HM*α.

Two kinds of controlling element models: OSHIMA and TAKANO (1971) and HARASHIMA, NOGI and OSHIMA (1974) proposed that *HM***a** and *HM*α are controlling elements analogous to those of maize, and that association of the *HM***a** element or the *HM*α element with the mating-type locus leads to an α or **a** cell, respectively. Several specific proposals can be made for the nature of the controlling element. In the cassette model, *HM***a** and *HM*α are silent copies of *MAT*α and *MAT***a** information, respectively. That is, *HM***a** and *HM*α contain the structural genes for the various functions of the mating-type loci, but are expressed only when placed into proper position at a site of the mating-type locus (Figure 2a). In another kind of model, the "transposable regulatory site" (TRS) model, *HM***a** and *HM*α are not structural genes, but are regulatory sites that can associate with the mating-type locus, which has the structure shown in Figure 2b. Association of *HM***a** with the mating-type locus would direct expression of the α regulatory information, and association of *HM*α with the mating-type locus would direct expression of the **a** regulatory information. Three

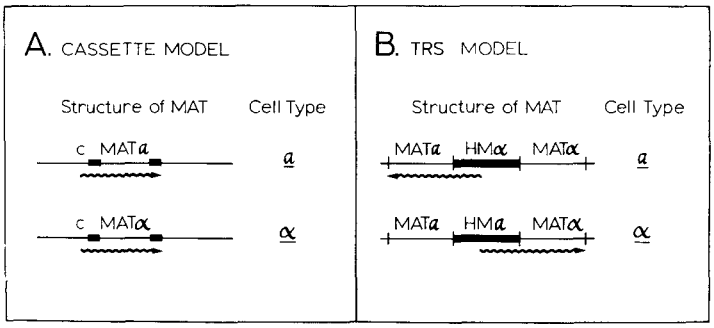

FIGURE 2.—(A) According to the cassette model, the mating-type locus (*MAT*) contains either *MAT***a** or *MAT*α genes, but not both. Insertion of a *MAT***a** cassette adjacent to the controlling site, *c*, leads to the expression of **a** cell type. Association of *MAT*α with the controlling site, *c*, leads to expression at the α cell type. The solid rectangles indicate hypothetical recognition sites involved in mating-type interconversion.

(B) According to the TRS model, the mating-type locus (*MAT*) contains both *MAT***a** and *MAT*α genes. The particular gene(s) being expressed is determined by a transposable regulatory site coded for by *HM***a** or *HM*α. Association of *HM*α with *MAT* leads to the **a** cell type; association of *HM***a** with *MAT* leads to the α cell type.

lines of evidence lead us to favor the cassette model explanation: (1) Heterothallic strains can switch from **a** to α by formation of a circular chromosome *III* (STRATHERN *et al.* 1979). According to the cassette model, a single intrachromosomal recombination event deletes the active *MAT***a** information at the mating-type locus and fuses the silent *MAT*α information at *HM***a** to a controlling site at the mating-type locus. It is not readily apparent from the TRS model how juxtaposition of *HM***a** and the mating-type locus by circle formation would lead to a switch from **a** to α. (2) Mutations in the mating-type loci (*mat*α*1*, *mat*α*2*, and *mat***a***-1*) are all efficiently healed by mating-type interconversion. The cassette model predicts that all mutations within the mating-type locus should be healable. The TRS model can also account for healing, but only of mutations that affect the regulatory element itself, but not mutations in the **a** and α structural information. (3) The *sir1–1* mutation allows *HM***a**-dependent suppression of mutations at the α mating type locus. According to the cassette model, the *MAT*α information at *HM***a** is no longer silent and can provide functions not produced by the defective α mating-type locus. The observations on *sir1–1* cannot be explained by the TRS hypothesis, which requires that *HM***a** information be transposed into the mating-type locus to confer the α cell type.

Mechanism of action of SIR*:* Because the various actions of *sir1–1* are recessive to *SIR*$^+$, we propose that *SIR1* codes for or controls synthesis of a negative regulator of expression of *HM***a** and *HM*α. Several kinds of negative regulators can be imagined:

(1) *SIR1* might not be specifically concerned with control of *HM***a** and *HM*α expression, but, for example, might code for an analogue of the *E. coli* transcription-termination protein, rho (ROBERTS 1970). On this hypothesis, transcription

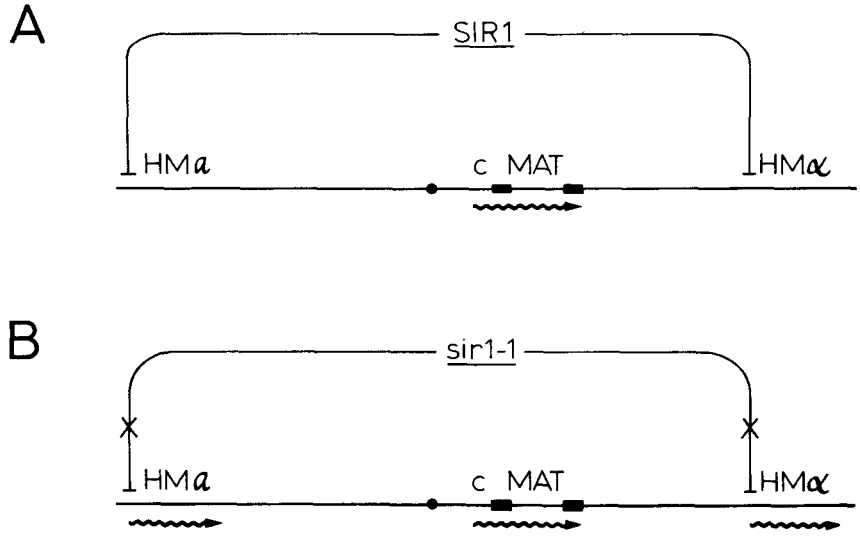

FIGURE 3.—A model for the action of SIR1 gene product.

(A) In *SIR1* cells, SIR product acts to inhibit expression of *MATα* and *MAT***a** information at *HM***a** and *HMα*, but not at the mating-type locus.

(B) The mutation *sir1–1* partially abolishes the activity of the *SIR1* gene product. The mutation allows the *in situ* expression of *HM***a** and *HMα*. The level at which *SIR1* regulation occurs is not yet known.

of *HM***a** and *HMα* in the *sir1–1* mutant would occur by read-through of transcription initiated on chromosome *III*, which is allowed to continue into *HM***a** and *HMα*. This kind of hypothesis would be favored if *sir1–1* is found to affect expression at loci other than *HM***a** and *HMα*.

(2) *SIR1* might code for a specific inhibitor of the products of *HM***a** *and HMα*, being analogous to the proposed action of the negative regulator *GAL80* (MATSUMOTO, TOH-E and OSHIMA 1978; PERLMAN and HOPPER 1979). According to this hypothesis, the RNA or protein products of *HM***a** and *HMα* must be different from those produced by the mating-type locus.

(3) *SIR1* might code for specific repressor that acts at operator sites adjacent to *HM***a** and *HMα*, which are absent at the mating-type locus (see Figure 3). According to this hypothesis, transposition of a copy of *HM***a** *or HMα* information into the mating-type locus activates these genes by removing them from a site of negative control.

The possibility that *SIR1* specifically regulates *HM***a** and *HMα* raises the question of whether these loci are ever expressed *in situ* in heterothallic cells, due to some event leading to inactivation of *SIR1* product.

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