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An *RME1*-Independent Pathway for Sporulation Control in *Saccharomyces cerevisiae* Acts Through *IME1* Transcript Accumulation

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ABSTRACT

The *RES1-1* mutation was isolated on the basis of its ability to allow *MAT***a***/MATα* diploid *Saccharomyces cerevisiae* cells to express a late sporulation-regulated gene, *SPR3*, in the presence of excess copies of *RME1*. *RME1* is a repressor of meiosis that is normally expressed in cells that lack the **a**1/α2 repressor encoded by *MAT*. The *RES1-1* mutation also supports sporulation in *mat*-insufficient diploids. This phenotype does not result from a failure to express *RME1* and is not due to activation of the silent copies of mating type information. *RES1-1* activates sporulation by allowing *IME1* accumulation in all cell types, irrespective of the presence of the *MAT* products. *IME1* is still responsive to *RME1* in *RES1-1* cells, since double mutants (*rme1 RES1-1*) that are deficient at *MAT* can sporulate better than either single mutant. *RES1-1* is not an allele of *IME1*.

SPORULATION in the yeast, *Saccharomyces cerevisiae*, is a profound cellular response to a combination of physiological and genetic cues. The process includes a modified round of DNA replication, high levels of recombination, pairing and segregation of chromosomes, and, finally, encapsulation of the haploid meiotic products into progeny ascospores (reviewed by ESPOSITO and KLAPHOLZ 1981).

The genetic signal for sporulation is the result of the activities of the *MAT***a**1 and *MATα*2 products of the mating type locus (STRATHERN, HICKS and HERSKOWITZ 1981). Diploids are capable of sporulating because they express both of these products simultaneously. Haploids, or diploids carrying defective *MAT* alleles, cannot normally sporulate unless they also contain mutations that bypass the need for both *MAT* products (*i.e.*, *rme1* or *csp1* mutations; HOPPER and HALL 1975; KASSIR and SIMCHEN 1976; RINE, SPRAGUE and HERSKOWITZ 1981) or if they express the normally silent *HML* and *HMR* loci (reviewed by HERSKOWITZ and OSHIMA 1981). One role of the *MAT* products is to shut off the expression of *RME1*, a repressor of meiosis that is expressed in haploids and *mat*-insufficient diploids. Overexpression of *RME1* represses sporulation even in *MAT***a***/MATα* diploids (MITCHELL and HERSKOWITZ 1986).

Sporulation also requires the appropriate nutritional conditions. Initiation of the process is controlled, at least in part, by the ability of the *RAS2* product to regulate adenylate cyclase activity, with consequent modulation of the cAMP-dependent protein kinase; mutants that lack *RAS2* sporulate precociously in nutrient-replete medium whereas those that contain activated *RAS2*^{val19} alleles are incapable of doing so under any conditions. Many other observations are also consistent with the idea that high activity of the cyclic AMP-dependent protein kinase is inhibitory to sporulation (UNO, MATSUMOTO and ISKIKAWA 1982; TODA *et al.* 1987a,b; CANNON and TATCHELL 1987). Mutations that activate the kinase constitutively because of the absence of the regulatory subunit (*bcy1*) or the presence of activated catalytic subunits (*e.g.*, *TPK1* or *SRA3*), cause sporulation defects. These observations implicate a phosphorylated molecule as a negative regulator of sporulation but do not preclude the existence of an additional *RAS2*-coupled process to activate sporulation as well.

The target of the nutritional and cell type response pathways that is of most relevance to sporulation is *IME1* (KASSIR, GRANOT and SIMCHEN 1988). *IME1* is an essential activator that is expressed at high levels in sporulating diploids and is probably the singular target of *RME1* activity. Artificial overexpression of *IME1* bypasses *MAT* control of sporulation and also overrides nutritional control to some degree (GRANOT, MARGOLSKEE and SIMCHEN 1989). *IME1* was identified based on its ability to activate recombination (as a monitor of sporulation) in *mat*-insufficient diploid cells. Although the biochemical nature of the *IME1* product is not known, its activity is necessary to induce *IME2* and other sporulation-specific genes (SMITH and MITCHELL 1989; MITCHELL, DRISCOLL and SMITH 1990).

Although it is clear that mating type control of sporulation is mediated by *RME1*, there is evidence from several laboratories that other functions are also involved. One long-standing and relevant observation is that the absence of *RME1* is not sufficient to pro-

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mote normal diploid levels of sporulation in *mat*-insufficient cells. This was attributed to potential leakiness of the original *rme1-1* allele but is now known to be a property of null alleles as well (MITCHELL and HERSKOWITZ 1986). Conversely, *RME1* never represses sporulation completely in diploids, even when its expression is driven by strong *ENO* or *GAL* promoters (G. SIMCHEN, personal communication; A. MITCHELL, personal communication; our unpublished observations).

The available evidence is consistent with the idea that the target of additional *MAT*-dependent regulators is *IME1* and that the reduced sporulation promoted by *rme1* mutations is the result of limited expression or activity of this gene. Overexpression of *IME1* in *mat*-insufficient diploids supports nearly full levels of sporulation, suggesting that adequate levels of *IME1* are sufficient for a complete bypass of *MAT*-dependence. That these levels are limiting when sporulation is dependent on *rme1* is suggested by the observation that *IME1* transcript is slower to appear and reaches lower steady-state levels under these conditions (KASSIR, GRANOT and SIMCHEN 1988).

The work described below is an effort to identify genetic functions that regulate sporulation in diploid *S. cerevisiae* cells. The *RES1* (*RME1*-escape) gene was defined by a dominant allele (*RES1-1*) that allows full levels of sporulation to occur in diploids that harbor excess copies of *RME1*. A related approach has also been used to isolate plasmids that elicit high levels of recombination under similar conditions (SMITH and MITCHELL 1989).

The *RES1-1* mutation confers phenotypes on all of the *S. cerevisiae* cell types. It allows *MAT***a**/*MAT*α diploids to escape *RME1* repression, and also allows *mat*-insufficient diploids to express *IME1* and to sporulate, although not at full levels. Haploids carrying this mutation do not sporulate, but do express early (*IME1*) and late (*SPR3:lacZ*) sporulation-specific genes at low levels. These phenotypes of *RES1-1* strains do not depend on the silent mating type information and are not the result of a failure in *RME1* expression. Although both *RES1-1* and *rme1* mutations support sporulation in *mat*-insufficient cells, they do so in different ways; the two mutations together confer higher sporulation on *mat*-insufficient cells than does either alone. Thus, unlike *RME1*, the *RES1* product appears to play a role that is relevant to the induction of sporulation in *MAT***a**/*MAT*α diploid cells. This role is to regulate the induction or stability of the *IME1* mRNA in such cells.

MATERIALS AND METHODS

Media and growth conditions: *Saccharomyces cerevisiae* strains were grown in YEPD or in supplemented minimal medium according to the methods described by SHERMAN, FINK and HICKS (1986). Respiratory adaptation of the cells prior to use in sporulation experiments was accomplished by growth in YEPA as described previously (HOLAWAY *et al.* 1985). Sporulation medium was 1% potassium acetate supplemented as required by the strain. Progress through sporulation was routinely monitored by light microscopy and also, in some experiments, by staining the cells with the nuclear stain DAPI (4,6-diamidino-2-phenylindole; WILLIAMSON and FENNEL 1975) using a Nikon Labophot microscope equipped for epifluorescence. *S. cerevisiae* strains were transformed using the method of ITO *et al.* (1983) as described by SHERMAN, FINK and HICKS (1986).

Construction and description of *S. cerevisiae* strains: The strains used are listed in Table 1. Most strains were constructed and analyzed by standard genetic techniques (SHERMAN, FINK and HICKS 1986). The procedure of BREEDEN and NASMYTH (1987) was used for crosses between strains containing *HO*. The two strains to be crossed were transformed separately with either YCp50 (*URA3*; SHERMAN, FINK and HICKS 1986) or pGK25 (*LEU2*, see below) and sporulated. The spores were mixed and plated on minimal medium that lacked both supplements to select for diploids that had acquired a second prototrophy by mating.

The parent strain, GKY5, was derived from a cross between SCMS7-1 (SMITH *et al.* 1988) and a haploid derivative of DK337 (GOTTLIN-NINFA and KABACK 1986) called DK10a (HOLAWAY *et al.* (1987). SCMS7-1 was sporulated and the spores were mated to the DK10a haploid by micromanipulation. GKY5 was a segregant from one such diploid; GKY5 retains the *HO* marker from the SCMS7-1 parent and contains all of the desired auxotrophic markers (see Table 1). Because of the presence of *HO*, GKY5 is homozygous for all of its genes except for the mating type alleles. All strains derived from GKY5 are thus isogenic to each other as well as to the GKY5 parent.

Haploid derivatives of GKY5 and mutant strains were constructed by transplacement (ROTHSTEIN 1983) of an *ho:HIS3* null allele into the GKY5 diploid, sporulation of the His$^+$ diploid transformants and screening of the segregants for the production of mating pheromones (CHAN and OTTE 1982). The source of the DNA for transplacement was pGK28. This plasmid contained an internal fragment of *HO* into which the *HIS3* gene had been inserted as a 1.7-kb *Bam*HI fragment between the *Bam*HI and *Bgl*II sites at approximately +710 and +1100 within the open reading frame of *HO* (RUSSELL *et al.* 1986). This was constructed in the pUC118 vector beginning with a 2.1-kb *Pst*I-*Eco*RI fragment from YCp50-*HO* (very kindly provided to us by IRA HERSKOWITZ; RUSSELL *et al.* 1986) which contains the region of the *HO* ORF spanning approximate positions +340 to +2510 bp, cloned between the *Eco*RI and *Pst*I sites in the polylinker region of pUC118. *HIS3* was then inserted as above. For use in disruption experiments, pGK28 was restricted with *Sca*I, which cleaves *HO* at approximately +565 and +1330. The structure of the *HO* locus was verified by Southern blot analysis (SOUTHERN 1975) of "miniprep" DNA prepared from His$^+$ transformants (SHERMAN, FINK and HICKS 1986). The *ho:HIS3* segregants from the His$^+$ diploids were retained as haploids or mated to form diploids that were isogenic to the original GKY5 transformant except for the alleles at *MAT* and *HO*.

Strains isogenic to GKY5 and defective at *MAT* were constructed similarly using pGK29, a derivative of the ax50 allele of *MAT***a**1. Plasmid ax50 contains a mutant *mat***a** cassette in the shuttle vector YRp7; this was modified by the addition of a 2.2-kb *Sal*I-*Xho*I fragment containing *LEU2* at the *Xho*I site which marks the location of the original linker insertion allele (NASMYTH and TATCHELL 1980; ASTELL *et al.* 1981; TATCHELL *et al.* 1981). This construction, designated pGK29, was used to inactivate the chromosomal *MAT* alleles by digestion with *Hin*dIII followed by selection of

TABLE 1

Strains used in this study

Strain	Genotype
GKY5	*MATa/MATα RES1/RES1 HO/HO leu2/leu2 ura3/ura3 trp1/trp1 his3/his3*
GK22	*MAT***a** *RES1 ho:HIS3 leu2 ura3 trp1 his3*
GK25	*MAT***a** *RES1 ho:HIS3 leu2 ura3 trp1 his3*
GK31	*MATα RES1 ho:HIS3 leu2 ura3 trp1 his3*
GK10	*MATα RES1 ho:HIS3 leu2 ura3 trp1 his3*
GK56	*MATa/MATα RES1/RES1* Cross between GK25 and GK31
GK53	*MATa/MATα RES1/RES1* Cross between GK22 and GK31
125	*MATa/MATα RES1-1/RES1-1 HO/HO leu2/leu2 ura3/ura3 trp1/trp1 his3/his3*
125B-2c	*MATa/MATα RES1-1/RES1-1 HO/HO leu2/leu2 ura3/ura3 trp1/trp1 his3/his3*
125-2	*MAT***a** *RES1-1 ho:HIS3 leu2 ura3 trp1 his3*
125-8	*MAT***a** *RES1-1 ho:HIS3 leu2 ura3 trp1 his3*
125-20	*MATa/MATα RES1-1/RES1-1* Cross between 125-2 and 125-10
125-6	*MATα RES1-1 ho:HIS3 leu2 ura3 trp1 his3*
125-10	*MATα RES1-1 ho:HIS3 leu2 ura3 trp1 his3*
125-18	*MATa/MATα RES1-1/RES1-1* Cross between 125-8 and 125-10
125-12	*MATa/MATα RES1-1/RES1-1* Cross between 125-2 and 125-6

All strains were constructed for this study.

yeast transformants for leucine protrotrophy. Either *MAT* allele could be disrupted with this construction, since the sequences flanking *MAT* are common to the two loci. *MAT* disruptants were identified by their ability to produce α factor (genotype *mat***a**1:*LEU2/MATα*) or **a** factor (genotype *MAT***a**/*mat***a**1:*LEU2*). The ax50 allele and corresponding wild type *MAT* genes were very kindly provided by KELLY TATCHELL.

Construction of *rme1, ime1* and *mat*-defective derivatives of the parent and mutant strains was accomplished similarly. Plasmids containing null alleles of *IME1* were kindly provided by YONA KASSIR and AARON MITCHELL. The allele of *RME1* used for disruption was derived from pAM232 (MITCHELL and HERSKOWITZ 1986). This contained *LEU2* inserted at a unique *Bgl*II site within the gene as described by MITCHELL and HERSKOWITZ (1986) to form pGK24. This DNA was restricted with *Pvu*II and *Sph*I for use in transformation experiments.

IME1 was disrupted using the *ime1:TRP1* allele present in pAM506 (kindly provided by AARON MITCHELL). This contains *TRP1* inserted at a *Cla*I site internal to the *IME1* transcription unit. Disruption was accomplished by transforming *S. cerevisiae* cells with pAM506 DNA that had been digested with *Bgl*II and *Bam*HI, selecting for Trp^+ transformants. The wild type *IME1* allele used in this study was contained in pM4B. This was obtained from a GKY5-derived library constructed in a YCp50 vector. pM4B was obtained by hybridization of *E. coli* transformants to a 350-bp *Eco*RI fragment internal to the *IME1* coding region. The latter was obtained from pAM506. The cloned fragment complements an *IME1* null mutation and its restriction map is compatible with published maps of the *IME1* region.

Strains that lacked both *RME1* and *MAT* activities were constructed somewhat differently. *RME1* was disrupted in haploid strains GK22, GK31, 125-2, 125-8 and 125-10. Leu^+ transformants were selected and the expected disruption events confirmed by SOUTHERN (1975) blotting. The desired haploids were mated to form diploids that lacked *RME1*. *MAT* was disrupted in the resulting diploids by cotransformation of pGK29 DNA that had been cleaved with *Hin*dIII along with intact YCp50 DNA. Ura^+ transformants were purified and screened for mating pheromone production as above.

Strains lacking *HMLα* were constructed as suggested to us by ALAN ROSE and JAMES BROACH, using pAR65 to remove all sequences distal to the *HML* I-site. pAR65 contains a 1.1-kb *Hin*dIII-*Eco*RI fragment homologous to sequences that are just centromere proximal to *HMLα*. The vector contains a telomere in a backbone containing the *URA3* and *SUP11* genes for selection and screening in yeast. Digestion of pAR65 with *Eco*RI and *Not*I linearized the plasmid and exposed the telomere and *HML*-adjacent sequences. Stable Ura^+ transformants were screened by SOUTHERN (1975) blotting for replacement of *HMLα* with vector sequences. *MAT*-insufficient diploids that lacked *HML* were made by mating haploid transformants and disrupting *MATα* with pGK29 as above. Correct disruptions were verified using Southern blots as described by MANIATIS, FRITSCH and SAMBROOK (1982), using a 4.2-kb *Hin*dIII fragment from YCp50-*MAT***a** as a probe. This plasmid was constructed by RITA BASU. The source of the *MAT***a** allele was pBR322-*MAT***a**; this was kindly provided by KELLY TATCHELL.

Mutagenesis and screen: Mutagenesis of GKY5 was accomplished with EMS using the method of OSHIMA and TAKANO (1980). Three ml of free spore suspension (5×10^7 spores per ml) was incubated with EMS until approximately 50% of the spores were unable to form colonies upon subsequent plating. Approximately 10^5 viable mutagenized spores were spread on each of three YEPD plates and grown at 30° for 5–7 generations so that the spores could germinate, switch mating types and mate clonally. This property of *HO* strains has been used previously by several other laboratories to obtain diploids that are homozygous for recessive mutations (ESPOSITO and ESPOSITO 1969; ROCKMILL and ROEDER 1988; SMITH *et al.* 1988). The cells were scraped off each plate, resuspended in 10 ml of YEPD at an initial density of $1-2 \times 10^6$ cells per ml and allowed to grow with shaking at 30° for 6–8 hr, at which time the cell density had reached $4-7 \times 10^7$ cells per ml. The cells were then washed and transformed with the pGK23 repressor plasmid or the pGK21 control. Approximately 25,000 total transformants obtained in two separate experiments were then replica-plated twice onto minimal medium, once onto YEPA plates and finally onto SPM plates supplemented with X-gal (5-bromo-4-chloro-3-indolyl-3-D-galactoside). Transformants containing pGK23 which turned blue at the same time as pGK21 controls were retained for further analysis. In total, eleven apparently mutant strains were obtained from 160 plates of transformants.

Plasmid constructions and maintenance: *Escherichia coli* strains were grown and maintained in LB medium, and transformed by standard procedures (MANIATIS, FRITSCH and SAMBROOK 1982). All cloning experiments utilized DNAs that had been isolated from CsCl gradients (DAVIS, BOTSTEIN and ROTH 1980) and, in many cases, further purified from low melting or standard agarose gels that were poured and run in Tris-acetate buffer (MANIATIS, FRITSCH and SAMBROOK 1982). Ligation, fill-in, and end-labeling reactions and restriction enzyme digestions were performed as recommended by the suppliers of the relevant enzymes (BRL or New England Biolabs).

The sporulation reporter construction has been described previously (CLANCY *et al.* 1983; HOLAWAY *et al.* 1987; KAO *et al.* 1989). This contains sequences corresponding to the 5′ end and first 46 amino acids of a late sporulation gene, *SPR3*, fused to the coding region of a *lacZ* derivative (from pMC1871; CASADABAN *et al.* 1983) that had been deleted at its N terminus. For routine monitoring of sporulation, a derivative of this fusion was introduced into a centromere-containing vector that also contained the *LEU2* and *URA3* selectable markers for yeast (pGK16; HOLAWAY *et al.* 1987).

The same fusion was also inserted at the unique *Pvu*II site of the high copy two micron circle-derived vector, YEp24, using an 8.4-kb *Hin*dIII-*Bgl*II fragment derived from pGK11 (HOLAWAY *et al.* 1987) that had been blunt ended prior to ligation to the YEp24 vector. Expression from the resulting construction, pGK21, was assumed to reflect the normal activity from the *SPR3:lacZ* construct in high copy.

To monitor levels of *SPR3:lacZ* expression in the presence of *RME1*, the same fusion was cloned into a derivative of pAM232 (MITCHELL and HERSKOWITZ 1986) to form pGK23 (the repressor plasmid). pAM232 was first modified by the addition of an *Xho*I linker at the unique *Pvu*II site. The sporulation-regulated fusion was then introduced as an *Xho*I-*Sal*I fragment derived from pGK11 after modification of a unique *Sma*I site by the addition of an *Xho*I linker. The *Sal*I endpoint was within the sequences originating in the pBR322 vector. The final pGK23 plasmid therefore contained the *RME1* gene, the sporulation-regulated *SPR3:lacZ* fusion, the origin of replication derived from the yeast two micron circle and markers for selection in yeast and *E. coli*.

pGK25 contains *CEN3* from pFW14 (kindly provided by GEORGE BOGUSLAWSKI) cloned as a *Bam*HI-*Hin*dIII fragment between these same sites as YEp13. A *lacZ* fusion to *RME1* was constructed using the Tn*10::LUK* system described by HUISMAN *et al.* (1987). Plasmid pNK629 and phage λ1227 were used as *in vivo* sources of Tn*10* transposase and the mini Tn*10* fusion, respectively. These were kindly provided by NANCY KLECKNER. The *RME1*-containing target plasmid, pGK26, was a 4.0 kb *Bam*HI-*Pvu*II fragment from pAM232 cloned into pGK25 (see above). The *RME1*-containing fragment consisted of the complete coding region of the gene, adjacent 3′ and vector sequences and approximately 1.5 kb of upstream DNA. The resulting plasmid contains *LEU2* and *CEN3* for selection and maintenance in yeast, and the *bla* gene and *ColE1* replication origin to provide these functions in *E. coli*.

pGK26 (Ap^r) and pNK629 (Tc^r) were transformed simultaneously into *E. coli* strain CC118 (su^0), selecting for ampicillin resistance and subsequently screening for tetracycline resistance. Transposase production from pNK629 was induced by growing the cells in the presence of IPTG (isopropyl thiogalactoside, 1 mM) for 2 or 4 hr, as suggested by HUISMAN *et al.* (1987), but using LB (MANIATIS,. FRITSCH and SAMBROOK 1982) containing ampicillin (100 μg/ml) and tetracycline (12.5 μg/ml) rather than λYM. The primary pool of transposition products was obtained by infecting approximately 3×10^8 cells with λ1224 (m.o.i. of 0.3), growing them for an additional 90 min and plating on LB containing 50 μg/ml kanamycin. Purified DNA from this pool (approximately 8000 transformants) was then retransformed into CC118, selecting for kanamycin and ampicillin resistance in the presence of 1.25 mM sodium pyrophosphate. This eliminated plasmids containing insertions in pNK629 and selected against phage genomes which may have contaminated the initial DNA preparation. Approximately 1300 transformants were used to yield the final DNA preparation.

This pool was introduced into the haploid *S. cerevisiae* strain GK10, selecting for the *URA3* marker on the transposon. Yeast transformants were screened for β-galactosidase production using liquid nitrogen to permeabilize the cells, as described to us by L. BUCKINGHAM and R. E. ESPOSITO. Plasmids that could direct activity in yeast were recovered in *E. coli* and characterized by restriction mapping. Insertions that appeared to be in or near the *RME1* gene were transformed into isogenic haploid and diploid strains, to determine whether β-galactosidase expression was cell type regulated as expected. One plasmid which exhibited the correct behavior (pHW2) was retained for further studies. This plasmid contained a Tn*10::LUK* insertion approximately 220–240 bp 5′ to the *Bgl*II site in *RME1*. This placed the transposon approximately 180 bp downstream of the *RME1* initiation codon (SMITH and MITCHELL 1989).

β-Galactosidase determinations: Cell extracts were prepared from 1–3 ml culture samples ($0.6–1.0 \times 10^8$ cells) by a slight modification of the method of ROSE and BOTSTEIN (1983), as described previously (HOLAWAY *et al.* 1987). Activity is expressed as nmol ONPG (*o*-nitrophenyl-β-D-galactoside) hydrolyzed/min per milligram protein, using a value of 0.0045 $O.D._{420}$ per nmol of *o*-nitrophenol in one ml of assay mixture. Values were corrected for the absorbance of the extract. Protein was assayed by the method of BRADFORD (1976) using commercially available reagent (Bio-Rad) as recommended by the supplier. Bovine IgG was used as standard.

RNA preparation and hybridization conditions: RNA was prepared from vegetative and sporulating cells essentially as described by MACDONALD *et al.* (1987). Approximately 10^9 cells were suspended in 3.0 ml of GTC (guanidinium thiocyanate) buffer and broken by vortexing the suspension for approximately four minutes on the highest setting of the vortex. The extract was removed and the beads were rinsed with approximately 2 ml of additional GTC buffer so that the final volume of the extract was 5.0 ml. Precipitation and extraction of RNA were accomplished essentially as described, except that extractions following the second precipitation with ethanol and potassium acetate used phenol and chloroform (MANIATIS, FRITSCH and SAMBROOK 1982) instead of chloroform and butanol. The RNA was stored at −56° in 70% ethanol and TE at a final concentration of approximately 1 mg/ml.

Preparation of samples for Northern blot analysis was conducted by the methods described by AUSUBEL *et al.* (1987), except that the blots were washed at 42° in 0.2% SDS and 2 × SSC. The gels were poured and run in 20 mM MOPS (3-*N*-morpholino-propanesulfonic acid) containing 1.1% formaldehyde. The nitrocellulose filters (THOMAS 1983) were hybridized to nick-translated DNAs prepared as described by MANIATIS, FRITSCH and SAMBROOK (1982). Alternatively, labeling was accomplished using random primers from Pharmacia according to the specifications of the manufacturer. Approximately 0.3–0.5 mg of total RNA was obtained from 10^9 cells.

RESULTS

Isolation of the *RES1-1* mutation: We screened for mutants that had lost sensitivity to excess copies of *RME1*, by looking for the ability of mutagenized cells to express a late sporulation gene, *SPR3*, carried on the same high copy plasmid as a wild-type allele of *RME1*. We expected that such mutations would define genes that function along with *RME1* and the *MAT* products to activate or repress sporulation, either in concert with these molecules or in response to them. This screen exploited the earlier observation of MITCHELL and HERSKOWITZ (1986) that *RME1* represses sporulation in *MAT***a**/*MAT*α diploids under similar conditions.

The test plasmid, pGK23, contained *RME1* and an *SPR3:lacZ* fusion. The expression of the latter gene is dependent on early events in the meiotic process and serves as a downstream monitor of the ability of the cells to escape the repressive effects of *RME1*. Colonies of wild type *MAT***a**/*MAT*α cells containing this plasmid remained white on sporulation medium supplemented with X-gal, whereas controls containing pGK21, which carries only the *SPR3* fusion, turned bright blue within 24 hr after plating. Cells containing pGK23 did not express *SPR3:lacZ* because *RME1* prevented entry into meiosis in the bulk of the cells of the population (data not shown) and those that had become sporulation-proficient due to plasmid loss during growth did not contribute to the colony color because they lacked the *SPR3:lacZ* fusion as well.

Approximately 25,000 transformants containing pGK23 were screened for blue color after pregrowth on YEPA and exposure to sporulation medium, as described in MATERIALS AND METHODS. Eleven blue colonies were obtained, and ten of these were cured and retested with fresh pGK23 and pGK21 to eliminate plasmid-borne mutations from further consideration. It was assumed that the mutant phenotype of the eleventh strain was the result of insertion of portions of pGK23 into the genome of the transformation recipient, because the Ura^+ phenotype of this strain was stable to prolonged growth in nonselective media. All ten of the remaining strains appeared to carry chromosomal mutations, since they retained the mutant phenotype upon re-transformation with pGK23. None appeared to elevate the rate of plasmid loss.

One isolate (strain 125) was chosen for further analysis and the mutant allele that it contained was designated *RES1-1*. We first examined β-galactosidase activity directed by pGK21 and pGK23 in cell extracts prepared from wild-type and mutant cells grown under conditions which favor vegetative growth or sporulation (Table 2). Activity was significantly repressed in the wild type strain carrying pGK23 relative to the pGK21 control, whereas the mutant exhibited comparable levels of activity under the two conditions. Sporulation was also restored by the *RES1-1* mutation; the wild type strain was moderately to severely repressed (2–40-fold) in the presence of excess copies of *RME1* in this and other experiments, whereas the mutant was relatively insensitive to the repressor. Thus, the blue color originally detected on the SPM plates was a reflection of the higher levels of expression of β-galactosidase due to the sporulation competence of the *RES1-1* cells, rather than an artifact of their altered permeability to X-gal.

The data in Table 2 also show that the mutant strain retained the normal regulation of *SPR3* by the sporulation process; no activity was detected in either the mutant or the wild type during vegetative growth in either PSP or YEPA. When the cells were exposed to sporulation medium for 24 hours, *SPR3* expression was induced normally by both the mutant and wild type strains when they carried the control plasmid, pGK21.

The data in Table 2 also demonstrate that the *RES1-1* mutation is dominant to its wild type allele and that it segregates 2:2 in a cross with wild type, as expected of a nuclear gene. Diploids heterozygous for the *RES1-1* mutation were constructed by forced mating of homothallic spores to the isogenic wild type parent (see MATERIALS AND METHODS). The heterozygote and segregants were analyzed for their ability to express *SPR3:lacZ* and to sporulate in the presence of the high copy *RME1*-containing plasmid. The heterozygote expressed the activity and sporulated at mutant rather than wild type levels, indicating that the *RES1-1* lesion is dominant or semi-dominant to its wild type allele. The more pronounced repression of β-galactosidase expression relative to sporulation in this experiment is partly due to plasmid loss (data not shown).

RES1-1* phenotypes in *MAT*-insufficient cells:** The experiments described above demonstrated that the *RES1-1* diploid sporulates in the presence of excess copies of the *RME1* gene. This phenotype, which can be assayed only in *MATa**/*MAT*α diploids, suggested that *RES1* might function as a target of *RME1* or as a modulator of *RME1* activity. Alternatively, *RES1* could define an *RME1*-independent modulator of sporulation. We expected that if any of these possibilities were correct, the *RES1-1* allele might allow sporulation in the absence of complete *MAT* information; the **a**1/α2 repressor would not be needed in *RES1-1* cells, because such cells would not require the absence of *RME1* for sporulation to occur.

To determine whether the *RES1-1* mutation could alleviate the requirement for both **a** and α information, we constructed haploid derivatives of the original *RES1-1* strain and the wild-type parent, as well as an isogenic series of strains in which either the **a** or α allele at *MAT* had been inactivated by transplacement. These were then examined for their ability to express *SPR3:lacZ* and to sporulate.

TABLE 2

Growth of wild-type and mutant cells

Strain	Markers present[b] on high copy plasmid	β-Galactosidase activity[c] (*SPM* (*PSP*))	Percent sporulation[d]	Inferred genotype
GKY5	*SPR3:lacZ*	229 (0.64)	44 (<0.1)	
	SPR3:lacZ RME1	18 (0.29)	27 (<0.1)	
125	*SPR3:lacZ*	408 (0.60)	59 (<0.01)	
	SPR3:lacZ RME1	350 (0.13)	60 (<0.01)	
125 × GKY5	*SPR3:lacZ*	225	64	
	SPR3:lacZ RME1	109	58	
2a	*SPR3:lacZ*	334	54	Wild type
	SPR3:lacZ RME1	29	18	
2b	*SPR3:lacZ*	463	55	Wild type
	SPR3:lacZ RME1	36	22	
2c	*SPR3:lacZ*	546	64	Mutant
	SPR3:lacZ RME1	331	53	
2d	*SPR3:lacZ*	452	67	Mutant
	SPR3:lacZ RME1	431	61	
4a	*SPR3:lacZ*	489	70	Mutant
	SPR3:lacZ RME1	526	72	
4b	*SPR3:lacZ*	554	68	Mutant
	SPR3:lacZ RME1	382	58	
4c	*SPR3:lacZ*	489	55	Wild type
	SPR3:lacZ RME1	21	22	
4d	*SPR3:lacZ*	571	57	Wild type
	SPR3:lacZ RME1	40	30	

[a] The genotypes for 125 and GKY5 are shown in Table 1. The heterozygous strain (125 × GKY5) was constructed by forced mating of homothallic spores from the two strains as described above. 2a, 2b, etc. are segregants from the heterozygous strain shown.

[b] Cells contained either pGK21 (*SPR3:lacZ*) or pGK23 (*SPR3:lacZ* and *RME1*).

[c] Activity was determined in extracts prepared from 3 ml of growing or sporulating cells as described above. The values shown are averages of two determinations each from two independent transformants of each strain.

[d] Percent sporulation was determined by light microscopy. At least 200 cells were counted for each determination.

Table 3 shows that the *RES1-1* mutation supports low to moderate levels of sporulation in *mat*-insufficient backgrounds. These varied from less than 1% in some experiments to over 20% in others; we do not understand the reason for this amount of variation. In addition, *mat*-insufficient diploid derivatives of the mutant strain formed blue colonies on SPM supplemented with X-gal, when they carried the *SPR3:lacZ* fusion in single copy in the genome or on a YCp50-derived plasmid.

The ability to sporulate was presumed to be characteristic of the *RES1-1* mutation, since analogous *mat*-insufficient derivatives of the wild-type strain did not produce asci and did not express detectable levels of *SPR3:lacZ*. In addition, *mat*-insufficient diploids that were formed by mating wild-type and *RES1-1* haploids were able to sporulate (Table 3). This indicated that **a**1/α2-independent sporulation was a dominant phenotype of the *RES1-1* strains, as was the ability to overcome *RME1* overexpression, and suggested that these two related phenotypes were the result of the same mutation.

Haploids containing the *RES1-1* mutation did not produce detectable asci. They did, however, express low levels of β-galactosidase from an integrated or plasmid-borne *SPR3:lacZ* fusion. These levels were substantially lower than for the corresponding *MAT*-insufficient diploid strains but well above the detection limits of this assay (specific activities of 0.5–1.0 *vs.* 10–15). Mutant haploids that contained such constructions formed pale blue colonies after prolonged incubation on sporulation medium supplemented with X-gal, whereas those derived from the wild type GKY5 parent remained white. This provided a convenient assay for the presence of the *RES1-1* mutation in segregants from genetic crosses (see below). These phenotypes of the *RES1-1* haploids are similar to those of strains containing *rme1* null mutations; the latter mutation does not support sporulation in haploid strains in some genetic backgrounds, even in the presence of the *spo13* mutation (Wagstaff, Klapholz and Esposito 1982), although it appears to do so in others (Margolskee 1988).

Interactions with *MAT*, *HML* and *HMR*: The ability of *RES1-1* strains to sporulate does not require the presence of either *MAT* allele from the original mutant strain, since we have recovered the *RES1-1* allele from segregants of both mating types. Moreover, *RES1-1* does not segregate as an allele of *MAT* in genetic crosses. Thus, *RES1-1* is not an allele of the mating type locus.

We wanted to determine whether, like *rme1*, the *RES1-1* mutation could bypass the normal requirement for both *MAT* alleles; alternatively, the mutation

TABLE 3

Sporulation of *RES1-1*

Strain	Relevant genotype	Percent sporulation[a]
GK56	*MAT***a**/*MAT*α *RES1*/*RES1*	65.7
GK22	*MAT***a** *RES1*	0.0
GK31	*MAT*α *RES1*	0.0
GK56(*mat***a**)	*mat***a***1*:*LEU2*/*MAT*α *RES1*/*RES1*	0.0
125-12	*MAT***a**/*MAT*α *RES1-1*/*RES1-1*	68.0
125-8	*MAT***a** *RES1-1*	0.0
125-6	*MAT*α *RES1-1*	0.0
125-12(*mat***a**)	*mat***a***1*:*LEU2*/*MAT*α *RES1-1*/*RES1-1*	11.7
125-18(*mat***a**)	*mat***a***1*:*LEU2*/*MAT*α *RES1-1*/*RES1-1*	13.0
GK22 × 125-6(*mat***a**)	*mat***a***1*:*LEU2*/*MAT*α *RES1*/*RES1-1*	3.5
GK31 × 125-8(*mat***a**)	*mat***a***1*:*LEU2*/*MAT*α *RES1*/*RES1-1*	4.6

[a] The percentage of sporulated cells was determined by DAPI staining of cells which had been exposed to sporulation medium for approximately 72 hr. The values given reflect the total of tetranucleate cells plus asci visible in the cultures at this time. At least 500 cells from each of two independent cultures of each strain were examined in the particular experiment shown.

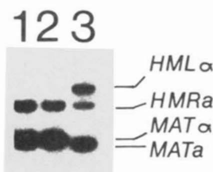

FIGURE 1.—Southern analysis of strains lacing *HML*α. Genomic DNA from a wild-type *MAT***a** haploid (lane 3) and from two *MAT*α Ura+ transformants (lanes 1 and 2) was digested with *Hin*dIII and hybridized to a *MAT***a** probe. This was a 4.2-kb *Hin*dIII fragment from YCp50-*MAT***a** that contained the wild type *MAT***a** allele. The positions of *MAT***a**, *MAT*α, *HML* and *HMR* are indicated.

could allow sporulation by activating the silent copies of *MAT* information located at *HML* and *HMR*. To distinguish these possibilities, we asked whether the *RES1-1* mutation could support sporulation in diploid strains that contain only **a** information anywhere in their genomes.

The required strains were constructed by deleting *HML*α from haploid *RES1-1* strains and wild-type *RES1* controls (see MATERIALS AND METHODS). This was accomplished by transforming the desired strains with a linearized plasmid (pAR65) that contained a telomere at one end and sequences just centromere-proximal to *HML* at the other. Ura+ transformants were screened by Southern blotting for the desired event, *e.g.*, removal of all chromosome *III* sequences distal to the site of integration of pAR65, including *HML*. A Southern blot confirming the absence of *HML* from the transformant recipients is shown in Figure 1. The resulting *RES1-1* haploids that lacked *HML* were able to express *SPR3*:*lacZ* at low levels, suggesting that these strains were like the original *RES1-1* strains in their ability to express sporulation functions as haploids.

The *MAT*α locus was then disrupted in diploids that were formed by mating. The relevant disrupted strains, genotypically *MAT***a**/*mat***a***1*:*LEU2*, *HMR***a**/*HMR***a**, *hml*:*URA3*/*hml*:*URA3*, were examined for their ability to sporulate. The results shown in Table 4 show that the *RES1-1* mutation bypassed the requirement for both mating type alleles; strains that lacked α information but carried the *RES1-1* mutation could sporulate to an appreciable extent (22% in the experiment shown). This level was comparable to that observed in isogenic strains that contained *HML*, as well as to other *RES1-1* strains that were defective at *MAT*. Controls that carried the wild type *RME1* allele did not sporulate in the absence of complete *MAT* information. We conclude that *RES1-1* does not allow *mat*-insufficient cells to sporulate by activating the silent copies of *MAT* information at *HML* and *HMR*.

Interactions between *RME1* and *RES1-1*: The following interactions were observed.

Expression of RME1 in RES1-1 background: One explanation for the apparent insensitivity of the *RES1-1* mutant to pGK23 might be that the mutant fails to express *RME1*. A mutation of this type would be insensitive to excess copies of *RME1* and could also allow sporulation in the absence of complete *MAT* information. To determine whether this was the basis for the observed *RES1-1* phenotypes, we tested the mutant strains for their ability to express and regulate *RME1* normally.

We measured the activity of β-galactosidase in extracts from wild type and mutant cells that had been transformed with pHW2, a *CEN*-containing plasmid which carries *RME1* fused to the *lacZ* gene of *E. coli* (MATERIALS AND METHODS). The data in Table 5 demonstrate that *RES1-1* mutant strains expressed *RME1*:*lacZ* at levels comparable to otherwise isogenic wild type strains, and that *RME1* was appropriately regulated by cell type in these strain backgrounds. As expected, activity was readily detected in *MAT***a** and *MAT*α strains, whereas it was substantially diminished in *MAT***a**/*MAT*α diploids (approximately 10-fold for both wild type and *RES1-1* strains). *mat*-insufficient strains also expressed *RME1*:*lacZ* at equivalent levels in the presence and absence of the *RES1-1* mutation; the specific activities were lower than in the haploid

TABLE 4

Independence of *RES1-1* dependent sporulation from information at *HML*

Strains	Percent sporulation[a]
Strains containing *HMLα*	
*MAT***a***/MATα RES1/RES1*	60.5
*mat***a***1:LEU2/MATα RES1/RES1*	0.0
*MAT***a***/mat***a***1:LEU2 RES1/RES1*	0.0[b]
*mat***a***1:LEU2/MATα RES1-1/RES1-1*	17.0
*MAT***a***/mat***a***1:LEU2 RES1-1/RES1-1*	17.2
Strains lacking *HML*	
*mat***a***1:LEU2/MATα RES1/RES1 hm1:URA3/hm1:URA*	0.0
*MAT***a***/mat***a***1:LEU2 RES1/RES1 hm1:URA3/hm1:URA3*	0.0
*mat***a***1:LEU2/MATα RES1-1/RES1-1 hm1:URA3/hm1:URA3*	12.3
*MAT***a***/mat***a***1:LEU2 RES1-1/RES1-1 hm1:URA3/hm1:URA3*	22.3

[a] Sporulation was monitored by DAPI staining the cells after 48 hr of incubation in sporulation medium. The values shown are averages of at least 300 cells from each of two or more isolates from each strain.

[b] This value was obtained as above from a separate experiment.

strains, presumably because the cells were larger. We conclude from these experiments that the phenotypes of the *RES1-1* mutation are not the result of defective *RME1* expression.

Epistatic interactions with RME1: Since mating type control of sporulation is not manifested solely through *RME1*, it was possible that *RES1-1* might define a second pathway rather than a target or effector of *RME1* activity. To examine this possibility, we first asked whether the sporulation ability of the *RES1-1* mutant strain could be enhanced by the presence of an *rme1* null mutation in the same strain. The rationale was that if *RES1* acts on the same pathway as *RME1*, the double mutant would sporulate no better than either single mutant, whereas if *RES1-1* and *rme1* acted on different or convergent pathways, the presence of both mutations might allow higher levels of sporulation than either would alone. Using the same techniques as above, we constructed *rme1:LEU2* null mutations in wild type and *RES1-1* mutant backgrounds. The strains also contained the null *mat***a**1:*LEU2* allele, to allow *MAT*-independent sporulation to be scored.

The results in Table 6 demonstrate that *RES1-1* and *RME1* define genetically different pathways. The *rme1:LEU2* mutation allowed approximately 5% sporulation to occur in *mat*-insufficient derivatives of our parent strain, irrespective of whether the disrupted *MAT* allele was *MAT***a** or *MATα*. The *RES1-1* mutation supported reproducibly higher levels than this (9–18%) in experiments when these were compared directly. Strains containing both the *rme1* and *RES1-1* mutations sporulated better than those containing either mutation alone. These results suggest that *RES1-1* mutants retain their sensitivity to *RME1*, and that the sporulation observed in *mat*-insufficient strains results from activation of functions that are unrelated to *RME1*.

Interactions between *RES1* and *IME1*: We envisioned two formal possibilities for the interaction between *RES1* and *IME1*. *RES1-1* could act downstream of *IME1*, bypassing the requirement for *IME1* activity to promote sporulation. Alternatively, *RES1-1* could act by activating *IME1* in the absence of appropriate *MAT* information. This could occur as a result of a mutation in a *trans*-acting modulator of *IME1* expression or activity, or by an alteration in *IME1* itself.

We first asked whether *RES1-1* would allow sporulation in the absence of *IME1* activity. Strains homozygous for null alleles of *IME1* were constructed using a plasmid-borne allele in which a *TRP1* selectable marker had been introduced at a *Cla*I site internal to the gene. The transformation recipients were the homothallic wild-type (GKY5) and *RES1-1* (125B-2c) strains, so that segregants could be examined directly for their ability to sporulate. Transformants that were heterozygous for the expected disruption were sporulated and approximately 40 segregants from each strain were obtained. In all cases, Trp^+ segregants failed to sporulate while Trp^- ones did so normally. Thus, neither wild type nor *RES1-1* diploids could sporulate when they lacked functional *IME1*. Similar strains that also contained the *SPR3:lacZ* fusion carried on a plasmid remained white on sporulation medium supplemented with X-gal. We conclude that the *RES1-1* mutation does not bypass the need for *IME1* for sporulation or for *SPR3:lacZ* expression.

To determine whether *IME1* regulation was normal in *RES1-1* mutants, we examined the steady-state levels of *IME1* transcripts in wild type and mutant strains (Figure 2) relative to the *URA3* transcript and to total RNA. As reported previously, *IME1* transcripts were undetectable in glucose-grown cells but present in YEPA-grown cells of all types, reaching high levels in stationary phase cultures (panels A–D, Figure 2; KASSIR, GRANOT and SIMCHEN 1988; data not shown). As expected, levels of RNA homologous to the *IME1* probe were very high in wild-type *MAT***a***/MATα* dip-

TABLE 5

***RME1:lacZ* activity in wild-type and *RES1-1* strains**

Genotype[a]	Wild type		RES1-1	
	Specific activity[b]	Relative activity[c]	Specific activity	Relative activity
*MAT***a**/*MATα*	0.011		0.019	
*mat***a***1:LEU2/MATα*	0.154	13.5	0.089	5.1
*mat***a***1:LEU2/MAT***a**	0.056	4.9	0.113	6.4
*MAT***a**	0.108	9.5	0.202	11.5
MATα	0.118	10.4	0.215	12.2

[a] Wild-type strains were GKY5 (diploid), GK25, GK31 and *mat*-insufficient derivatives of a cross between GK25 and GK31. *RES1-1* strains were 125B-2c, 125-8, 125-10, and *mat*-insufficient derivatives of a cross between 125-8 and 125-10.

[b] Specific activity was defined as nmole ONPG hydrolyzed/min/mg protein in extracts prepared from cells carrying pHW2. The *RME1* gene in pHW2 was fused to β-galactosidase as described in MATERIALS AND METHODS. Extracts were prepared from 5.0 ml of YEPD-grown cells.

[c] Relative activity is the level of β-galactosidase produced in each haploid or *mat*-insufficient strain by the level observed in isogenic *MAT***a**/*MATα* diploids.

TABLE 6

Epistatic interactions between *RES1-1* and *RME1*

Strains	Percent sporulation[a]
Strains that are wild type at *MAT*[b]	
*MAT***a**/*MATα RES1/RES1*	65.5
*MAT***a**/*MATα RES1-1/RES1-1*	64.9
mat-insufficient strains that are wild type for *RES1*[c]	
*mat***a***1:LEU2/MATα RME1/RME1*	0.0
*MAT***a**/*mat***a***1:LEU2 RME1/RME1*	0.0
*mat***a***1:LEU2/MATα rme1:LEU2/rme1:LEU2*	4.7
*MAT***a**/*mat***a***1:LEU2 rme1:LEU2/rme1:LEU2*	5.1
mat-insufficient strains that contain the *RES1-1* mutation[d]	
*mat***a***1:LEU2/MATα RME1/RME1*	9.0
*MAT***a***1/mat***a***:LEU2 RME1/RME1*	19.5
*mat***a***1:LEU2/MATα rme1:LEU2/rme1:LEU2*	18.6
*MAT***a**/*mat***a***1:LEU2 rme1:LEU2/rme1:LEU2*	39.5

[a] Percent sporulation was determined by light microscopy after 72 hr of incubation in sporulation medium. Values shown are averages for at least two isolates of each genotype for three independent experiments.

[b] The wild type *MAT* strains are GKY5 and 125B-2c, respectively.

[c] *mat*-insufficient Rme⁺ strains were made by disrupting *MAT* in GK53. Comparable Rme⁻ strains were made by disrupting *rme1* in GK22 and GK31, mating and disrupting *MAT* in the diploid (see MATERIALS AND METHODS).

[d] Rme⁺ strains were made by disrupting *MAT* in 125-18. Comparable Rme⁻ strains were constructed by disrupting *RME1* in 125-10 and 125-2 and disrupting *MAT* in the resulting diploid.

loids that had been exposed to sporulation medium for three to eight hours (panel A), relative to those observed in isogenic *mat*-insufficient diploids and haploids (Figure 2, panels B, C and D). Wild type and *RES1-1* strains behaved identically with respect to their ability to regulate *IME1* during growth in glucose and acetate; *IME1* levels were indistinguishable under conditions of vegetative growth, as was the apparent induction during the early phase of sporulation (Figure 2 and not shown).

Unlike the wild-type strains, however, all *RES1-1* mutant strains accumulated high levels of *IME1* transcripts shortly after they had been shifted to sporulation medium, irrespective of their ploidy or *MAT* genotype (panels B, C and D). In all of the *RES1-1* strains, the increase in *IME1* RNA in *RES1-1* strains relative to wild-type controls was clearly evident by 3 hr after the shift and high levels were sustained until at least 8 hr in this and other experiments. These levels were lower than those attained by *MAT***a**/*MATα* diploids, to a degree that appeared to be consistent with the relatively modest sporulation promoted by the *RES1-1* mutation. We conclude that *RES1-1* strains can sporulate in the absence of complete *MAT* information because they are capable of inducing *IME1* under such conditions.

Segregation of *RES1* and *IME1* in genetic crosses: Because the *RES1-1* mutation is dominant, it was not possible to examine its allelism to *IME1* by complementing the *RES1-1* defect with a wild-type *IME1* allele. Instead, we examined the segregation of *RES1-1* relative to a marked *IME1* locus using genetic crosses. We expected that haploid segregants carrying *RES1-1* could be distinguished from those containing the wild-type allele, by the ability of the former to produce pale blue colonies on sporulation medium supplemented with X-gal. To confirm that *SPR3:lacZ* expression in haploids was a reliable assay for the *RES1-1* mutation, we analyzed the pattern of color production in segregants from a diploid that was heterozygous for *RES1-1* and contained *lacZ* integrated at both chromosomal *SPR3* loci. Indeed, blue color segregated 2:2 in all eight complete tetrads examined, as expected (see also above).

To examine possible allelism between *RES1-1* and *IME1*, we constructed *MAT***a**/*MATα* diploids from which we could also monitor segregation of *IME1*. One parent was a haploid carrying the *RES1-1* mutation, and the other type was wild type for *RES1* but contained an allele of *IME1* that was tagged by the insertion of *TRP1* marker in tandem with the resident allele. This was accomplished by integrating pAM506 at a unique *Nco*I site approximately 500 bp 5′ to the *IME1* transcript start. This insertion also introduced the small deletion present in the pAM506 allele into a region immediately adjacent to the *Nco*I site. This complicated subsequent analysis somewhat, since the

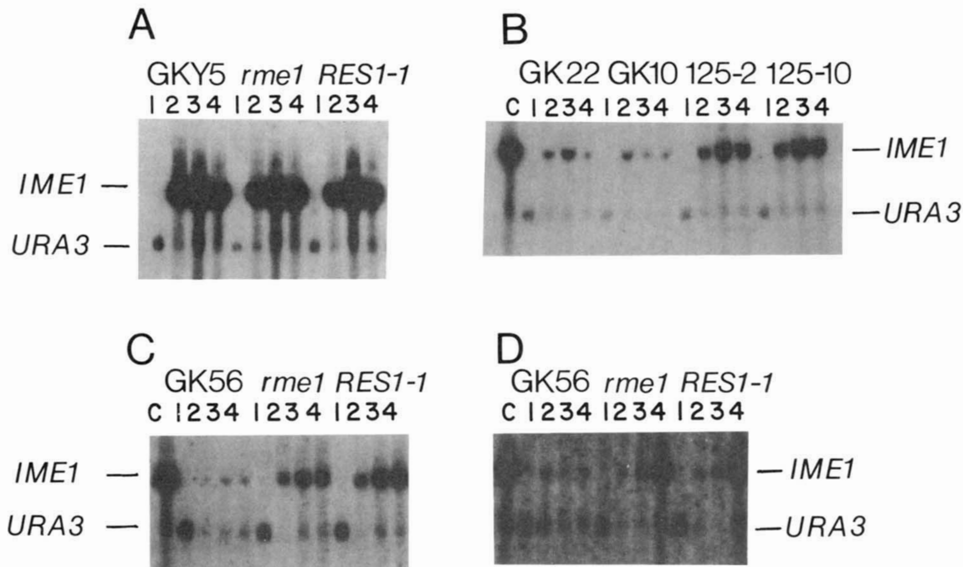

FIGURE 2.—Northern blot analysis of *IME1* transcript accumulation in wild-type and *RES1-1* strains. Total RNA was isolated from *S. cerevisiae* strains, fractionated through agarose gels in the presence of 1.1% formaldehyde and blotted to nitrocellulose. *IME1* transcript was detected using a 0.4-kb *Eco*RI fragment internal to the gene. The source of this fragment was pAM506 (kindly provided by AARON MITCHELL). *URA3* was detected using a 1.1-kb *Hin*dIII fragment from YEp24 that had been cloned into pBLUESCRIPT. The latter was the gift of TOM PUGH. The locations of each of these transcripts are shown. Lanes 1 contain approximately 10 μg of total RNA that was prepared from YEPD-grown cells. Lanes 2 are from cells that had been grown to saturation in YEPA. Lanes 3 and 4 contain RNA from cells that were incubated in sporulation medium for 3 and 8 hours, respectively. Lanes marked C indicate RNA from diploid *MAT***a**/*MATα* cells that had been incubated in sporulation medium for 3 hr. Panel A, Abundance of *IME1* RNA in *MAT***a**/*MATα* diploids that contain the wild type *RES1* or mutant *rme1* or *RES1-1* alleles. Wild type is indicated as GKY5; *rme1* indicates that the RNA was prepared from *rme1:LEU2* derivatives of GK56; lanes marked as *RES1-1* contain RNA prepared from 125-18. Panel B, Abundance of *IME1* RNA in wild type haploids GK22 and GK10 (*MAT***a** and *MATα*, respectively) and in corresponding *RES1-1* mutant strains (125-2 and 125-10). Panels C and D, Abundance of *IME1* RNA in *MAT***a**/*mat***a***1:LEU2* (panel C) and *mat***a***1:LEU2/MATα* (panel D) diploid strains. These strains were otherwise wild type (GK56), defective for *RME1* (*rme1*), or defective for *RES1* (*RES1-1*) as above. The *RES1-1* strains were isogenic to 125-18 except for the disruptions at *MAT*.

marked allele was only partially functional and therefore masked the *RES1-1* phenotype in double mutant strains.

We anticipated, therefore, that if *RES1-1* were allelic to *IME1*, each tetrad would yield two blue Trp⁻ segregants and two white Trp⁺ segregants. Alternatively, if *RES1-1* and *IME1* segregated independently of each other, one or both of the *RES1-1* segregants in any tetrad could be white, due to the presence of the crippled *IME1:ime1:TRP1* allele. In this case, blue color would segregate 2:2 (PD), 1:3 (TT) or 0:4 (NPD), in a ratio of 1:4:1. The results we obtained were consistent with the latter hypothesis; of the eighteen complete tetrads examined, four showed a 2:2 pattern of segregation, three were 0:4 and 11 were 1:3. All tetrads segregated 2:2 for tryptophan prototrophy, and for the **a** and α alleles at *MAT*.

To confirm the expected *RES1-1* genotypes of the Trp⁺ segregants from the 0:4 tetrads, we introduced a plasmid-borne wild-type allele of *IME1* (pM4B) into these strains. The *RES1-1* allele was revealed under these conditions; as expected, all four Trp⁺ segregants examined became blue when exposed to sporulation medium. We also tested the two Trp⁺ segregants from each of three tetrads in which blue color had segregated 1:3; in each case, one of the previously white haploids became blue, whereas the other remained white. This confirmed that *RES1-1* had segregated 2:2 in these tetrads. We conclude that *RES1* is not allelic to *IME1*.

DISCUSSION

We have screened for mutations that allow diploid *Saccharomyces cerevisiae* cells to express a late sporulation-regulated gene (*SPR3*) in the presence of excess copies of *RME1*. By screening in *MAT***a**/*MATα* cells, we hoped to define functions that are relevant to the activation of sporulation in diploid cells. Such mutations could act by enhancing *IME1* transcription, mRNA stability or activity, or they could release downstream functions from their normal requirement for high levels of *IME1*. Related screens in other laboratories have successfully identified chromosomal mutations and high copy plasmids that alter both of these levels of regulation. Defects in *UME* genes (STRICH, SLATER and ESPOSITO 1989) and overexpression of *IME2* (SMITH and MITCHELL 1989; MITCHELL, DRISCOLL and SMITH 1990) allow expression of early sporulation functions and some sporulation, respectively, in the absence of *IME1* activity; these genes therefore define functions that act downstream of *IME1*. Conversely, *IME3*, which was identified in the

same screen that yielded *IME2*, appears to function upstream of *IME1* (AARON MITCHELL and LENORE NEIGEBORN, personal communication).

The *RES1-1* mutation allows full levels of *SPR3:lacZ* activity and restores sporulation to diploid cells that contain excess *RME1*. *RES1-1* is semidominant to its wild type *RES1* allele. Although we detected *RES1-1* in *MAT***a**/*MAT*α diploids, the mutation confers phenotypes on all cell types; *RES1-1* diploid cells can sporulate in the absence of complete *MAT* information and the corresponding haploids can express *SPR3:lacZ* at low levels. These phenotypes are due to the ability of *RES1-1* strains to express *IME1* at high levels upon shift to starvation medium, irrespective of the presence of the *MAT* products. These phenotypes are not the result of activation of the silent *HML* and *HMR* loci, since *RES1-1* cells that contain only **a** information retain their ability to sporulate. Neither are they the result of a failure to express *RME1* or to respond to the *RME1* product.

Our ability to interpret these results in terms of the normal regulation of the *RES1* product is hampered by a lack of information about the nature of the *RES1-1* allele and the phenotype of the null mutant. One possibility that is of particular concern is that the *RES1-1* allele is a spurious dominant such as might result from a transposon insertion or other gross rearrangement. A deregulated allele of this type could produce all of the phenotypes that we see but would not be informative of the regulation that occurs in wild-type cells. Because of this, we are reluctant to speculate on the role of the wild-type *RES1* product or its relationship to mating type control of sporulation; although the mutant allele confers phenotypes on all cell types, it is not at all clear whether the wild type product is normally present in all cells.

Nonetheless, we can make some conclusions. Since the *RES1-1* allele enhances sporulation in all cell types, we suggest that the wild type functions in *MAT***a**/*MAT*α diploid cells to regulate *IME1*. In this respect, *RES1-1* defines a different sort of regulator from *RME1*, which is relevant only to *mat*-insufficient cells and haploids. This eliminates any potential activities for *RES1* that would be restricted to *mat*-insufficient cells. For example, one function that might be expected to exist, based on the limited sporulation promoted by *rme1* null mutations, is an additional repressor that acts in concert with *RME1* to regulate sporulation; inactivation of a molecule of this sort could explain the *RES1-1* phenotype in *mat*-insufficient cells but not in diploids, because, like *rme1*, such a molecule would normally be absent from *MAT***a**/*MAT*α diploids. This argument assumes, of course, that the low levels of *RME1* normally produced by *MAT***a**/*MAT*α diploids are not physiologically relevant, as has been suggested (MITCHELL and HERSKOWITZ 1986).

Because *IME1* plays a crucial role in promoting meiosis, it is likely to be subject to complex regulation, at the transcriptional and post-transcriptional levels. Indeed, *IME1* transcription is mediated by an unusually long upstream region that contains distinct targets for cell type and nutritional information (GRANOT, MARGOLSKEE and SIMCHEN 1989; YONA KASSIR, personal communication). Response to *RME1* is governed by an element that is located approximately 2.5 kb upstream of the *IME1* transcript start site. Downstream of this element are negatively acting sites that respond to the environment. These sequences are distinct from the UAS region (−220 to −500), defined by its ability to promote complementation of *ime1* defects in *MAT***a**/*MAT*α diploids. The recent reports (YOSHIDA *et al.* 1990; L. NEIGEBORN and A. MITCHELL, personal communication) that *IME2* (*SME1*) and *IME3* have homology to protein kinases also support the notion that control of the early events of meiosis is not mediated exclusively at the transcriptional level.

Our genetic evidence indicates that *RES1-1* and *RME1* control *IME1* accumulation in distinct ways. The *RES1-1* mutation supports levels of sporulation in *mat*-insufficient cells that are higher than those supported by *rme1*. In addition, *mat*-insufficient strains that contain both mutations sporulate better than either single mutant and in some cases approach diploid levels. These results show that *RES1-1* defines a pathway that is genetically distinct from that controlled by *RME1* and consequently, that *RES1-1* is not an insensitive target of *RME1* activity. They also show that *IME1* accumulation remains sensitive to *RME1* in *RES1-1* strains but imply that *RME1* is not sufficient to repress *IME1* fully in such strains. Although we have not examined the possibility directly, these results also confirm the expectation (from the initial screen) that the *RES1-1* is not an allele of *RME1*.

There are several possible models for *RES1* activity that are equally compatible with our data. One attractive role for *RES1* (and the *RES1-1* allele) is that of a regulator of *IME1* whose activity is modulated in diploids in response to conditions that promote sporulation. The existence of such an activator would explain the observation that diploids are not fully repressed by *RME1*; the levels of *IME1* produced in the presence of *RME1* would be high enough to allow some sporulation, since activator and repressor activities would antagonize each other. This model for *RES1* demands that the *RES1-1* allele be hyperactivated in diploids as well as *mat*-insufficient cells, to explain the phenotypes in the various cell types. This could result from artificial overexpression of the wild type *RES1* gene in all cell types, as discussed above, or as the result of a mutation in the coding region of the gene that causes the protein to respond aberrantly to signals that normally regulate its activity.

We have no evidence for any hyperactivating activity of the *RES1-1* allele in diploids, except for their

ability to escape *RME1* repression. Our Northern analysis of the *IME1* transcript indicates that the gene responds identically to nutritional signals and that the levels of *IME1* accumulated during sporulation are essentially the same in *RES1-1 vs.* wild-type cells. The only aberration that we could detect was that *mat*-insufficient *RES1-1* cells accumulated high levels of *IME1* transcript during starvation, whereas wild type cells did not. That regulation of *IME1* should appear normal in *MAT*a/*MAT*α diploids is not too surprising, however, given the leakiness of the *RME1* repression in these cells; an increase of 20–30% in *IME1* activity due to the *RES1-1* mutation could drive sporulation in the blocked cells but might not be apparent by Northern analysis. Similarly, relief of one control (*i.e.*, constitutive activation of *RES1*) would not be expected to cause constitutive activation of *IME1*, since additional controls that respond to carbon source and other stimuli would remain operative.

It is also possible that the wild type *RES1* product functions as a negative regulator of *IME1* that normally functions in all cell types. One activity for *RES1* that would be consistent with such a role is that of a direct repressor of *IME1* transcription. Alternatively, *RES1* could act at the protein level to restrain an activator of *IME1*. If either of these models is correct, the phenotypes that result from the dominant *RES1-1* allele could result from reduced levels of the wild-type product such that *IME1* is not fully repressed in heterozygous strains (*i.e.*, haplo-insufficiency). Alternatively, the product of the *RES1-1* allele could interfere with the activity of the corresponding wild-type protein (*i.e.*, an antimorph).

Another equally likely model for the activity of *RES1* is that it participates in modulating the stability of *IME1* mRNA. *RES1-1* could inactivate a system for negative control of *IME1* that normally serves to damp *IME1* accumulation. There is some evidence for a "feedback" system that down-regulates *IME1* in cells that have accumulated sufficient *IME2* (SMITH and MITCHELL 1989), and it is possible that *RES1* is a component of this system.

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