a/α-Specific Repression by *MATα2*

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Manuscript received February 29, 1988
Revised copy accepted May 21, 1988

ABSTRACT

The product of the *MATα2* gene is a DNA-binding protein that acts as a repressor of two different sets of cell type-specific genes. In α cells, the α2 protein represses the transcription of several **a**-specific genes. In **a**/α cells, the α2 protein acts together with the product of the *MAT***a***1* gene, the **a**1 protein, to repress several genes used by haploids in the mating process. In addition to the *matα2* mutations that result in defects in both types of regulation, other *matα2* alleles have been described that result in defects in the repression of **a**-specific genes but that do not affect the ability of the α2 and **a**1 proteins to interact to repress the haploid-specific genes. We report here the isolation of a new class of *matα2* mutations that do not affect the ability of the α2 protein to repress **a**-specific genes, but that interfere with the ability of the α2 protein to interact with the **a**1 protein to repress the haploid-specific genes and establish the **a**/α cell type. These mutations may help determine the means by which the **a**1 protein interacts with α2 to expand the set of genes under its control.

THE α2 protein, encoded by *MATα2*, is a negative regulator of the expression of genes of the sexual cycle in the yeast *Saccharomyces cerevisiae* (STRATHERN, HICKS and HERSKOWITZ 1981). The α2 protein is particularly interesting because it is involved in the repression of two different sets of cell type-specific genes. In α cells, α2 has the role of a repressor of **a**-specific genes such as *MF***a***1* and *MF***a***2* [genes encoding a pheromone involved in coordinating mating (BRAKE *et al.* 1985)], *STE2* [the gene encoding the receptor for the pheromone produced by α cells, α-factor (JENNESS *et al.* 1983)], *BAR1* [the gene encoding a protease that degrades α-factor (SPRAGUE and HERSKOWITZ 1981; CIEJEK and THORNER 1979)], and *STE6* [an **a**-specific gene involved in **a**-factor biogenesis (WILSON and HERSKOWITZ 1984; CHAN *et al.* 1983)]. A consensus DNA sequence required for regulation by α2 has been identified in the 5′ noncoding region of these genes (MILLER, MACKAY and NASMYTH 1985). Specific DNA-protein interaction between α2 protein and the consensus sequence at the *STE6* gene has been demonstrated (JOHNSON and HERSKOWITZ 1985). Although such DNA-protein binding can even be demonstrated for α2 protein made in *Escherichia coli*, it remains possible that proper regulation of these **a**-specific genes requires a second yeast protein *in vivo*.

In its second role, as a repressor of "haploid-specific" genes, α2 protein requires the **a**1 protein, the product of the *MAT***a***1* gene. These haploid-specific genes include *HO* [the gene for an endonuclease that initiates homothallic switching (JENSEN, SPRAGUE and HERSKOWITZ 1983; KOSTRIKEN and HEFFRON 1984)], *MATα1* [encodes a positive regulator of α-specific genes (KLAR *et al.* 1981; NASMYTH *et al.* 1981; JARVIS, HAGEN and SPRAGUE 1988)], *STE4, STE5* and *STE12* [genes required for mating in both **a** and α cells (unpublished observations cited in HALL and JOHNSON 1987)] and *SST2* [a gene involved in pheromone response (DIETZEL and KURJAN 1987)]. The repression of these genes in **a**/α cells is also mediated through a consensus sequence found upstream of their transcribed regions (MILLER, MACKAY and NASMYTH 1985; SILICIANO and TATCHELL 1986; DIETZEL and KURJAN 1987). This sequence is related to, but different from, the sequence required for the repression of **a**-specific genes by α2 protein. The mechanism by which **a**1 protein modifies or cooperates with α2 protein to broaden the spectrum of genes that it represses has not been determined.

Most *matα2* mutations have effects on the phenotypes of both α and **a**/α cells. For example, the *matα2-1* allele results in sterility in a haploid α cell and cannot support sporulation in a *matα2-1*/*MAT***a** diploid (MACKAY and MANNEY 1974a, b; STRATHERN, HICKS and HERSKOWITZ 1981). This is the phenotype of the *matα2* null mutation.

One approach to determining the features of α2 responsible for its two different roles in cell type regulation has been to isolate mutants in which these functions are dissociated. The *matα2-4* mutation, which comes from the sterile α strain VP1 isolated by MACKAY and MANNEY (1974a, b), results in sterility due to a defect in repression of **a**-specific genes, but is able to support sporulation in a *matα2-4*/*MAT***a** diploid (SPRAGUE, RINE and HERSKOWITZ 1981).

TABLE 1

Yeast strains

Strain	Genotype	Source
DC14	*MAT***a** *his1*	G. FINK
DC17	*MATα his1*	G. FINK
DC122	*matΔ leu2 trp1 his5 ura3 ade6*	This study
JSS3-2A	*MATα leu2 trp1 ura3*	This study
JSS3-3A	*MAT***a** *leu2 ura3*	This study
JSS56-11B	*MAT***a** *his3 leu2 trp1 ura3*	This study
JSS82-22B	*MAT***a** *his3 leu2 ura3 can1^r cyh2^r*	This study
294	*MATα his3 leu2 trp1 ura3*	J. BROACH
JH727	*MATα HML***a** *HMR***a** *mar2-1 leu2 lys2 his4*	This study
JH807	*matα2d-807 HML***a** *HMR***a** *mar2-1 leu2 lys2 his4*	This study

Hence, the α2 protein encoded by the *matα2-4* mutation is still capable of interacting with the **a**1 protein to repress haploid-specific genes. We will refer to such mutations as *matα2h* to reflect the defect in the α haploid phenotype. Similar *matα2h* mutations, capable of conferring the **a**/α phenotype but not of giving the α phenotype, have been isolated by linker insertion mutagenesis (TATCHELL *et al.* 1981) and by site-directed mutagenesis (PORTER and SMITH 1986).

We report here the isolation of *matα2* mutants that retain the ability to regulate **a**-specific genes but that cannot support the **a**/α phenotype. As haploids these *matα2* mutants have no apparent defect and mate like α cells. However, as *matα2*/*MAT***a** diploids they fail to sporulate, and rather than being nonmaters, they mate as if they are α cells. Thus, they have separated the roles of the α2 protein in the opposite sense of the *matα2h* mutations. This novel class of mutations is designated *matα2d* to denote their defect in the **a**/α diploid phenotype. One such recessive mutation is near the "homeobox-DNA binding domain." Such *matα2d* mutations may be useful in determining the mechanism of interaction of the α2 and **a**1 proteins.

MATERIALS AND METHODS

Yeast strains: The genotypes of the yeast strains employed in this study are listed in Table 1.

Plasmids: The high copy number yeast vector YEp213 (HICKS *et al.* 1982) carries the *LEU2* gene and the 2-micron origin of replication. It was derived from YEp13 (BROACH, STRATHERN and HICKS 1979) by inverting the 2.2 kbp *Sal*I to *Xho*I fragment containing *LEU2* (HICKS *et al.* 1982). The plasmid YEp213:MATα has the 4.3-kbp genomic *Hin*dIII fragment carrying the *MATα* allele inserted into YEp213 (Figure 1). GRMAT1 is similar to YEp213:MATα but has a deletion of the *MAT* W, X, Y, and Z1 sequences (HICKS *et al.* 1982). A unique *Xho*I site separates the remaining portions of the *MAT Hin*dIII fragment.

Plasmid mutagenesis: The YEp213:MATα plasmid was mutagenized by propagating the plasmid in the *mut*D *E. coli* bacterial strain, LE30 (COX 1976). Plasmid DNA was prepared from a pool of about 1000 primary LE30 transformants. One reflection of the level of mutagenesis is that the pool of plasmids included about 1% that were defective in *LEU2* as judged by their inability to complement the *E. coli* mutation *leuB*. A second indication of successful mutagenesis was that when this pool was transformed into a yeast strain lacking *MAT* (DC122), 6 of the 480 Leu$^+$ strains tested had plasmid-borne mutations resulting in sterility. These mutants were not further classified as *matα1* or *matα2*.

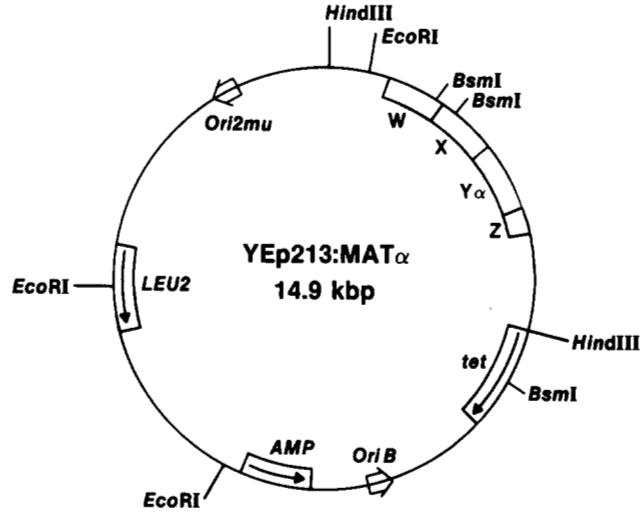

FIGURE 1.—The *MATα* locus cloned as a *Hin*dIII fragment into the cloning vector YEp213. The 480-base *Bsm*I fragment that includes the *matα2d-807* mutation is indicated.

Yeast genetics: The media and culture techniques described in the Cold Spring Harbor Yeast Course Manual (SHERMAN, FINK and HICKS 1986) were utilized. Mating proficiency was tested by the restrictive mating protocol, which involves spreading 0.2 ml of the mating tester strain (DC14 or DC17) suspended in YEPD on synthetic minimal medium plates. The colonies to be tested were replicated onto these plates and scored for the ability to form prototrophic diploids. The mating type of strains carrying the various alleles of *MATα* on YEp213 were tested by a similar protocol using JSS3-2A and JSS3-3A as tester strains. In these matings, the Leu$^+$ phenotype was selected so that only the mating phenotype of cells that retained the plasmid would be scored. The sporulation ability of the diploids was determined after growth on complete SPOR medium for 4–6 days. The cells were then scored microscopically for asci. In addition, these diploids were heterozygous for recessive drug resistance mutations at the *can1* and *cyh2* genes. Hence, although the diploid is sensitive to either drug, one quarter of the meiotic progeny are resistant to both drugs. Therefore, the ability to give rise to cells resistant to both

drugs is a sensitive assay for sporulation. After growth on sporulation medium, the diploids to be tested were replica-plated to plates containing cycloheximide and canavanine.

Transformations: Transformations were performed by the glusulase-spheroplast technique (HINNEN, HICKS and FINK 1978; BROACH, STRATHERN and HICKS 1979). To screen the transformants containing the mutagenized *MATα* plasmid the primary transformation plates were homogenized and the resulting cell suspension diluted and replated again selecting Leu$^+$. The resulting colonies were then tested for mating phenotype. To place the *matα* mutations into the genome at the normal *MAT* position on chromosome *III*, a yeast strain in which the *MAT* locus has been deleted (DC122) was transformed with the *Hin*dIII fragment carrying the mutation. To facilitate the screen for *MAT* substitutions, the cells were co-transformed with the *TRP1* containing replicating plasmid YRp7 (STRUHL *et al.* 1979). Trp$^+$ transformants were screened for loss of the *matΔ* phenotype.

Bacterial transformations were done using competent DH5 cells from Bethesda Research Laboratories, Gaithersburg, Maryland.

Sequencing: DNA sequencing was done using a chain-termination protocol (SANGER, NICKLEN and COULSON 1977) modified to allow the use of double stranded DNA (P. SUTRAVE, personal communication). The oligonucleotide primers corresponding to about 200 base intervals on both strands of *MATα2* were used in the sequencing reactions.

RESULTS

Previously described *matα2* mutations cause defects only in the repression of the **a**-specific genes in haploid α cells (*matα2h*), or cause defects in both the repression of **a**-specific genes and the repression of haploid-specific genes found in **a**/α diploids (*matα2* null). As a step toward understanding how the **a**1 protein interacts with the α2 protein to establish the **a**/α state of repression, we isolated *matα2* mutants that were defective in **a**/α diploid regulation (*matα2d*) but retained the ability to repress the **a**-specific genes in haploid α cells.

Mutagenesis of *MATα*: The 4.3-kbp *Hin*dIII fragment carrying the *MATα* locus is sufficient to confer the α phenotype on cells that have had the *MAT* locus deleted (*matΔ*). For example, when that fragment is carried on a high copy number plasmid such as YEp213:MATα (Figure 1) in a yeast strain that has *matΔ*, the yeast strain will mate efficiently as an α. Thus, it will complement both the *matα1* and *matα2* defects of the *matΔ* deletion. Similarly, this *MATα* plasmid confers the **a**/α phenotype on cells that also have a good *MAT***a** allele. Thus, an **a**/**a** diploid carrying one of these plasmids does not mate as an **a** or as an α but it can be induced to undergo meiosis and sporulation.

The basis of the screen for mutations of the desired *matα2d* type was to transform a pool of mutagenized YEp213:MATα plasmids into a *MAT***a** yeast strain (JSS56-11B) and then screen for cells that were able to mate like α cells. Plasmid mutagenesis was by passage through a bacterial mutator strain as described in MATERIALS AND METHODS. About 5000 transformants were screened, of which 5 (D50–D54) showed the α mating phenotype. This phenotype was shown to be dependent on the plasmid by demonstrating that Leu$^-$ segregants that had lost the plasmid regained the **a** mating phenotype. This level of analysis left the possibility that the defect was in the *MAT***a** chromosomal locus. That is, if for some reason the transformants had a *mat***a** mutation (KASSIR and SIMCHEN 1976), they would have had the α phenotype when the plasmid was present, and the **a**-like phenotype characteristic of *mat***a** mutants when the plasmid was lost. Therefore, the five plasmids were recovered from the mutants into *E. coli* and reintroduced into *MAT***a** cells. All five again conferred the α phenotype. When these plasmids were transformed into an **a**/α diploid (294 mated to JSS56-11B), the strains had the nonmating phenotype of an **a**/α strain, indicating that the *matα* mutations on the plasmid were recessive to *MATα* on the chromosome. Given that **a**/α diploids do not express *MATα1* (KLAR *et al.* 1981; SILICIANO and TATCHELL 1984), the difference between the phenotype of the plasmid in an **a** strain and the phenotype in an **a**/α strain should be due to the presence of the functional *MATα2* gene product. This strongly suggests that the mutations are in the *MATα2* gene.

To test the phenotype of these mutations in single copy, they were placed in the yeast genome at the normal position of *MAT* on chromosome *III*. For this purpose, a strain with the *MAT* locus deleted was used (DC122). This strain has the **a**-like phenotype (STRATHERN, HICKS and HERSKOWITZ 1981) caused by the inability to turn on α-specific genes (*matα1*), and the inability to turn off **a**-specific genes (*matα2*). This strain was transformed with the *MAT Hin*dIII fragment and screened for cells that did not have the **a**-like phenotype (see MATERIALS AND METHODS). The presence of the intact *Hin*dIII fragment at *MAT* was confirmed by restriction endonuclease digestion and blotting analysis (data not shown).

All five mutants (DC122 *matα2d-50*, DC122 *matα2d-51*, DC122 *matα2d-52*, DC122 *matα2d-53* and DC122 *matα2d-54*) analyzed by this protocol were sterile when tested at 30° (Table 2). Therefore, the mating competency of the original mutants (D50–D54) required both a *matα* mutation and its presence on a high copy number vector. However, DC122 *matα2d-50* and DC122 *matα2d-51* were able to mate as α cells at 22° (Table 2). This allowed the construction of isogenic *MATα/MAT***a**, *matα2d-50/MAT***a** and *matα2d-51/MAT***a** diploids by mating to strain JSS82-22B. The mutant diploids had a weak α phenotype at 22° (Table 2) but could not sporulate at any temperature.

***matα2-807*:** An additional *matα2d* mutant was ob-

TABLE 2

Mating competency

Strain	With **a**, with α 22°		With **a**, with α 25°		With **a**, with α 30°	
DC122 *matΔ*	−	+++	−	+++	−	+++
DC122 *MATα*	+++	−	+++	−	+++	−
DC122 *matα2d-50*	++	−	+	−	−	−
DC122 *matα2d-51*	++	−	+	−	−	−
DC122 *matα2d-52*	−	−	−	−	−	−
DC122 *matα2d-53*	−	−	−	−	−	−
DC122 *matα2d-54*	−	−	−	−	−	−
DC122 *matα2d-807*	+++	−	+++	−	+++	−
*MATα/MAT***a**	−	−			−	−
*matα2d-50/MAT***a**	+	−			−	−
*matα2d-51/MAT***a**	+	−			−	−
*matα2d-807/MAT***a**	+++	−			+++	−

tained by screening a *MATα HML***a** *HMR***a** *mar2* strain (JH727) for cells capable of mating like an α. Because of the *mar2* (also known as *sir3*) mutation, this strain expresses the **a**1 protein from *HML***a** and *HMR***a** as well as α2 from *MATα* and hence, has the **a**/α phenotype. A similar screen was used in the isolation of suppressors of the *mar* mutation (KLAR *et al.* 1985). The screen of JH727 for cells that could mate like an α yielded one mutant (JH807) that was unique in that, when mated to a *MAT***a** haploid, it gave a diploid that mated like an α cell and that could not sporulate.

The phenotype of strain JH807 was consistent with either a mutation in *MATα* or a dominant mutation elsewhere that kept **a**1 from functioning. Analysis of the DNA in JH807 had confirmed that the *HML***a** and *HMR***a** alleles were intact (data not shown). To determine if the mutation was in *MATα*, the *MAT* locus was cloned from strain 807 by the gapped plasmid technique (HICKS *et al.* 1982). Strain 807 was transformed with the GRMAT1 plasmid cut with *Xho*I to create a gapped plasmid spanning the *MAT* locus. About 100 Leu$^+$ transformants were pooled and DNA isolated. Plasmid DNA was recovered by transformation into *E. coli* (see MATERIALS AND METHODS) and confirmed to be YEp213 with a complete *MAT Hin*dIII fragment, designated YEp213:MATα807.

YEp213:MATα807 was shown to carry a *matα2d* mutation by transforming the plasmid into a *MAT***a** strain JSS56-11B. The resulting transformant had the α mating phenotype in contrast to the control JSS56-11B strain with YEp213:MATα, which has the nonmating **a**/α phenotype. As shown below, this mutation is in the *MATα2* gene and is designated *matα2d-807*. A *MAT***a**/*MATα* strain (294 mated to JSS56-11B) was transformed with YEp213:MATα or YEp213:MATα807. Both transformed diploids had the **a**/α nonmating phenotype. Therefore, even when the *matα2d-807* mutation is present on a high copy number plasmid, it is recessive to a *MATα* allele.

The *matα2d-807* mutation was placed into the genome of DC122 at *MAT* by the technique described above and shown to be sufficient in single copy to confer the *matα2d* phenotype. That is, DC122 *matα2d-807* could mate efficiently as an α (Table 2), but the resulting *MAT***a**/*MATα* diploids could not sporulate and mated like α cells.

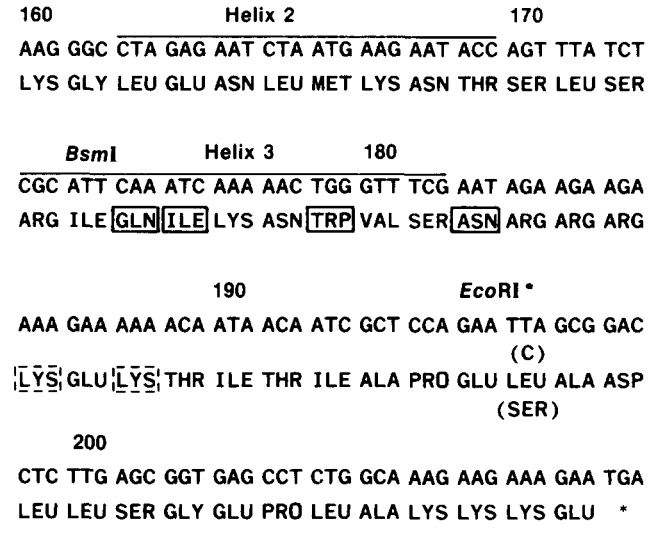

FIGURE 2.—The C-terminal end of the *MATα2* open reading frame. The postulated helices of the homeobox (LAUGHON and SCOTT 1984), and the base change and corresponding amino acid substitution in the *matα2d-807* mutation at codon 196 are indicated in parentheses below the nucleotide and amino acid sequences. Changes at codons 175, 176, 179 and 182 are *matα2* null mutations (solid boxes) and changes at codons 186 and 188 are *matα2h* mutations (dashed boxes) (PORTER and SMITH 1986).

Sequencing of the *matα2d-807* coding region identified only one base change between the published sequence of the wild-type gene (ASTELL *et al.* 1981) and *matα2d-807* (Figure 2). The prediction from the sequencing that the mutation destroys an *Eco*RI* site was confirmed (data not shown). To confirm that this mutation was responsible for the defect, the *Bsm*I fragment (480 bp) containing just the last 110 bases of the 3′ end of the coding region of the *MATα2* gene was exchanged between the wild-type and mutant clones. For this experiment, a YEp213:MATα plasmid with the *MAT Hin*dIII fragment in the opposite orientation was used. The different orientation of the *Hin*dIII fragment had no effect on the phenotype but made unambiguous the demonstration that the *Bsm*I fragments had been exchanged. The transferred mutant *Bsm*I fragment was sufficient to alter the phenotype from wild-type to mutant, and the wild-type *Bsm*I fragment was sufficient to alter he mutant to wild type.

The *matα2d-807* mutation results in a substitution of a serine for a leucine at position 196 of the predicted protein. This position in the α2 protein is next to the "homeobox domain" and sequences involved in DNA binding as discussed below.

DISCUSSION

We have isolated and characterized mutant alleles of the *MATα2* gene that are defective in the regulation of the **a**/α diploid phenotype while retaining the ability to support the α haploid mating phenotype. These have been designated *matα2d* alleles to reflect their defect in the diploid functions and to distinguish them from previously reported alleles defective only in the α haploid phenotype (designated here *matα2h*) or *matα2* null alleles defective in both aspects of cell type regulation by the α2 protein.

Mutations in the *MAT***a***1* gene have no effect on the mating ability of **a** cells (KASSIR and SIMCHEN 1976). This led to the conclusion that the **a**1 protein had no role in the repression of α-specific genes in **a** cells. Similarly, the failure of **a**/α cells to mate has been attributed to the repression of the haploid specific genes by the combined action of the **a**1 and α2 proteins. It has been demonstrated that the *STE3* gene is expressed in *MAT***a**/*matα2* diploids and thus that **a**1 is not sufficient to repress that gene (SPRAGUE, JENSEN and HERSKOWITZ 1983). The ability of the *MAT***a**/*matα2d-807* diploids to mate suggests that the *MAT***a***1* gene product has no direct role in the repression of any gene required for mating by α cells.

The properties of the *matα2d* mutants described here support the view that the α2 protein has separable functions involved in repression of **a**-specific genes in α haploids (defective in *matα2h* mutants) and **a**/α-specific repression in diploids (defective in *matα2d* mutants). The *matα2h* point mutations made by site-directed mutagenesis have been interpreted as identifying a region of α2 required for repression of **a**-specific genes but not for **a**/α-specific repression (PORTER and SMITH 1986). The *matα2d-807* mutation in amino acid 196 may identify a region of α2 protein required specifically for **a**/α repression.

Genes such as *STE5, HO*, and *MATα1* are transcriptionally repressed in **a**/α diploids but can be expressed in haploids. Both the **a**1 and the α2 proteins are required for this repression. The mechanism by which **a**1 and α2 together turn off these genes is as yet unknown. It would seem reasonable that the role of α2 in this regulation is that of a repressor, directly binding to DNA as it does in α cells to repress **a**-specific genes (JOHNSON and HERSKOWITZ 1985). The recognition site for α2 protein was deduced from a consensus sequence in the 5′-regulatory region of several **a**-specific genes (MILLER, MACKAY and NASMYTH 1985). A single copy of this sequence is sufficient to make expression of a promoter sensitive to repression by the α2 protein (JOHNSON and HERSKOWITZ 1985). Specific binding to this sequence has been demonstrated for α2-β-galactosidase-fusion proteins (JOHNSON and HERSKOWITZ 1985). A consensus sequence for genes repressed by α2-**a**1 has also been determined (MILLER, MACKAY and NASMYTH 1985; SILICIANO and TATCHELL 1986; ERREDE *et al.* 1985) and short fragments carrying this sequence were shown to be sufficient to confer **a**/α-specific repression on a heterologous gene (MILLER, MACKAY and NASMYTH 1985). The observation that the α2 and **a**1/α2 recognition sequences are related, although different, supports the view that the α2 protein has a sequence-specific binding role in **a**/α regulation. However, the α2 protein alone does not bind to the **a**1/α2 operator (JOHNSON cited in MILLER, MACKAY and NASMYTH 1985). These observations argue that the role of the **a**1 protein is to alter the sequence specificity of the α2 protein, not merely to bind independently to the DNA (MILLER, MACKAY and NASMYTH 1985).

A priori, several different models for the interaction of the **a**1 and α2 proteins can be imagined. Broadly, they can be classified as models invoking (1) a catalytic modification of α2 by **a**1 that alters the DNA recognition specificity of α2, (2) the stoichiometric binding of **a**1 to α2 to again alter the sequence recognition of α2, (3) the binding of **a**1 to α2 to form a heteromultimer in which both the **a**1 and α2 components have DNA sequence recognition roles, and (4) the independent binding of α2 and **a**1 proteins to sequences upstream of the haploid-specific genes, and that their combined effects result in repression. The observation that **a**1 protein, like α2 protein, has homology to the "homeobox" sequence of the DNA-binding domains of several regulatory proteins (LAUGHON and SCOTT 1984) would seem to support the latter two models that invoke direct DNA interaction by **a**1 protein.

A 68 amino acid domain corresponding to residues 136–204 of the 210 amino acid α2 protein has been demonstrated to be sufficient for sequence-specific DNA binding to the α2 consensus operator (HALL and JOHNSON 1987). This region has similarity to proteins encoded by several *Drosophila* homeotic genes (GEHRING 1987), and includes the helix-turn-helix motif involved in DNA binding by prokaryotic repressors (LAUGHON and SCOTT 1984). PORTER and SMITH (1986) made specific codon alterations in this interval of *MATα2* and obtained both complete null mutations and mutations that cause defects only in repression of the **a**-specific genes (*matα2h* mutations). It is interesting to note that they obtained *matα2h* alleles at codons 186 and 188, very near the *matα2d-807* mutation at codon 196. Further, they were able to make substitutions in codons 189, 190 and 191 without affecting either regulatory role of α2 protein.

HALL and JOHNSON (1987) have defined a region of α2 protein involved in binding to **a**1 protein. This conclusion was based on *matα2* mutations that were dominant to *MATα* for a defect in regulation of an *HO-lacZ* fusion gene when the mutation was present on a high copy number vector in a *MAT***a**/*MATα*

strain. This was interpreted as a reflection of a competition between the mutant and functional α2 proteins for binding to **a**1 protein. Mutations that did not affect the ability of the α2 protein to bind the α2 operator DNA *in vitro* but lost the dominant phenotype were interpreted as having lost sequences required to interact with **a**1. The region defined in that approach included amino acids 20 to 62. These results contrast with the recessive nature of the *matα2d-807* mutation in amino acid 196 on a similar high copy number vector when tested for mating. If the model that a heterodimer between α2 and **a**1 protein is formed is correct, *matα2d-807* may define another region of α2 important for that interaction. Alternatively, the *matα2d-807* mutation could define an extended region of α2 involved in sequence-specific recognition required for **a**/α-specific repression but not for repression in α cells. A third view is that the α2 proteins in **a**/α cells are in equilibrium between an α2 homomultimer (functional in repression of **a**-specific genes) and a heteromultimer formed with **a**1 protein (involved in **a**/α-specific repression). The α2-807 proteins might have higher affinity for each other than for **a**1 proteins so that little of the **a**1/α2 repressor is formed and the *matα2d* phenotype would result. Based on *matα2* mutations with dominant defects in the repression of *MF***a**2, HALL and JOHNSON (1987) have concluded that the region of the protein near the homeobox (amino acids 140–189) is involved in α2-α2 interaction. The *matα2-807* mutation in codon 196 could increase the affinity of that interaction. Biochemical characterization of the α2-807 protein may allow tests of the predictions of these different views and help elucidate mechanism by which the α2 and **a**1 proteins combine to regulate the **a**/α cell type in yeast.

Research sponsored by the National Cancer Institute, Department of Health and Human Services, under contract NO1-CO-74101 with Bionetics Research, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

We would like to thank STUART AUSTIN, DAVE GARFINKEL, STEVE HUGHES and KEITH WEINSTOCK for their helpful review of this paper, PRAMOD SUTRAVE and CHRIS PETROPOULOS for advice on the sequencing protocols, and SYLVIA LUCAS for preparation of the manuscript.

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Communicating editor: E. W. JONES