Requirement for *RGR1* and *SIN4* in RME1-Dependent Repression in *Saccharomyces cerevisiae*

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ABSTRACT

RME1 is a zinc-finger protein homolog that functions as a repressor of the meiotic activator *IME1*. RME1 is unusual among yeast repressors in two respects: it acts over a considerable distance (2 kbp) and it can activate transcription from a binding site separated from its natural flanking region. To identify genes required for RME1 to exert repression, we have selected mutants with improved RME1-dependent activation. One rare mutant was defective in RME1-dependent repression of an artificial reporter gene as well as the native *IME1* gene. The mutation permits sporulation of **a**/**a** diploids, which express *RME1* from its natural promoter, and of **a**/α diploids constructed to express *RME1* from the *GAL1* promoter. The mutation also causes temperature-sensitive growth and a methionine or cysteine requirement. Analysis of a complementing genomic clone indicates that the mutation lies in a known essential gene, *RGR1*. Prior studies have indicated a functional relationship between *RGR1* and *SIN4* (also called *TSF3*); we have found that a *sin4* null mutation also causes a defect in RME1-dependent repression and a methionine or cysteine requirement. The *rgr1* and *sin4* mutations do not cause a reduction of RME1 polypeptide levels. The defect in RME1-dependent repression may result from effects of *sin4* and, presumably, *rgr1* on chromatin structure.

THE yeast *RME1* gene specifies an inhibitor of meiosis and spore formation (KASSIR and SIMCHEN 1976; MITCHELL and HERSKOWITZ 1986; reviewed in HONIGBERG *et al.* 1993; MITCHELL 1994). *RME1* is expressed in **a** and α cells, which are unable to enter meiosis, and is repressed in **a**/α cells, which enter meiosis in response to starvation (MITCHELL and HERSKOWITZ 1986). RME1 blocks meiosis by preventing expression of *IME1* (KASSIR *et al.* 1988), a positive regulator of many or all genes that are expressed primarily during meiosis (ENGEBRECHT and ROEDER 1990; SMITH and MITCHELL 1989; SMITH *et al.* 1990). In vegetative cells, *IME1* is expressed at low levels in the presence or absence of RME1; in starved cells, *IME1* is expressed at much higher levels only in the absence of RME1 (COVITZ *et al.* 1991; KASSIR *et al.* 1988; SMITH and MITCHELL 1989).

RME1, a zinc-finger protein homolog (COVITZ *et al.* 1991), acts directly as a repressor of *IME1*. RME1 binds to a site that lies 2 kbp upstream of the *IME1* gene (see Figure 1A; COVITZ and MITCHELL 1993). Deletion of the binding site partially relieves RME1-dependent repression of *IME1*, so the site is required for full repression. The binding site is contained within a 404-bp DNA segment, called the repression cassette, that confers RME1-dependent repression of the heterologous *CYC1* promoter when inserted adjacent to the *CYC1* upstream activation sequences (Figure 1B; COVITZ and MITCHELL 1993). Binding of RME1 to DNA is necessary but not sufficient for repression, as indicated by three observations. First, a 233-bp segment of the repression cassette adjacent to the RME1 binding site is essential for repression; we refer to this segment as the modulation region. Second, a small DNA segment that includes the RME1 binding site activates transcription. Activation by this segment (which we call the RME1 response element) depends on overexpression of RME1. Third, repression by the repression cassette is exerted in only one orientation (COVITZ 1993). These observations indicate that repression by RME1 is more complex than predicted by a simple repressor-operator interaction. We have suggested that repression and activation by RME1 reflect alternative protein complexes at the repression cassette, or that repression may depend on precise spacing between RME1 and the target promoter (COVITZ and MITCHELL 1993).

The fact that binding of RME1 to DNA can have opposite effects, depending on the context of the binding site, implies that additional gene products may be required for RME1-dependent processes. Thus far, the only known mutations that impair RME1-dependent repression or activation lie in *RME1* or the RME1 binding site. In this study, we have made use of the properties of the repression cassette to select a mutant defective in repression. Our results implicate two global regulatory genes, *RGR1* and *SIN4*, in RME1-dependent repression.

MATERIALS AND METHODS

Yeast strains and genetic methods: Yeast strains are listed in Table 1. Strains JSHα and JSH125α were provided by

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M. CLANCY (KAO *et al.* 1990); strain 1446-2C was derived from JSHα by *HO*-initiated mating type interconversion (HERSKOWITZ and JENSEN 1991). Strains 150 and 251, which were used to score *RES1-1* (see below), resulted from crosses between mapping strains (SHERMAN and WAKEM 1991). All other strains were constructed through standard genetic manipulations (ROSE *et al.* 1990) of rapidly sporulating SK-1 strains (ALANI *et al.* 1987; KANE and ROTH 1974).

Auxotrophic markers have been described (ALANI *et al.* 1987; SMITH *et al.* 1990). The *sin4::TRP1* mutation was introduced by transformation, as described by JIANG and STILLMAN (1992). *RME1* alleles have been described in detail (COVITZ *et al.* 1991); their relevant properties are summarized here. *rme1Δ5::LEU2* is a complete deletion of the *RME1* coding region. *rme1::P_{GAL1}-S53-RME1::TRP1* replaces the *RME1* promoter with the *GAL1* promoter and modifies the N terminus of RME1 to include the S53 epitope. This allele, which we refer to in the text as *P_{GAL1}-RME1*, is functional. *rme1::P_{GAL1}-S53-rme1-213::TRP1* is a missense allele of *P_{GAL1}-RME1* causing a substitution in the the second RME1 zinc finger motif. This allele, which we refer to as *P_{GAL1}-rme1-213*, abolishes RME1 site-specific DNA binding (COVITZ and MITCHELL 1993) but not polypeptide accumulation (COVITZ *et al.* 1991).

Our SK-1 strains fail to ferment galactose. We have introduced a *gal80* mutation in many strains to permit expression of genes from the *GAL1* promoter (MITCHELL *et al.* 1990; SMITH *et al.* 1990). In *gal80* mutants, the *GAL1* promoter is expressed in the absence of glucose, such as in media with galactose or acetate as sole carbon source (JOHNSTON 1987; TORCHIA *et al.* 1984).

Most **a**/**a** diploids were constructed from **a**/α diploids after mild UV irradiation (90% survival) by screening for mating ability with tester lawns. To construct strain 1398, the α parent was first transformed with an integrating *URA3 MAT*α plasmid (YIp5-*MAT*α). After mating, purified **a**/α diploids were plated on 5-fluoro-orotic acid medium to select for Ura$^-$ segregants (ROSE *et al.* 1990). Two of four independent Ura$^-$ segregants displayed ability to mate with α strains and were assumed to be **a**/**a** diploids.

The *rgr1-100* mutant was isolated as follows. A washed, saturated YPD culture of strain 1343 was spread on galactose medium without histidine. Approximately 2×10^8 cells were plated on each of 20 15-cm Petri dishes. The plates were UV-irradiated to yield 20% survival and incubated at 30°. His$^+$ papillae arose after 4–5 days. They were purified on galactose medium lacking histidine before further characterization. The *rgr1-100* mutation causes several phenotypes. First, there are defects in RME1-dependent repression detailed in the Results section. Second, there is a temperature-sensitive growth defect. On synthetic glucose medium, *rgr1-100* strains form small colonies at 30° and pinpoint colonies at 37°. On YPD medium, *rgr1-100* strains form small colonies at 30° and 37°, and become inviable after a temperature shift during exponential growth from 30° to 37°. The cells arrest with unseparated daughter cells that are removed by zymolyase treatment, consistent with incomplete septation (SAKAI *et al.* 1990). Third, there is a requirement for either methionine or cysteine (20 mg/liter) at 30° in synthetic glucose medium. Fourth, there is slight clumpiness, which is most apparent from the settling of cells in liquid culture. However, all SK-1-derived strains are relatively clumpy, so this mutant phenotype is subtle.

The dominant *RES1-1* mutation was followed in crosses through its stimulation of sporulation in the presence of a multi-copy *RME1* plasmid (KAO *et al.* 1990). Each spore colony from a *RES1-1/RES1* diploid was mated to tester strains 150 and 251 (both *RES1*) carrying multi-copy *RME1* plasmids pAM232 (MITCHELL and HERSKOWITZ 1986) or pAM226R-2μ (SMITH and MITCHELL 1989), which carry *URA3* and *LEU2* genes, respectively. Diploids from matings to the testers were selected on SC-Ura-His or SC-Leu-His and then incubated on Spo plates for 5 days at room temperature. The Spo plates were then replica-plated to synthetic medium to select Ura$^+$ His$^+$ or Leu$^+$ His$^+$ progeny resistant to both canavanine and cycloheximide. Diploids able to sporulate (*RES1-1/RES1*) when carrying the *RME1* plasmid yielded numerous drug-resistant papillae, whereas diploids unable to sporulate (*RES1/RES1*) did not.

Growth media: Standard growth media included YPD (2% glucose, 2% Bacto-peptone, 1% Difco yeast extract), YPAc (2% potassium acetate, 2% Bacto-peptone, 1% Difco yeast extract), and synthetic media containing either 2% glucose or 2% galactose as carbon source (ROSE *et al.* 1990). Media were solidified with 2% agar when necessary. Galactose fermentation ability was determined on galactose indicator plates (ROSE *et al.* 1990) or by following the Leu$^+$ phenotype conferred by the *gal80::LEU2* disruption (TORCHIA *et al.* 1984). Sporulation ability was tested after incubation on Spo plates (SMITH and MITCHELL 1989). The level of resistance of strains to 3-aminotriazole was determined by their ability to form single colonies on galactose medium lacking histidine and containing 0 mM, 5 mM, 10 mM, 20 mM, 40 mM, 60 mM, or 100 mM 3-aminotriazole.

***HIS3* and *CYC1-lacZ* hybrid genes:** The *HIS3* reporter genes diagrammed in Figure 1C are derived from plasmid pD1509 (provided by M. GWADZ and D. SHORE), which contains the *HIS3* coding region and 5′ sequences up to −53 from the transcription start site (STRUHL 1986) in the vector pRS314 (SIKORSKI and HIETER 1989). These 5′ sequences include the TATA region but lack activation sequences (STRUHL 1986), so this gene is designated *ΔUAS-HIS3*. *IME1* regulatory sequences were inserted as *Apa*I-*Eco*RI fragments (from inserts within a pBluescript II polylinker) between the *Apa*I and *Eco*RI sites of pD1509 to create episomal *RC-HIS3*, *RRE-HIS3*, *MR-HIS3* and *RRE-MR-HIS3* genes (Figure 1C). The *RC-HIS3* hybrid gene was transferred as a *Xho*I-*Bam*HI fragment into the *Xho*I and *Bam*HI sites in the integrating vector pRS306 (SIKORSKI and HIETER 1989) to form plasmid pWS6-4. Integration of pWS6-4 was directed to the chromosomal *ura3* gene by digestion with *Stu*I. We note that strains carrying the plasmid-borne *RC-HIS3* gene displayed weak His$^+$ growth, whereas strains carrying the integrated *RC-HIS3* gene were His$^-$. We assume that this difference arises from increased gene dosage of the plasmid-borne gene.

The *CYC1-lacZ* reporter gene is carried in plasmid pKB112 (BOWDISH and MITCHELL 1993). Insertion of the repression cassette into pKB112 yielded the *RC-CYC1-lacZ* reporter gene (see Figure 1D) in plasmid pAC153-4 (COVITZ and MITCHELL 1993). The *ΔUAS-CYC1-lacZ* reporter gene is carried in plasmid pLGΔ312SΔSS (GUARENTE and MASON 1983). Insertion of the RME1 reponse element into pLGΔ312SΔSS yielded the *RRE-CYC1-lacZ* reporter gene (Figure 1D) in plasmid pAC110-6 (COVITZ and MITCHELL 1993).

Construction of *RGR1* plasmids: The *RGR1* plasmid pS7-2 was isolated from a yeast genomic library in plasmid pRS314 (SU and MITCHELL 1993b). Subclones of pS7-2 were constructed by standard methods of restriction enzyme digestion, fragment isolation, and ligation. pAC303, pAC306 and pAC307 are inserts in plasmid pRS314 (SIKORSKI and HIETER 1989). Several smaller subclones could not be constructed in pRS314, suggesting that *RGR1* sequences might be toxic in one orientation. Thus pAC313, pAC320, pAC320ΔBG and pRS414-RGR were constructed in pRS414, in which the polylinker sequences are reversed. pAC313 was constructed by

TABLE 1

Yeast strains

Strain	Genotype[a]
SK-1 derivatives:	
107	a *GAL80*
714	a *rme1Δ5::LEU2 his3 met4*
931	a/a *his4-G/his4-N arg6/ARG6 ade3/ADE3*
1040	α *rme1Δ5::LEU2*
1122	α *rme1::P_{GAL1}-S53-RME1::TRP1*
1124	α *rme1::P_{GAL1}-S53-rme1-213::TRP1*
1323	α *rme1::PGAL1-S53-RME1::TRP1 rgr1-100 his3*
1342	a *ura3::RC-HIS3::URA3 GAL80 his3*
1343	α *rme1::P_{GAL1}-S53-RME1::TRP1 ura3::RC-HIS3::URA his3*
1343r1	α *rme1::P_{GAL1}-S53-RME1::TRP1 ura3::RC-HIS3::URA3 his3 rgr1-100*
1350	a *ura3::RC-HIS3::URA3 rgr1-100 GAL80 his3*
1357	α *rgr1-100 TRP1 LEU2 GAL80*
1360	a/a *ura3/ura3::RC-HIS3::URA3 rgr1-100/rgr1-100 GAL80/GAL80 his3/his3 trp1/TRP1 leu2/LEU2*
1362	a/a *rme1Δ5::LEU2/rme1Δ5::LEU2 gal80::LEU2/GAL80 his3/HIS3 trp1/TRP1*
1363	a *rme1::P_{GAL1}-S53-RME1::TRP1 ura3::RC-HIS3::URA3 GAL80 his3*
1365	a *ura3::RC-HIS3::URA3 GAL80 his3*
1366	a *ura3::RC-HIS3::URA3 his3*
1368	a *ura3::RC-HIS3::URA3 rgr1-100 his3*
1369	a *rme1::P_{GAL1}-S53-RME1::TRP1 rgr1-100 his3*
1370	α *rme1::P_{GAL1}-S53-rme1-213::TRP1 rgr1-100*
1377	a *rgr1-100 his3*
1379	a *rgr1-100 his3*
1380	α *rme1Δ5::LEU2 his3*
1396	α *rme1::P_{GAL1}-S53-RME1::TRP1 sin4::TRP1 arg6*
1397	α *rme1::P_{GAL1}-S53-rme1-213::TRP1 sin4::TRP1 arg6*
1398	a/a *rgr1-100/rgr1-100 his3/HIS3*
1399	a *rme1::P_{GAL1}-S53-RME1::TRP1 sin4::TRP1 his3*
Derivatives of other genetic backgrounds:	
JSHα	α *ura3 leu2 trp1 lys2 his3 ho::HIS3 spr3-lacZ*
JSH125α	α *ura3 leu2 trp1 lys2 his3 ho::HIS3 spr3-lacZ RES1-1*
1446-2C	a *ura3 leu2 trp1 lys2 his3 ho::HIS3 spr3-lacZ*
150	α *ura3 trp1 leu2 his4 can1 cyh2*
251	a *ura3 trp1 leu2 his7 tyr1 can1 cyh2*

[a] All SK-1 derivatives carried the mutations *leu2::hisG trp1::hisG lys2 ura3 ho::LYS2 gal80::LEU2*, except as indicated.

insertion of the 4.7-kbp *Eco*RI-*Bgl*II fragment containing *RGR1* into the *Eco*RI and *Bam*HI sites of pRS414. Oligonucleotide mutagenesis of pAC320 was used to introduce the sequence GATCTA (a *Bgl*II site) between *RGR1* codons 1081 and 1082, yielding plasmid pAC320. pAC320ΔBG was constructed by deletion between *Bam*HI and *Bgl*II sites in pAC320, leaving six codons of *RGR1* and one codon specified by the *Bgl*II half-site. pRS414-RGR was constructed by insertion of the *Eco*RI-*Bgl*II fragment from pAC320 into the *Eco*RI and *Bam*HI sites of pRS414; it results in fusion of *RGR1* at codon 1081 to the plasmid-encoded *lacZ* α-fragment. The integrating plasmid pAC300 was made by transferring the 3.1-kbp *Bgl*II fragment from plasmid pS7-2 into the *Bam*HI site of plasmid YIp5 (Rose *et al.* 1990). Integration of a *URA3* marker at the *RGR1* locus was targeted by transformation of strain 107 with *Xho*I-digested pAC300 DNA.

Immunoblots: Cells from a 50-ml exponential culture in synthetic galactose medium were extracted in 0.1 M Tris (pH 7.4), 0.2 mM phenylmethylsulfonyl fluoride, 0.7 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 1 μg/ml aprotinin by vortexing with 0.45-mm glass beads. Debris was pelleted in a microcentrifuge, and 100 μg of extract protein (estimated with a Bio-Rad protein assay kit) were fractionated on a 12.5% polyacrylamide sodium dodecyl sulfate gel. Proteins were electrophoretically transferred to Immobilon P paper and visualized with rabbit anti-S53 immunoglobulin G and peroxidase-conjugated goat anti-rabbit antibodies, as described previously (Covitz *et al.* 1991).

Miscellaneous methods: β-Galactosidase assays were conducted as described elsewhere (Covitz and Mitchell 1993) with at least three independent cultures of each strain. Procedures for preparation of RNA, electrophoresis and Northern blot analysis have been described previously (Smith and Mitchell 1989). Probes for *IME1* (Mitchell *et al.* 1990) and a control transcript, carried on plasmid pC4/2 (Law and Segall 1988; Su and Mitchell 1993a), were labeled with a random-primed synthesis labeling kit (Boehringer).

RESULTS

Properties of *HIS3* reporter genes containing *IME1* regulatory sequences: Previous studies of the *IME1* promoter had identified a region, called the repression cassette, that could confer RME1-dependent repression on the heterologous *CYC1* promoter. The repression cassette comprises two functional units: the RME1-response element and the modulation region (Figure 1B). Neither of these segments alone can confer RME1-dependent repression on the *CYC1* promoter. The RME1-response element, which includes an RME1 binding site, permits RME1-dependent activation of an adjacent promoter which lacks upstream activation sequences of its own. The modulation region, when adjacent to the RME1-response element, both weakens

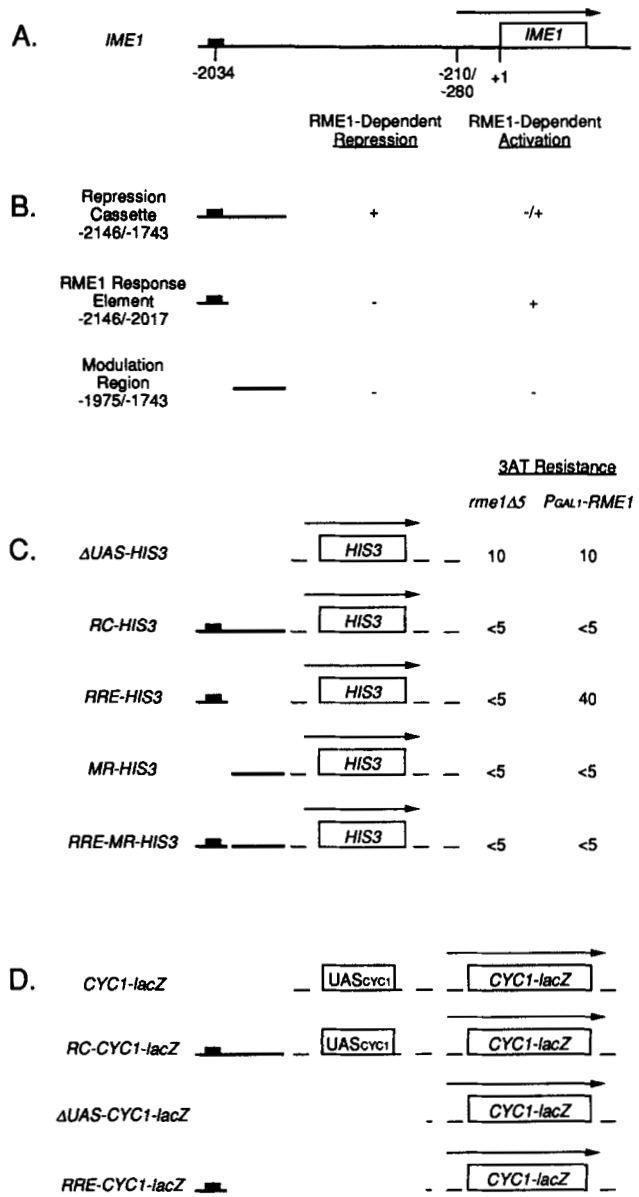

FIGURE 1.—Relevant segments of the *IME1* regulatory region and their properties. (A) The *IME1* gene. The deduced *IME1* open reading frame begins at base pair +1; RNA start sites lie between -210 and -280 (SHERMAN *et al.* 1993; Smith *et al.* 1990). The black rectangle represents the one known binding site for the repressor RME1, centered at -2034 (COVITZ and MITCHELL 1993); other binding sites are thought to exist (COVITZ 1993; COVITZ and MITCHELL 1993; GRANOT *et al.* 1989). (B) Properties of regions required for RME1-dependent repression (based on COVITZ and MITCHELL 1993). Repression is assayed through a downstream *CYC1-lacZ* reporter gene that includes its own UAS. Activation is assayed through a downstream *CYC1-lacZ* reporter gene that has no UAS. RME1-dependent activation has only been observed in cells that overexpress RME1 from a P_{GAL1}-*RME1* hybrid gene. (C) Properties of *HIS3* reporter genes. The *HIS3* segment (dashed line) includes the entire coding region, RNA start sites, and TATA region (STRUHL 1986). This segment is fused to vector sequences (Δ*UAS-HIS3*) or to *IME1* upstream sequences. Numbers at the right indicate the minimum millimolar concentration of 3-aminotriazole that blocks growth of strains 714 (*rme1Δ5*) and 1052 (P_{GAL1}-*RME1*), carrying the respective *HIS3* plasmids, in galactose medium without histidine. (D) *CYC1-lacZ* reporter genes. *CYC1-lacZ* sequences (dashed line) include the *CYC1* UAS region and a *CYC1-lacZ* fusion, as indicated. *IME1* sequences include the repression cassette (*RC-CYC1-lacZ*) or the RME1 response element (*RRE-CYC1-lacZ*).

RME1-dependent activation and is required for RME1-dependent repression (COVITZ and MITCHELL 1993). These aspects of RME1-dependent activation were reflected by expression of hybrid *HIS3* genes (Figure 1C). Segments of the *IME1* regulatory region were placed 5′ to a derivative of the *HIS3* gene that lacks other upstream activation sequences. Expression of these hybrid genes was compared in a strain lacking RME1 (*rme1Δ5*) and in one that expresses RME1 from the *GAL1* promoter (P_{GAL1}-*RME1*). *HIS3* expression was determined semiquantitatively by the level of resistance to 3-aminotriazole, which inhibits activity of the *HIS3* product (WOLFNER *et al.* 1975). The *HIS3* reporter gene alone (Δ*UAS-HIS3*) conferred resistance to 10 mM 3-aminotriazole; its expression presumably depended on vector sequences. Insertion of either the repression cassette or the modulation region reduced resistance to 3-aminotriazole (*RC-HIS3* and *MR-HIS3*), perhaps by increasing the distance between vector sequences and *HIS3*. Insertion of the RME1-response element also reduced resistance in the *rme1Δ* strain but increased resistance in the P_{GAL1}-*RME1* strain (*RRE-HIS3*). RME1-dependent activation was blocked by inclusion of the modulation region (*RRE-MR-HIS3*). These results indicate that RME1 can activate expression of *HIS3* through the RME1-response element; presence of the modulation region reduces or abolishes RME1-dependent activation.

Isolation of a repression-defective mutant: Our attempt to identify mutations that relieve RME1-dependent repression was based on the idea that repression and activation by RME1 might represent alternative states or activities. Thus loss of ability of RME1 to repress might permit RME1 to activate through the repression cassette. A His^- strain carrying an integrated *RC-HIS3* hybrid gene and P_{GAL1}-*RME1* (strain 1343) was plated on galactose medium without histidine. Among 32 His^- isolates, 22 were His^+ on glucose medium, in which the P_{GAL1}-*RME1* gene is not expressed. The mutations apparently relieved the need for RME1 to activate *RC-HIS3* and were discarded. Another five isolates grew too poorly to permit characterization. The remaining five isolates were tested for defects in RME1-dependent repression by comparing expression of *CYC1-lacZ* reporter plasmids that differed by presence of the repression cassette (Figure 1D). Four of the isolates, like the parent strain, expressed *RC-CYC1-lacZ* at 10–25-fold lower levels than *CYC1-lacZ*. One isolate, 1343r1, expressed *RC-CYC1-lacZ* at only 2-fold lower levels than *CYC1-lacZ*. These results suggested that mutant 1343r1

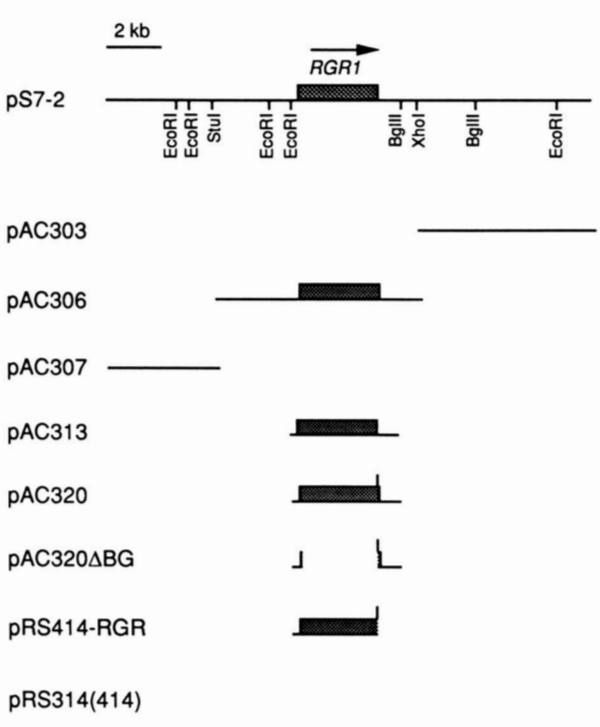

FIGURE 2.—*RGR1* plasmids and functional activity. The *RGR1* insert in plasmid pS7-2 and relevant sublcones are diagrammed. Lines represent segments present in each plasmid. The shaded box represents the *RGR1* coding region, which is oriented with its 5′ end to the left. All inserts are carried in the low copy vectors pRS314 or pRS414. Plasmids pAC320, pAC320ΔBG and pRS414-RGR include a *Bgl*II site introduced by oligonucleotide mutagenesis at the 3′ end of *RGR1*. Plasmid complementation of the Ts⁻ and Met⁻ phenotypes of strain 1350 (**a** *rgr1-100*) are summarized in the 37° and SC-Met columns, respectively. Levels of sporulation observed in transformants of strain 1398 (**a**/**a** *rgr1-100/rgr1-100*) after incubation for 5 days on Spo plates are summarized in the Spo column. Numbers are the mean and standard deviation for at least four independent transformants with each plasmid.

was defective in repression through the repression cassette.

Meiotic analysis indicated that a single mutation in strain 1343r1 permitted *RC-HIS3* expression. 44 tetrads were analyzed from a cross of the mutant to strain 1363 (*RC-HIS3* P_{GAL1}-*RME1 GAL80*). The *GAL80* allele prevented P_{GAL1}-*RME1* expression and thus allowed the diploid to sporulate. Among 88 *gal80* segregants, 46 were His^+ and 42 were His^- on galactose medium. These results match the predicted 1:1 His^+:His^- ratio for a single gene trait. All *gal80* segregants were His^- on glucose medium, in which P_{GAL1}-*RME1* is not expressed. 16 tetrads were analyzed from a cross of 1343r1 to strain 1342 (*RC-HIS3 RME1 GAL80*). Among 12 P_{GAL1}-*RME1 gal80* segregants, six were His^+ and six were His^- on galactose medium. These results confirm that a single mutation permits *RC-HIS3* expression. In addition, recovery of P_{GAL1}-*RME1 gal80* segregants that are phenotypically His^- indicates that the mutation is not linked to the P_{GAL1}-*RME1* gene.

Cloning of *RGR1*: The mutant 1343r1 had two unanticipated phenotypes: temperature-sensitive growth (Ts^- phenotype) and a methionine or cysteine requirement (Met^- phenotype). These two phenotypes segregated 2+:2– and cosegregated with one another in crosses of the mutant to wild-type strains. In the cross of the mutant to strain 1363 (*RC-HIS3* P_{GAL1}-*RME1 GAL80*), the Ts^- and Met^- phenotypes cosegregated with the P_{GAL1}-*RME1*-dependent His^+ phenotype in 28 half-tetrads analyzed. (Only the *gal80* segregants could be analyzed for a P_{GAL1}-*RME1*-dependent phenotype.) Both the Ts^- and Met^- phenotypes were recessive, so we used these phenotypes to clone the corresponding gene by complementation. A meiotic segregant carrying the Ts^-/Met^- mutation, strain 1350, was transformed with a low copy vector-based genomic library and plated on selective medium at 37°. Three transformants acquired a plasmid-dependent Ts^+ phenotype, and plasmid DNA retrieved from each could confer Ts^+ and Met^+ phenotypes after re-transformation into strain 1350. Restriction enzyme digestions indicated that the three plasmids were identical, and one (designated pS7-2) was analyzed further.

We used linkage analysis to determine whether the pS7-2 plasmid insert included DNA from the locus of the original mutation. The non-replicating plasmid pAC300 was constructed from a segment of the pS7-2 insert and the *URA3*-bearing plasmid YIp5. Transformation of strain 107 (Met^+ Ura^-) with pAC300 resulted in integration of the *URA3* marker at the chromosomal locus of the cloned DNA, as confirmed by a Southern blot. A Ura^+ transformant (Met^+ Ura^+) was crossed to strain 1357 (Met^- Ura^-) and Met and Ura phenotypes were followed in 36 tetrads. Thirty-four tetrads were parental ditypes and two tetrads were tetratypes. Thus the site of integration is tightly linked to the Ts^-/Met^- mutation. We conclude that the pS7-2 insert contains DNA from the locus of the Ts^-/Met^- mutation.

Plasmid complementation of the Ts^- and Met^- phenotypes of strain 1350 delimited a 4-kbp functional region of the pS7-2 insert, carried in plasmid pRS414-RGR (Figure 2). This low copy plasmid could fully complement the Ts^- phenotype and partially complement the Met^- phenotype. The sequence of three DNA segments within this interval, totalling 400 bp, was identical to portions of the *S. cerevisiae* gene *RGR1* (SAKAI *et al.*

TABLE 2

Effect of P_{GAL1}-*RME1* and *rgr1-100* on sporulation

Strain	P_{GAL1}-*RME1* expression[a]	*RGR1* alleles	Sporulation (%)[b]
1122/1366	+	+/+	<0.5
1122/1365	-	+/+	88
1323/1368	+	*rgr1-100/rgr1-100*	70
1323/1350	-	*rgr1-100/rgr1-100*	69
1122/1368	+	+/*rgr1-100*	<0.5
1122/1350	-	+/*rgr1-100*	86

[a] P_{GAL1}-*RME1* was expressed in *gal80/gal80* diploids and repressed in *GAL80/gal80* diploids.

[b] Sporulation ability was determined by microscopic examination after incubation for 2 days on Spo plates. Values are the mean of three determinations; standard deviations were less than 10% of the mean.

TABLE 3

Sporulation of a/a diploids

Strain	Relevant genotype	Sporulation (%)[a]
931	**a/a** *RGR1/RGR1 RME1/RME1*	<0.1
1362	**a/a** *RGR1/RGR1 rme1Δ5/rme1Δ5*	84
1360	**a/a** *rgr1-100/rgr1-100 RME1/RME1*	13

[a] Sporulation ability was determined by microscopic examination after incubation for 5 days on Spo plates. Values are the mean of three determinations; standard deviations were less than 20% of the mean.

1990). The insert in plasmid pRS414-RGR includes only the RGR1 coding region (fused at codon 1081 to plasmid *lacZ* sequences) and 291 bp of *RGR1* 5′ sequences. We conclude that the Ts⁻/Met⁻ mutation is a defect in the *RGR1* gene. We designate the mutation *rgr1-100*.

Disruption of RME1-dependent repression by *rgr1-100*: We first used assays of sporulation ability to determine whether *rgr1-100* caused a defect in RME1-dependent repression. Sporulation was measured for **a**/α diploid strains of genotype *RGR1/RGR1*, *RGR1/rgr1-100*, or *rgr1-100/rgr1-100* that differed in expression of *RME1* from the *GAL1* promoter (Table 2). Expression of P_{GAL1}-*RME1* inhibited sporulation over 100-fold in both *RGR1/RGR1* and *RGR1/rgr1-100* diploids. In contrast, expression of P_{GAL1}-*RME1* had no effect on sporulation in *rgr1-100/rgr1-100* diploids. These observations indicate that *rgr1-100* is a recessive mutation that relieves inhibition of sporulation by the P_{GAL1}-*RME1* gene.

Characterization of *rgr1-100* had thus far relied upon properties of the P_{GAL1}-*RME1* hybrid gene. We also examined the effects of the *rgr1-100* mutation in **a**/**a** diploids, which are normally unable to sporulate because they express RME1 from its natural promoter (Table 3). The control **a**/**a** diploid (*RME1/RME1 RGR1/RGR1*) was unable to sporulate, as expected. An **a**/**a** diploid that lacked RME1 (*rme1Δ5/rme1Δ5 RGR1/RGR1*) yielded 84% sporulation. An **a**/**a** diploid homozygous for *rgr1-100* (*RME1/RME1 rgr1-100/rgr1-100*) yielded 13% sporulation. Therefore, the *rgr1-100* mutation permits sporulation when *RME1* is expressed from the natural *RME1* promoter. We conclude that the *rgr1-100* phenotype does not depend on artifactual expression of *RME1* from the *GAL1* promoter.

RME1 blocks sporulation in **a** and α cells by preventing *IME1* transcript accumulation in response to starvation. Thus *RME1* strains fail to accumulate *IME1* RNA; *rme1* mutants are able to accumulate *IME1* RNA after starvation. If *rgr1-100* prevents RME1-dependent repression at the native *IME1* gene, then an *rgr1-100* mutant should also accumulate *IME1* RNA during starvation. We tested this prediction through Northern blot analysis (Figure 3). Haploid **a** or α strains were constructed that had either *RME1* or *rme1Δ5* and either *RGR1* or *rgr1-100* alleles. RNA was prepared from these strains during vegetative growth (lanes 1, 6, 11 and 16) or at various times after transfer to sporulation medium. *IME1* RNA levels were undetectable in the *RME1 RGR1* strain and increased after starvation of the *rme1Δ5 RGR1* strain, as expected. *IME1* RNA levels also increased after starvation of both the *RME1 rgr1-100* and *rme1Δ5 rgr1-100* strains. Both *rgr1-100* strains had lower *IME1* RNA levels than the *RGR1 rme1Δ5* strain, but higher levels than the *RGR1 RME1* strain. These observations indicate that *rgr1-100* permits *IME1* RNA accumulation in the presence of a wild-type *RME1* allele. Further, they show that *rgr1-100* does not relieve the need for starvation to stimulate IME1 expression.

To confirm that *rgr1-100* relieves repression at the repression cassette, we compared expression of the *RC-CYC1-lacZ* reporter gene in strains expressing functional or nonfunctional RME1 products (P_{GAL1}-*RME1* and P_{GAL1}-*rme1-213*, respectively; Table 4). In an *RGR1* background, RME1 repressed *RC-CYC1-lacZ* expression 15-fold (compare strains 1122 and 1124). In an *rgr1-100* background, RME1 repressed *RC-CYC1-lacZ* expression less than 2-fold (compare strains 1369 and 1370). We obtained similar results in assays of an integrated *RC-CYC1-lacZ* reporter gene (data not shown). We conclude that *rgr1-100* relieves repression through an effect on the repression cassette.

One trivial explanation for failure of RME1 to repress in *rgr1-100* strains is that the mutation causes a defect in RME1 protein accumulation. We used an immunoblot to visualize RME1 protein levels, as detected through an appended S53 epitope (Covitz *et al.* 1991). RME1 was identified as a M_r 36,000 protein present in an extract of a P_{GAL1}-*RME1* strain (Figure 4, lane 2) and not an *rme1Δ* strain (lane 1). The amount of M_r 36,000 RME1 was unaffected by the *rgr1-100* mutation (lane 3). A M_r 43,000 cross-reactive protein was more prominent in the *rgr1-100* extract than in the *RGR1* extract; this protein was also found in an extract of an *rgr1-100* strain expressing wild-type RME1 (lacking the S53 epitope) and is thus not related to *S53-RME1* expression (data

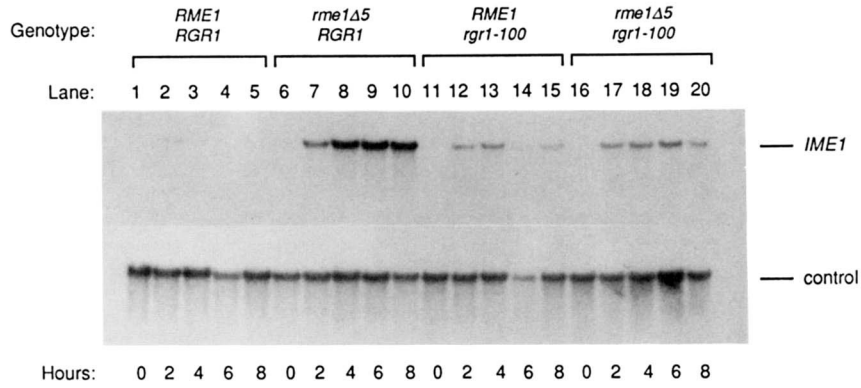

FIGURE 3.—Accumulation of *IME1* RNA in sporulation medium. Northern filters were prepared from cells grown in YPAc (lanes 1, 6, 11 and 16) or after a shift to sporulation medium for 2 hr (lanes 2, 7, 12 and 17), 4 hr (lanes 3, 8, 13 and 18), 6 hr (lanes 4, 9, 14 and 19), or 8 hr (lanes 5, 10, 15 and 20). Filters were probed for *IME1* RNA, stripped and reprobed with control plasmid pC4/2. Strains were 107 (*RME1 RGR1*; lanes 1–5), 1380 (*rme1Δ5 RGR1*; lanes 6–10), 1377 (*RME1 rgr1-100*; lanes 11–15), and 1379 (*rme1Δ5 rgr1-100*; lanes 16–20).

TABLE 4

Effects of *rgr1-100* on activity of the repression cassette

Strain	Relevant genotype	β-galactosidase activity[a]
1122	P_{GAL1}-*RME1*	130
1124	P_{GAL1}-*rme1-213*	2040
1369	*rgr1-100* P_{GAL1}-*RME1*	860
1370	*rgr1-100* P_{GAL1}-*rme1-213*	1580
1396	*sin4::TRP1* P_{GAL1}-*RME1*	830
1397	*sin4::TRP1* P_{GAL1}-*rme1-213*	1370

[a] Strains carrying the *RC-CYC1-lacZ* plasmid were grown to mid-log phase in synthetic galactose medium for β-galactosidase assays. β-Galactosidase activity, in Miller units, is the mean for four independent transformants. Standard deviations were less than 20% of the mean.

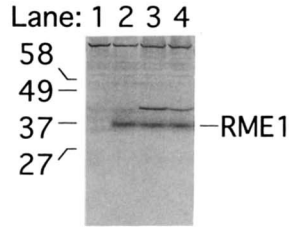

FIGURE 4.—Immunoblot analysis of RME1 polypeptide accumulation. RME1 was visualized in extracts of galactose-grown cells with anti-S53 anti-peptide antiserum through an S53 epitope specified by the P_{GAL1}-*RME1* gene. Mobilities of molecular weight markers are indicated on the left; the M_r 36,000 *S53-RME1* product is indicated on the right. Lane 1: strain 1040 (*rme1Δ5*); lane 2: strain 1122 (P_{GAL1}-*RME1*); lane 3: strain 1369 (P_{GAL1}-*RME1 rgr1-100*); lane 4: strain 1396 (P_{GAL1}-*RME1 sin4::TRP1*).

not shown). These results indicate that *rgr1-100* strains are not defective in accumulation of RME1.

The *rgr1-100* mutation might prevent repression by preventing binding of RME1 to DNA. The ability of RME1 to activate expression of the *RC-HIS3* reporter gene argues against this idea. To quantitate RME1-dependent activation, we compared expression of *CYC1-lacZ* reporter genes with no UAS (*ΔUAS-CYC1-lacZ*) and with the RME1-reponse element in place of the *CYC1* UAS (*RRE-CYC1-lacZ*; Table 5). *ΔUAS-CYC1-lacZ* expression was slightly greater in *rgr1-100* strains than in *RGR1* strains, but it was unaffected by presence of a functional P_{GAL1}-*RME1* allele. Similarly, *RRE-CYC1-lacZ* expression in the absence of *RME1* function was greater in *rgr1-100* strain (1370) than in the *RGR1* strain (1124). *RRE-CYC1-lacZ* expression was stimulated by a functional P_{GAL1}-*RME1* allele in both *rgr1-100* and *RGR1* strains (1369 and 1122, respectively). Because the *rgr1-100* mutation causes elevated expression of reporters with weak UAS activity, we cannot draw a quantitative conclusion concerning RME1-RRE binding *in vivo* in the *rgr1-100* background. However, these results confirm that *rgr1-100* does not block RME1 accumulation.

Anomalous complementation behavior of *RGR1* plasmids: In the course of cloning *RGR1*, we sought to determine whether *RGR1* plasmids would complement *rgr1-100* for sporulation ability. Sporulation ability of an **a**/**a** *rgr1-100/rgr1-100* diploid carrying various *RGR1* subclones was assessed microscopically (Figure 2). Presence of the vector alone or *RGR1* flanking regions (pAC320ΔBG) permitted the expected low level of sporulation (4–6%). Presence of a functional *RGR1* plasmid (pAC313, pAC320 or pRS414-RGR) stimulated sporulation two- or threefold. We detected no significant sporulation of **a**/**a** *RGR1/RGR1* diploids carrying these plasmids (<0.2%), so increased dosage of the wild-type *RGR1* gene alone does not relieve cell-type control of sporulation. There may be an interaction between the *RGR1* and *rgr1-100* products or a physiological basis for this phenomenon (see DISCUSSION).

Similarity between *rgr1-100* and a *sin4* null mutation: Studies on repression of the *HO* promoter indicate a functional relationship between *RGR1* and *SIN4* (STILLMAN *et al.* 1994). Therefore, we determined whether a *sin4* null mutation affected repression by RME1. Expression of an *RC-CYC1-lacZ* plasmid was measured in *sin4::TRP1* strains that contained either functional P_{GAL1}-*RME1* or nonfunctional P_{GAL1}-*rme1-213* alleles (Table 4). In the null *sin4::TRP1* background, RME1 repressed *RC-CYC1-lacZ* expression less than 2-fold (compare strains 1396 and 1397). We conclude that SIN4 is required for RME1-dependent repression.

To determine if the *sin4* mutation blocked repression by preventing RME1 protein accumulation, we visualized RME1 polypeptide levels on an immunoblot (Figure 4). The *sin4* mutant (lane 4) and wild-type strain

TABLE 5

Effects of *rgr1-100* on RME1-dependent activation

Strain	Relevant genotype	β-Galactosidase activity[a]	
		ΔUAS-CYC1-lacZ	*RRE-CYC1-lacZ*
1122	P_{GAL1}-*RME1*	<0.5	22
1124	P_{GAL1}-*rme1-213*	<0.5	1.6
1369	*rgr1-100* P_{GAL1}-*RME1*	1.7	226
1370	*rgr1-100* P_{GAL1}-*rme1-213*	2.3	45

[a] Strains carrying the *ΔUAS-CYC1-lacZ* or *RRE-CYC1-lacZ* plasmids were grown to mid-log phase in synthetic galactose medium for β-galactosidase assays. β-Galactosidase activity, in Miller units, is the mean for three independent transformants. Values over 20 units had standard deviations of less than 20% of the mean; other values had standard deviations of 30%.

(lane 2) expressed comparable levels of M_r 36,000 RME1. (The *sin4* mutant also expressed the M_r 43,000 cross-reactive protein.) We conclude that SIN4 is not required for accumulation of the RME1 polypeptide.

It seemed possible that the *sin4::TRP1* mutation, like *rgr1-100*, would cause a methionine or cysteine requirement. Our original *sin4::TRP1* transformant displayed an auxotrophy for either methionine or cysteine. Among 16 tetrads from a cross between *sin4::TRP1* and *SIN4* strains, the requirement for either methionine or cysteine cosegregated with *sin4::TRP1*. We conclude that SIN4 is required for synthesis of methionine and cysteine.

Given the similar phenotypes of *rgr1-100* and *sin4::TRP1* strains, we were surprised that the selection for *RC-HIS3* expression did not yield *sin4* mutants. To see whether *sin4::TRP1* would permit RME1-dependent activation of *RC-HIS3*, we analyzed 16 tetrads from a cross between strains 1343 (*RC-HIS3 SIN4* P_{GAL1}-*RME1 gal80*) and 1399 (*sin4::TRP1* P_{GAL1}-*RME1 GAL80*). None of eight *RC-HIS3 sin4::TRP1* P_{GAL1}-*RME1 gal80* segregants grew on galactose medium without histidine; all grew in the presence of histidine. Therefore, a *sin4* null mutation does not permit RME1 to activate *RC-HIS3* expression. Our initial assumption that mutants defective in RME1-dependent repression would display improved RME1-dependent activation through the repression cassette may have been in error.

Lack of linkage of *RGR1* or *SIN4* to *RES1-1*: The *RES1-1* mutation was identified as a dominant mutation that relieves repression by RME1 (KAO *et al.* 1990). The *RES1* gene has not been mapped or cloned, so we considered the possibility that *RES1-1* might be an *RGR1* or *SIN4* allele. We tested linkage between *RGR1* and *RES1-1* by integrating plasmid pAC300 (*RGR1::URA3*) into an **a** *RES1 ura3* strain (1446-2C), crossing the integrant to an α *RES1-1 ura3* strain (JSH125α), and scoring meiotic tetrads for segregation of *RGR1::URA3* and *RES1-1*. We found 6 PD, 1 NPD and 9 T tetrads among 16 tetrads with 4 viable spores. These results indicate that *RGR1* and *RES1-1* may be loosely linked (~50 cM), but are not allelic. We also tested linkage between *sin4::TRP1* and *RES1-1*. Spore viability was poor in this cross (<30%). We found 1 PD and 5 T tetrads among 6 tetrads with 4 viable spores. In addition, we found 20 recombinant spores and 22 parental spores among 19 other tetrads. These results indicate that *SIN4* and *RES1-1* are not allelic.

DISCUSSION

Our previous studies had suggested that binding of RME1 to DNA is necessary but not sufficient to exert repression (COVITZ and MITCHELL 1993). With these observations in mind, we looked for genes whose products may determine whether RME1 activity is positive or negative. We have found that two previously characterized genes, *RGR1* and *SIN4*, are required for RME1-dependent repression. Our results strengthen the proposal that RGR1 and SIN4 act in a single pathway and have implications with regard to the mechanism by which RME1 represses *IME1*.

Nature of the *rgr1-100* allele: It seems likely that *rgr1-100* causes a partial loss of *RGR1* function. A null *rgr1* mutation causes inviability (SAKAI *et al.* 1990), so the *rgr1-100* product must have some activity. The idea that *rgr1-100* causes decreased activity, rather than altered activity, is based on two observations. First, the phenotypes associated with *rgr1-100* (Ts^-, Met^-, and RME1 repression defects) are recessive in heterozygous diploids. Second, the phenotypes we have found associated with *rgr1-100* are similar, though less severe, than those associated with a C-terminal deletion allele, *rgr1Δ2* (SAKAI *et al.* 1990). Both alleles cause temperature-sensitive growth, incomplete cell separation (SAKAI *et al.* 1990), and a methionine or cysteine auxotrophy (A. MITCHELL, unpublished results). However, *rgr1Δ2* causes very slow growth even at permissive temperatures, whereas *rgr1-100* does not. (We have not assessed RME1-dependent repression in *rgr1Δ2* strains because of their slow growth.) The interpretation that *rgr1-100* is a mildly defective allele accounts for these observations.

Two of our observations cast some doubt on this interpretation. First, the *rgr1-100* mutation appears to be a rare kind of allele: it arose in a population of 10^9 mutagenized cells. We have since been unable to isolate a similar mutation in another 5×10^9 mutagenized cells (A. MITCHELL, unpublished results). We had expected impaired-function mutations to be more common. An

allele such as *rgr1-100* may be rare because the phenotypes require a precise reduction of RGR1 activity. Second, the *rgr1-100* allele was dominant in tests of *RGR1* plasmid complementation of **a/a** *rgr1-100/rgr1-100* sporulation ability. Dominance is often considered an indication of elevated or altered activity. In this case, dominance arises through combined expression of *RGR1* and *rgr1-100*, rather than independent functioning of the *rgr1-100* product, because sporulation is actually improved by presence of an *RGR1* plasmid. One explanation is that the wild-type and mutant products form a mixed oligomer with unique properties, in particular, when gene dosage is elevated. A second explanation is that sporulation may occur only in cells that have lost the *RGR1* plasmid; sporulation might be improved by the more vigorous growth in generations preceding loss of the plasmid and *RGR1* product. Although we are unaware of a precisely analogous observation in any other system, we note that both increases and decreases in histone H2A or H2B levels result in expression from defective promoters (CLARK-ADAMS *et al.* 1988). Thus the hypothesis that *rgr1-100* causes reduced RGR1 activity can accommodate these observations, but the hypothesis should be considered tentative.

rgr1Δ2 and *rgr1-100* apparently differ in their effects on sporulation: *rgr1Δ2* prevents sporulation, whereas *rgr1-100* permits sporulation in non-**a**/α cells. Why should mild and severe *rgr1* alleles have opposite effects on sporulation? The sporulation defect of *rgr1Δ2* strains may arise from poor growth or some other pleiotropic effect of the mutation, not from an effect on RME1-dependent repression. For example, SAKAI *et al.* (1990) reported that elevated cyclic AMP levels are found in *rgr1Δ2* strains; it has been shown that multiple defects in sporulation result from elevated cyclic AMP-dependent protein kinase activity (MATSUURA *et al.* 1990). If *rgr1-100* were to cause a slight increase in cyclic AMP synthesis, the phosphodiesterase-dependent feedback system would presumably compensate to maintain low cyclic AMP pools (NIKAWA *et al.* 1987).

rgr1Δ2 causes several phenotypes we have not examined in *rgr1-100* strains, including partial relief of glucose repression, reduced storage carbohydrate levels, and expression of *HO* in the absence of its activator SWI5 (JIANG and STILLMAN 1992; SAKAI *et al.* 1990). We infer that *rgr1-100* does not fully relieve glucose repression because *RC-HIS3* P_{GAL1}*-RME1 gal80 rgr1-100* strains were His^+ in galactose medium, not glucose medium. Understanding the relationship between RGR1 activity and the many mutant phenotypes will clearly require analysis of more *rgr1* alleles.

Relationship between *RGR1* and *SIN4*: The proposal that *RGR1* and *SIN4* have related functions is based on their common target genes and double mutant interactions (STILLMAN *et al.* 1994). Our observations support this idea. We found that *rgr1-100* and *sin4::TRP1* mutations cause defects in RME1-dependent repression and cause a methionine or cysteine auxotrophy, which presumably results from a defect in sulfate uptake or reduction (JONES and FINK 1982). [DAVID STILLMAN (personal communication) has observed that *sin4::TRP1* mutants are Met^+ in strains unrelated to ours.] We observed that *rgr1-100* permitted some expression of *ΔUAS-CYC1-lacZ*, and it has been shown that a *sin4Δ* mutation permits expression of genes lacking UAS regions (JIANG and STILLMAN 1992). Past studies have indicated that *rgr1Δ2* defects are more severe than *sin4* defects (STILLMAN *et al.* 1994), and suggest that RGR1 has a more central role than SIN4. Our finding that *rgr1-100*, and not *sin4::TRP1*, permits RME1-dependent activation of the *RC-HIS3* gene may be another example of the functional hierarchy.

The biochemical roles of RGR1 and SIN4 are unclear at present. Both deduced gene products are large (RGR1: 1082 residues; SIN4: 974 residues) and have no extensive homology to other known proteins (CHEN *et al.* 1993; Jiang and STILLMAN 1992; SAKAI *et al.* 1990). We have noticed that RGR1 has a possible leucine zipper (residues 812–833), which may serve as a region of protein-protein interaction. SIN4 has a possible zinc finger and a segment homologous to one nuclear targeting signal in the α2 protein (CHEN *et al.* 1993). SIN4 probably does not cause repression by binding to a specific DNA site, though, because a lexA-SIN4 fusion protein activates transcription from a lexA binding site (JIANG and STILLMAN 1992). Two observations suggest a more general role for SIN4 in chromatin structure (JIANG and STILLMAN 1992). First, a *sin4Δ* mutant expresses genes lacking UAS regions, as do strains depleted for histone H4 (HAN and GRUNSTEIN 1988). Second, a *sin4Δ* mutant has decreased plasmid superhelicity. Although chromatin in *rgr1* mutants has not been characterized, we found elevated expression of the *CYC1-lacZ* gene lacking its UAS region. Thus it seems reasonable that SIN4 and RGR1 might have a common role in chromatin structure. SIN4 and RGR1 may act together, or one protein may be required only for expression or activity of the other.

Models for repression by RME1: It possible that disruption of chromatin structure in *sin4* and (presumably) *rgr1-100* mutants causes a defect in RME1-dependent repression. One model is that wild-type chromatin structure may be required for RME1 to bind to DNA. We consider this particular explanation unlikely, though, because *rgr1-100* does not prevent RME1-dependent activation. A second model is that RME1 exerts repression through a local change in chromatin structure; SIN4 and RGR1 would be required to maintain or transmit this structural alteration. We consider this idea appealing because a chromatin structural alteration may permit repression to be exerted over a considerable distance, as is the case at the *IME1* gene. This model may be more rigorously tested if an RME1-dependent change in chromatin can be identified.

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