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# Identification of the DNA sequences controlling the expression of the *MATα* locus of yeast

(*Saccharomyces cerevisiae*/mating type/transcription)

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ABSTRACT **We have excised a 28-base-pair DNA fragment from the *MATα* intergenic region and tested its ability to direct diploid-specific transcriptional repression. This fragment (1643–1671, 5′- GCTTCCCAATGTAGAAAAGTACATCATA -3′) lies within a region required for the normal diploid-specific repression of the *MATα* transcripts. First, the fragment was inserted into a 53-base-pair *MATα* deletion that expresses α1 and α2 constitutively. Insertion of the fragment restores proper diploid regulation to the *MATα* transcripts: α1 mRNA is strongly repressed and α2 mRNA is reduced by a factor of ≈10 from its haploid level. The fragment works equally well in either orientation, and two copies of the fragment do not lead to stronger repression than a single copy. We also inserted the fragment at three sites upstream of the *CYC1–lacZ* fusion gene. Insertions placing the regulatory fragment between the *CYC1* upstream activator sequence (UAS) and the coding region make β-galactosidase efficiently in α haploids but produce 1/40th the enzyme in a/α diploids. This diploid-specific repression requires functional *MAT*a-*1* gene product. Insertion of the *MAT* fragment on the opposite side of the UAS (37 base pairs upstream of the UAS) also caused diploid repression of the fusion gene, but only by a factor of 7. When the regulatory fragment is inserted at a large distance on the far side of the UAS (375 base pairs), it has little if any effect on β-galactosidase expression. We postulate that this sequence is the operator recognized by the diploid-specific repressor.**

Three distinct cell types are found in the yeast *Saccharomyces cerevisiae*: haploid cell types **a** and α, and diploid cell type **a**/α. These cell types are distinguished by many cell-type-specific phenotypes including mating, pheromone production, and pheromone response (1, 2). Cell type is determined by regulating the expression of sets of unlinked genes. In **a** cells, a set of **a**-specific genes, such as the **a**-factor structural genes, is expressed. α cells express a set of α-specific genes, including the α-factor structural genes. Diploid cells express none of these genes but do activate a set of genes allowing meiosis and sporulation. Which set of genes is expressed is determined by a single genetic locus on chromosome III, the mating-type locus (*MAT*). Either of two alleles may reside at the *MAT* locus; haploid α cells carry *MATα*, while haploid **a** cells have *MAT***a**. Diploids resulting from the mating of α and **a** cells are heterozygous at *MAT*; *MATα*/*MAT***a**.

Strathern *et al.* (3) advanced the α1-α2 hypothesis to explain how *MAT* affects this control. The model proposes that in **a** cells, the **a**-specific genes are expressed without any action by *MAT***a**. In α cells, *MATα* encodes two functions: *MATα1* is an activator of α-specific genes, while *MATα2* is a repressor of **a**-specific genes. A set of haploid-specific genes, including genes necessary for mating in both haploid cell types, is expressed in both **a** and α cells. In **a**/α diploids, the products of the *MAT***a**-*1* gene and the *MATα2* gene act together to turn off the **a**-specific, the α-specific, and the haploid-specific genes, and to allow activation of the sporulation specific genes.

How are genes responding to mating-type control distinguished from the thousands of other genes in the cell? One potential means is a specific DNA sequence found at all genes responding to diploid-specific repression, identifying those genes to the control machinery.

Two lines of evidence argue for the existence of a specific DNA sequence that identifies genes under diploid-specific repression. First, a 14-base-pair (bp) deletion in the *MATα* intergenic region abolishes repression, indicating that a DNA sequences essential for control has been lost (4). Second, computer comparison of DNA sequences of genes responding to diploid-specific control reveals the consensus sequence $\begin{matrix}T\\C\end{matrix}C\begin{matrix}A\\G\end{matrix}TGTnn\begin{matrix}A\\T\end{matrix}nAnnTACATCA$ found in front of these genes (5). *HO*, encoding the endonuclease for homothallic switching, has 10 copies of the sequence; *STE5*, a gene required for mating in both haploid cell types, has 2 copies, and 1 copy is found at *MATα*. Two of the *HO* copies have been shown to be sufficient to confer diploid-specific repression (5). In addition, several Ty1 transposable elements cause the transcription of nearby genes to be repressed in diploids (6–8). Examination of the DNA sequence of these Ty elements reveals the presence of diploid repression consensus sequences (9).

In diploids, the combined action of **a**1 and α2 repress *MATα1*, the positive activator of the **a**-specific genes, at the level of transcription (10, 11). To identify DNA sequences that direct the repression of α-specific genes in diploids, we have isolated a small DNA fragment from the α1 5′ noncoding region. We show here that this fragment contains all the information needed for diploid-specific repression.

## MATERIALS AND METHODS

Restriction enzymes, T4 DNA ligase, and Klenow DNA polymerase were purchased from Bethesda Research Laboratories or New England Biolabs; $^{32}P$ was obtained from Amersham. All DNA constructions were sequenced: insertions into *MAT* were sequenced by a modified dideoxy method as described (4); insertions into pLGX1 and pLGΔ312 were sequenced by the same method using primers kindly provided by A. Johnson; insertions into pLGX2 were sequenced by the chemical technique from the *CYC1 Nde* I site, as suggested by A. Hinnebusch. Yeast transformation, integration of *MAT* mutants, and RNA blots were performed as described (4). β-Galactosidase assays were performed by the chloroform lysis method (12). All measurements were performed on five (Tables 1 and 2) or three (Table 3)

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Abbreviations: bp, base pair(s); UAS, upstream activating sequence.

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individual transformants and are shown as the mean activity with two standard deviations.

Yeast strain EG123 (**a** *leu2 trp1 ura3 his4*) and the congenic α derivative 246.1.1 (α *leu2 trp1 ura3 his4*) were used for the integrative transformations. The *CYC1–lacZ* plasmids were transformed into strains 246.1.1, MCY638 (**a** *his4-539 lys2-801 ura3-52*), and 23aX50 (*mata-1–50 leu2 trp1 ura3 his4*). Strain MCY638 was a gift of Marian Carlson.

Plasmid αX67 (100 μg), which has an *Xho* I linker inserted in the *MATα* sequences at position 1643 (numbering system from ref. 13) and a naturally occurring *Nde* I site at 1670, was digested to completion with *Nde* I. The ends were filled in with [α-$^{32}$P]dATP (50 μCi; 3000 Ci/mmol; 1 Ci = 37 GBq) and Klenow polymerase. *Xho* I linkers were then ligated to the filled-in ends and the DNA was digested with *Xho* I to release the desired fragment. The digestion mixture was separated on a 12% polyacrylamide gel and autoradiographed. The 34-bp band (28-bp fragment plus *Xho* I linker) was excised, electroeluted, and purified by DE-53 chromatography. DNA isolated in this way was used for all ligations into *Xho* I sites. For the pLGΔ312 constructions, αX67 was digested with *Xho* I and *Nde* I before filling in to create a blunt-ended fragment.

Derivatives of pLG669-Z with unique *Xho* I sites were prepared by partial digestion with *Xho* I, filling in the DNA ends with Klenow polymerase, and gel purifying the linear band. The DNA was religated, and resultant plasmids were screened for a single *Xho* I site, and the position of the site was mapped. The regulatory fragment was ligated into the unique *Xho* I sites of pLGX1 and pLGX2, and into the unique *Sma* I site of pLGΔ312.

## RESULTS

**A Fragment from the *MATα* Intergenic Region Restores Control to Constitutive Deletions.** *MATα* encodes two divergent transcripts, α1 and α2, whose 5′ ends are separated by only 230 bp. Transcription of both genes requires a bidirectional upstream activator sequence (UAS), located roughly half way between α1 and α2 (4). Deletion of the UAS abolishes the expression of both α1 and α2. A single regulatory region, necessary for the normal diploid-specific repression of both genes, is found on the α1 side of the UAS. Normally, *MATα* synthesizes both α1 and α2 transcripts in α haploids. In **a**/α diploids, however, α1 mRNA is not made and α2 message is present at only 10–20% of its haploid level. The diploid-specific repression of α1 and reduction in α2 requires the combined action of the *MAT***a**-*1* and *MATα2* gene products.

The *MATαR53* deletion (1643–1696) removes 53 bp from the right-hand portion of the intergenic region, including the sequences required for normal diploid-specific repression of *MATα* transcription (Fig. 1 *A* and *B*). The *MATαR53* deletion cannot be repressed by **a**-*1* and *α2* gene products and synthesizes the *MATα1* and *MATα2* transcripts constitutively (Fig. 2). Can the insertion of only a portion of the deleted sequences restore diploid-specific repression to *MATαR53*?

We isolated a restriction fragment [nucleotides 1643–1671 (5′-GCTTCCCAATGTAGAAAAGTACATCATA-3′)] from *MATαX67*. This fragment, from the linker *Xho* I site to a naturally occurring *Nde* I site, contains the DNA removed in constitutive deletion *MATαR14* (1643–1657). The sequence contains the entire consensus sequence for diploid-specific control, as defined by Miller *et al.* (5), plus an additional 8 bp flanking the consensus sequence. If this DNA sequence contains all the information needed to specify diploid repression, it should be sufficient to restore control to the constitutive *MATαR53* deletion.

The 28-bp *MATα-X67 Xho* I/*Nde* I fragment was ligated into the *MATαR53* deletion and four constructions were recovered (Fig. 1*B*). One copy of the fragment in its normal orientation is inserted in pR53+1, while pR53+2 has two

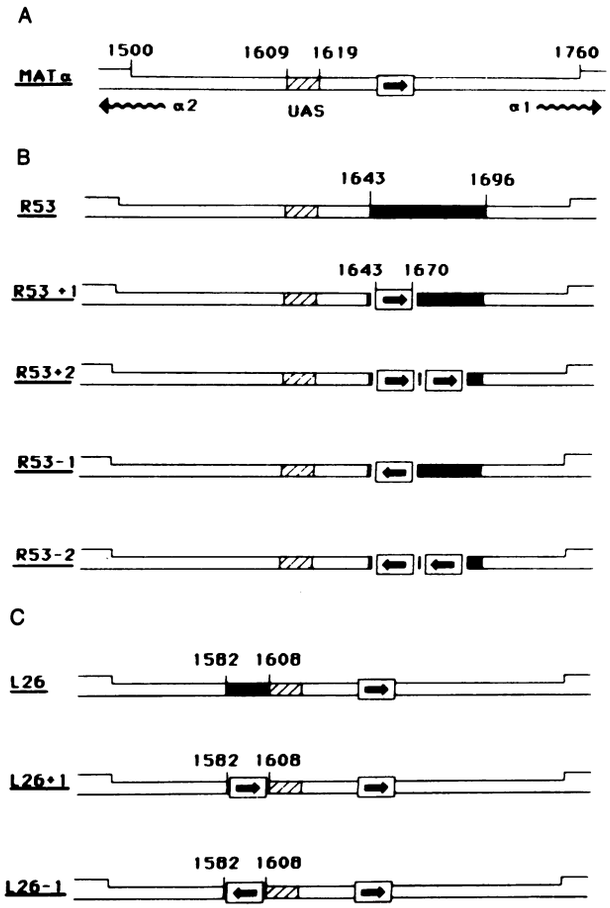


FIG. 1. Mating-type constructions. (*A*) The intergenic region of *MATα* is shown. The transcription initiation sites for α1 and α2 are indicated along with the location of the *MATα* UAS. The boxed arrow (1643–1670) represents the regulatory fragment. Numbering corresponds to ref. 13. (*B*) Insertions into pR53 are diagrammed. The dark box (1643–1696) represents the DNA deleted in pR53. Insertion constructions are shown below. The regulatory fragment is bordered on each side by an *Xho* I linker. (*C*) The pL26 deletion (1582–1608) and the insertions pL26+1 and pL26−1 are shown.

copies of the fragment, both in the normal orientation. Normal orientation refers here to the orientation with respect to *MATα1*. The constructions pR53−1 and pR53−2 have one and two copies of the fragment, respectively, inserted in the orientation opposite that normally found at *MATα1*.

These constructions were tested for regulation by integration into chromosome III at the *MAT* locus, as described (4). Integration of a single copy at the proper locus, with no vector sequences present, was confirmed by Southern blotting.

Diploid regulation of these mutants was tested by examining the α1 and α2 transcripts in haploid and diploid cells on RNA blots (Fig. 2). The parent deletion, *MATαR53*, makes wild-type amounts of both messages in an α haploid, and makes the same level of each transcript in diploid cells. This deletion, then, is fully constitutive for the synthesis of these mRNAs.

Insertion of one copy of the 28-bp fragment in its normal orientation, *MATαR53+1*, restores diploid repression of both transcripts. From this construction, both the α1 and α2 transcripts are synthesized in haploid cells at the wild-type levels. In **a**/α diploids, however, the α1 transcript is not detected and the α2 transcript is repressed to the same extent as seen in wild-type diploids. All the other constructions with the regulatory fragment inserted into *MATαR53* (*MATαR-*

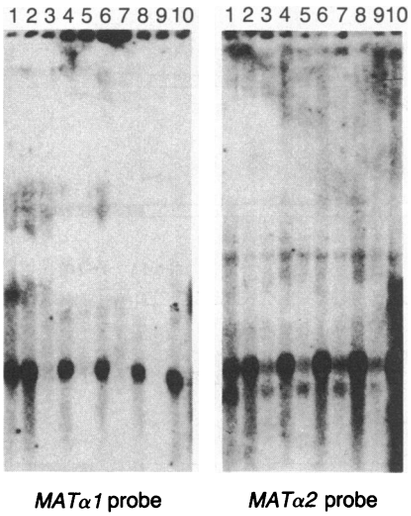


FIG. 2. Regulatory fragment restores control to *MATαR53*. RNA blot analysis shows restoration of diploid-specific repression when the regulatory fragment is inserted into pR53. (*Left*) Probed for α1; (*Right*) probed for α2. *MATαR53* synthesizes α1 and α2 mRNAs constitutively. Insertion of the regulatory fragment (*MATαR53+1, +2, −2, −1*) restores normal diploid control. No difference is detected between the normal and reversed orientations, or between one and two elements. The faster-migrating band found in the diploids is the *MATa-2* transcript, which is homologous to *MATα2* (11). The high background in lane 10 (*Left*) is due to hybridization from the adjacent lane. Lanes: 1, *MATa/MATαR53*; 2, *MATαR53*; 3, *MATa/MATαR53−1*; 4, *MATαR53−1*; 5, *MATa/R53−2*; 6, *MATαR53−2*; 7, *MATa/MATαR53+2*; 8, *MATαR53+2*; 9, *MATa/MATαR53+1*; 10, *MATαR53+1*.

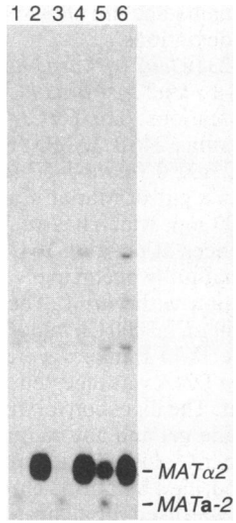


FIG. 3. Regulatory fragment in *MATαL26*. Insertion of the regulatory fragment into pL26 causes stronger repression of α2 transcription in diploids. This filter is probed for α2. *MATαL26* makes α1 and α2 transcripts subject to normal diploid repression. Insertion of the regulatory fragment into pL26 causes stronger repression of α2 in diploids. Lanes: 1, *MATa/MATαL26−1*; 2, *MATαL26−1*; 3, *MATa/MATαL26+1*; 4, *MATαL26+1*; 5, *MATa/MATαL26*; 6, *MATαL26*.

*53+2, MATαR53−1, MATαR53−2)* also restore diploid-specific repression to the *MAT* transcripts.

**Insertion of the 28-bp Fragment Between α2 and the UAS Causes More Severe Repression of α2 Transcription.** Deletion of 26 bp from the left-hand side of the intergenic region, *MATαL26*, does not affect *MAT* transcription (Fig. 3). To test the effect of having a diploid regulatory sequence between the *MATα2* gene and the UAS, the 28-bp fragment was also inserted into pL26 (Fig. 1*C*). Constructions were recovered with the fragment in both its normal orientation, pL26+1, and in the inverted orientation, pL26−1, and were integrated at the *MAT* locus. Both constructions make the α1 and α2 transcripts normally in haploid cells (Fig. 3), but α2 transcription in diploid cells carrying these mutations is repressed below the limit of detection. In addition, the α1 transcript is regulated normally in these constructions (data not shown).

The *MATαL26+1* and *MATαL26−1* diploids show very low levels of α2 mRNA. Thus, the regulatory fragment, when inserted between the *MAT* UAS and α2, causes more severe repression of the α2 transcript. These diploids sporulate at normal levels, indicating that the α2 gene product is present in amounts sufficient to confer the diploid-specific phenotype.

The α2 gene product is autoregulatory; it is required for its own repression. Presumably, when the level of α2 gene product drops too low, the gene is reactivated. Since the *MATαL26+1* and *MATαL26−1* diploids make greatly reduced levels of α2 mRNA and sporulate like normal diploids, we conclude that the level of α2 mRNA in wild-type cells is higher than necessary.

**The *MATα* Regulatory Fragment Can Put Heterologous Genes Under Mating Type Control.** If the 28-bp fragment contains all the DNA sequences necessary to identify a gene for diploid-specific repression, insertion of this fragment into a foreign gene should put that gene under diploid-specific control. We have tested this by placing the fragment in front of a *CYC1–lacZ* hybrid gene (14). This hybrid gene contains 1.1 kilobases of *CYC1* 5′ noncoding sequences, including all the DNA needed for the normal expression of *CYC1*, fused to the *Escherichia coli* β-galactosidase gene (Fig. 4*A*). Expression of the hybrid gene parallels the normal expression of *CYC1*; it is repressed by glucose, requires heme, and is not expressed in the absence of oxygen (15). The *CYC1* UAS present in the hybrid gene contains two subsites, named UAS1 and UAS2, each of which is active in promoting expression of *CYC1* (16). UAS1, centered approximately at −340 bp in Fig. 4, drives virtually all the *CYC1* expression during growth on glucose.

Derivatives of the hybrid *CYC1–lacZ* gene were constructed with unique *Xho* I sites at two positions in the *CYC1* 5′ noncoding region. Construction pLGX1 has a unique *Xho* I site at −245 bp, between the *CYC1* UAS and the hybrid gene coding region (Fig. 4*B*). Construction pLGX2 has a single *Xho* I site at −685 bp, upstream of the UAS (Fig. 4*D*). Both pLGX1 and pLGX2 were derived from pLG669-Z and contain 1.1 kilobases of *CYC1* upstream sequences.

Another derivative, pLGΔ312, was made by deletion from the *Sma* I site in the 3′ end of the *URA3* gene to the *Sma* I site at −375 bp in *CYC1* (Fig. 4*C*). This plasmid removes 705 bp of *CYC1* upstream DNA and brings in the 3′ end of the *URA3* gene. The *URA3* transcription unit does not influence the expression of the *CYC1–lacZ* hybrid gene (15). This derivative retains a unique *Sma* I site at −375 bp on the opposite side of the UAS from the hybrid gene.

The 28-bp fragment was cloned into pLGX1, between the *CYC1* UAS and the coding region, and the resultant constructions were tested for diploid-specific control. Plasmids were recovered containing one regulatory fragment inserted in the normal orientation (in reference to *MATα1*) in pLGX1+1 and one in the reverse orientation in pLGX1−1. Table 1 shows the expression of β-galactosidase from constructions carrying these insertions. The original hybrid gene, pLG669-Z, synthesizes equal amounts of enzyme in both haploid and diploid cells, as does the parent derivative, pLGX1. Insertion of the regulatory fragment does not affect the expression of the hybrid gene in haploids, but it causes a 40-fold decrease in expression when the gene is transcribed

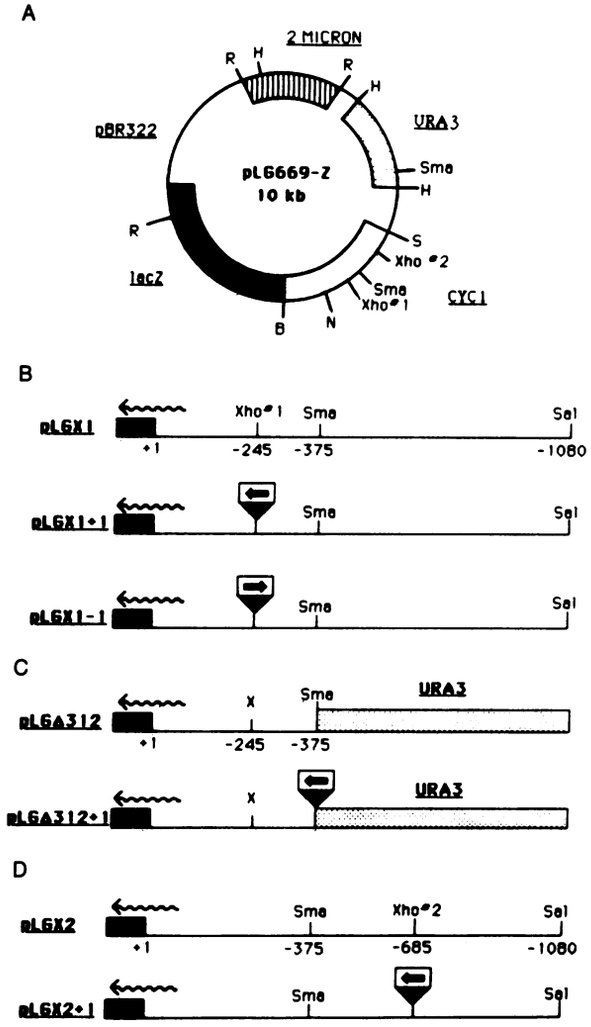


FIG. 4. *CYC1* upstream restriction map. (*A*) The plasmid pLG669-Z, the parent for all the *CYC1* constructions, is shown. The *CYC1* portion (open box) has been expanded for clarity. The locations of the *Xho* I and *Sma* I sites used in making the constructions are shown. Other restriction sites: B, *Bam*HI; H, *Hin*dIII; N, *Nde* I; R, *Eco*RI; S, *Sal* I. (*B*) The insertions of the regulatory fragment into pLGX1 are shown. The *CYC1* UAS is located between the *Xho* I #1 site and the *Sma* I site. (*C*) the pLGΔ312 and pLGΔ312+1 constructions are diagrammed. (*D*) pLGX2 and the insertion pLGX2+1 are shown.

in diploid cells. The fragment, then, contains all the information necessary to direct diploid repression. Again, the fragment functions equally in both orientations.

One possible explanation for the decreased expression in diploids is a diploid-specific decrease in the copy number of these plasmids. Although we have not measured copy number, we feel this is unlikely. pLGX1 is expressed at the same level in haploids and diploids—i.e., there is no indication of copy number changes in these vectors. It seems unlikely that the addition of 28 bp would cause a diploid-specific reduction in copy number of pLGX1+1. Miller *et al.* (5) note that no variations in copy number were found in a similar experiment.

Must the regulatory fragment lie between the UAS and the coding region? Insertion into the *Sma* I site of pLGΔ312 places the fragment ≈40 bp from the UAS on the far side of the coding region (Fig. 4*C*). A single regulatory fragment inserted here, pLGΔ312+1, causes a 6.6-fold repression of *lacZ* expression in diploids. The fragment, then, can direct

Table 1. β-Galactosidase levels of regulatory insertions

| Plasmid | β-Galactosidase units | |
|---|---|---|
| | Haploid | Diploid |
| Parents | | |
| pLG669-Z | 57.2 ± 13.8 | 60.0 ± 20 |
| pLG670-Z | <0.1 | 1.75 ± 1.1 |
| pLGX1 | 40.9 ± 0.8 | 57.4 ± 29 |
| PLGX2 | 56.4 ± 5.9 | 77.4 ± 5.9 |
| PLGΔ312 | 53.4 ± 7.8 | 54.6 ± 7.6 |
| Inserts | | |
| pLGX1+1 | 66.4 ± 3.4 | 1.60 ± 0.15 |
| pLGX1−1 | 64.2 ± 4.6 | 1.69 ± 0.17 |
| pLGΔ312+1 | 59.6 ± 3.0 | 8.98 ± 0.44 |
| pLGX2+1 | 87.0 ± 16 | 84.7 ± 19.9 |

β-Galactosidase activity was assayed as described (12). The haploid host strain was 246.1.1; for diploid measurements, 246.1.1 transformants were mated to MCY638. Two individual transformants of pLGX2+1 were assayed five times each to ascertain the variability within one transformant. Values for pLGX2+1.1 were 84.1 ± 4.7 and for pLGX2+1.6 were 91.6 ± 9.9. Since these values represent the inherent variability of the assay, some of the variability in the values shown in the table is probably due to differences between individual transformants. Plasmid pLG670-Z is derived from pLG669-Z by deletion of the 400-bp *Xho* I fragment, which contains UAS1 and UAS2. Without these, this plasmid synthesizes only very low levels of enzyme.

diploid-specific repression even when located upstream of the UAS.

Repression is not as strong with the fragment upstream of the UAS (pLGΔ312+1) as when it is inserted between the UAS and the coding region (pLGX1+1). These constructions mirror the control of the *MATα* transcripts: α1, with the regulatory element between the upstream promoter and the coding region, is repressed more strongly than α2, which is on the far side of the promoter from the regulator.

Can the regulatory element work over large distances? The fragment was inserted into the *Xho* I site of pLGX2 at −685 bp; this puts the fragment ≈375 bp upstream of the UAS (Fig. 4*D*). This construction, pLGX2+1, is not significantly repressed in diploids (Table 1), indicating that the regulator has little, if any, effect at this distance.

**The *CYC1* Control Mechanism Remains Intact.** Insertion of the *MATα* regulatory region places the hybrid gene under diploid-specific control. Does this insertion affect the response of the gene to its normal regulatory signals? We grew cells carrying the insertion constructions on lactate medium,

Table 2. Lactate derepression of regulatory inserts

| Plasmid | β-Galactosidase units | |
|---|---|---|
| | Haploid | Diploid |
| Parents | | |
| pLG669-Z | 203 ± 17 | 236 ± 42 |
| pLG670-Z | 3.05 ± 0.27 | 0.93 ± 0.19 |
| pLGX1 | 208 ± 33 | 328 ± 50 |
| pLGX2 | 235 ± 11 | 232 ± 30 |
| Inserts | | |
| pLGX1+1 | 156 ± 23 | 23.8 ± 9.8 |
| pLGX1−1 | 129 ± 18 | 20.0 ± 4.0 |
| pLGX2+1 | 217 ± 20 | 83.4 ± 43 |

β-Galactosidase levels were measured on five individual transformants as described (12). Cells were grown to stationary phase in medium containing lactate as described in ref. 16. Regulatory inserts into the *Xho* I site between the UAS and the gene, although still repressed, make significant levels of β-galactosidase. Inserts in the upstream *Xho* I (pLGX2) site appear repressed as well; the reason is unknown.

where transcription of *CYC1* is derepressed. As seen in Table 2, ≈5 times more β-galactosidase is produced from the parent plasmids under derepressing conditions. In diploids, constructions pLGX1+1 and pLGX1−1 are repressed by a factor of 6 but make significantly more β-galactosidase than when grown on glucose. This indicates that the upstream activator sequences of *CYC1* have not been altered such that they cannot be expressed in diploid cells. Insertion of the fragment adds another regulatory system to the normal *CYC1* control. In addition, these results suggest that as a gene becomes more active, the regulatory fragment is less able to cause repression.

**Repression Requires *MAT*a-*1* Function.** Repression of diploid-specific genes requires the *MAT*a-*1* and *MATα2* genes. If the regulatory fragment confers diploid-specific repression on the hybrid gene, this repression should require *MAT*a-*1* and *MATα2*. We transformed plasmids with regulatory insertions into strain aX50, which has a frameshift mutation within the *MAT*a-*1* coding region (17). This strain has the classical phenotype of *mat*a: it mates as an a, but the resultant diploid is unable to sporulate. Expression of hybrid gene constructions in *mat*a strains is shown in Table 3. Transcription of regulatory inserts is not repressed in a1$^-$/α diploids, indicating that this is bona fide mating-type control.

## DISCUSSION

A 28-bp DNA fragment from the intergenic region of *MATα* contains all the sequences necessary to signal a gene for diploid-specific repression. Deletions between the *MATα* UAS and the α1 coding region remove this sequence, resulting in mutant *mat* alleles that fail to repress the transcription of α1 and α2 in diploid cells. Reinsertion of this fragment in either orientation restores diploid-specific repression to constitutive *mat* mutants. This is the only sequence that controls the repression of α2, as no regulatory region is found on the α2 side of the *MATα* UAS.

Insertion of the regulatory fragment into a *CYC1–lacZ* hybrid gene places the fusion gene under mating-type control. Expression of the hybrid gene still obeys *CYC1* regulatory signals, and the diploid-specific repression requires functional *MAT*a-*1* gene product. The regulation of the *CYC1–lacZ* constructions closely parallels the normal *MATα* regulation. In the pLGX1+1 constructions, the regulator lies between the start of transcription and the UAS, ≈100 bp downstream from UAS1. This same configuration is found for *MATα1*, with the regulatory region lying ≈40 bp downstream of the *MATα* UAS. In both cases, the regulatory sequence exerts strong repression in diploids. In the case of both *MATα2* and pLGΔ312+1, the regulatory region lies ≈40 bp upstream of the UAS, and in each case expression is only reduced by a factor of 5–10 in diploids.

Table 3. Control of regulatory inserts requires *MAT*a-1

| Plasmid | β-Galactosidase units | |
|---|---|---|
| | aX50 | aX50/347 |
| pLG669-Z | 52.7 ± 3.7 | 66.2 ± 14 |
| pLG670-Z | 1.03 ± 0.04 | 2.32 ± 0.09 |
| pLGX1 | 35.6 ± 3.7 | 54.5 ± 11.9 |
| pLGX2 | 28.3 ± 2.5 | 43.6 ± 7.6 |
| X1+1 | 37.3 ± 5.1 | 46.9 ± 5.9 |
| X1−1 | 31.1 ± 1.1 | 30.6 ± 11.4 |
| X2+1 | 40.5 ± 4.4 | 53.3 ± 6.9 |

β-Galactosidase levels were measured in the haploid host strain aX50, a *mat*a-1 mutant isogenic to 246.1.1. For diploid measurements, aX50 transformants were mated to strain MCY347 (α *ade2-101 his4-539*), an α strain from the same genetic background as MCY638. Note that derivatives with regulatory insertions are not repressed in *mat*a-1/*MATα* diploids.

The regulatory fragment itself has no promoter activity. Many deletions that remove the *MAT* promoter but retain the regulator fail to transcribe either *MATα* gene (4). Also, a fusion of a promoterless *CYC1–lacZ* hybrid gene to the regulator fails to express β-galactosidase (unpublished observations).

Our results can be interpreted in terms of a classical operator/repressor model in which a repressor molecule binds at the *MATα* regulatory site. The *MATα2* and *MAT*a-*1* gene products are good candidates for such a repressor. Genetically, both are required for diploid repression (10, 11). Structural gene fusions of *MATα2* to *lacZ* are transported to the nucleus (18), and a purified α2–lacZ fusion protein binds to specific DNA sequences upstream of the a-specific gene *STE6* (19). These authors have demonstrated specific binding to a 32-bp sequence located 135 bases upstream from the *STE6* transcription initiation site. The fusion protein does not bind to *MATα* DNA (A. Johnson, personal communication), but repression of *MAT* transcription requires the combined action of α2 and a1, which has not yet been isolated. The interaction of the a-*1* gene product with α2 could change the base specificity of repressor binding as postulated (5, 19). Binding of a repressor at *MATα* could prevent the transcription of α1 directly, and impede the expression of α2 by steric hindrance. Such a mechanism would control transcription by controlling initiation. When inserted into the intron of an actin–*lacZ* fusion gene, the regulatory element was unable to repress transcription in diploids (data not shown). This suggests that the element normally acts by blocking transcription initiation.

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1. Herskowitz, I. & Oshima, Y. (1981) in *The Molecular Biology of the Yeast Saccharomyces*, Monograph 11A, eds. Strathern, J., Jones, E. & Broach, J. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 181–209.
2. Nasmyth, K. (1982) *Annu. Rev. Genet.* **16**, 439–500.
3. Strathern, J., Hicks, J. & Herskowitz, I. (1981) *J. Mol. Biol.* **147**, 357–372.
4. Siliciano, P. & Tatchell, K. (1984) *Cell* **37**, 969–978.
5. Miller, A., MacKay, V. & Nasmyth, K. (1985) *Nature (London)* **314**, 598–603.
6. Errede, B., Cardillo, T., Sherman, F., Dubois, E., Deschamps, J. & Wiame, J. (1980) *Cell* **25**, 427–436.
7. Williamson, V., Cox, D., Young, E., Russell, D. & Smith, M. (1983) *Mol. Cell. Biol.* **3**, 20–31.
8. Taguchi, A., Ciriacy, M. & Young, E. (1984) *Mol. Cell. Biol.* **4**, 61–68.
9. Errede, B., Company, M., Ferchak, J. D., Hutchison, C. A., III, & Yarnell, W. S. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 5423–5427.
10. Klar, A., Strathern, J., Broach, J. & Hicks, J. (1981) *Nature (London)* **289**, 239–244.
11. Nasmyth, K., Tatchell, K., Hall, B., Astell, C. & Smith, M. (1981) *Nature (London)* **289**, 244–250.
12. Guarente, L. (1983) *Methods Enzymol.* **101**, 181–191.
13. Astell, C., Ahlstrom-Jonasson, L., Smith, M., Tatchell, K., Nasmyth, K. & Hall, B. (1981) *Cell* **27**, 15–23.
14. Guarente, L. & Ptashne, M. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 2199–2203.
15. Guarente, L. & Mason, T. (1983) *Cell* **32**, 1279–1286.
16. Guarente, L., Lalonde, B., Gifford, P. & Alani, E. (1984) *Cell* **36**, 503–511.
17. Tatchell, K., Nasmyth, K., Hall, B., Astell, C. & Smith, M. (1981) *Cell* **27**, 25–35.
18. Hall, M., Hereford, L. & Herskowitz, I. (1984) *Cell* **36**, 1057–1065.
19. Johnson, A. & Herskowitz, I. (1985) *Cell* **42**, 237–247.