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Control of yeast α-specific genes: Evidence for two blocks to expression in *MAT*a/*MAT*α diploids

(eukaryotic gene control/yeast mating type/regulatory genes/promoter fusions)

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ABSTRACT **In yeast α cells, the product encoded by the *MATα1* gene of the mating-type locus is required for transcription of at least two genes, *STE3* and *MFα1*. To learn whether the lack of the *MATα1* product in a and a/α cells is sufficient to explain the failure to express *STE3* and *MFα1* in these cells, we have provided *MATα1* product via a hybrid gene that circumvents the normal regulation of the *MATα1* gene. We find by RNA blot analysis that provision of *MATα1* protein permits production of *STE3* and *MFα1* mRNA in a cells but not in a/α cells. These data suggest the existence of an additional regulatory mechanism that prevents expression of α-specific genes in a/α cells, even when *MATα1* product is present. This regulatory mechanism appears to control expression of *STE3* and *MFα1* at the transcriptional level rather than at the posttranscriptional level, because we show that *MFα1* mRNA supplied from a constitutive promoter is translated and processed in a/α cells to yield functional α-factor pheromone. This result shows further that a/α cells possess all the machinery necessary for pheromone maturation, even though these cells do not normally carry out these reactions.**

The *MATα* allele of the yeast mating-type locus encodes regulatory activities that impart an α mating type to haploid cells bearing that allele (refs. 1 and 2; reviewed in refs. 3 and 4). In particular, the *MATα1* product is a positive regulator necessary for expression of a class of genes that is uniquely required by α cells for mating (α-specific genes); expression of this class does not occur in the other two yeast cell types (**a** cells and a/α diploid cells that result from mating of **a** and α cells). Included among the α-specific genes are the structural genes for the α cell mating pheromone (α factor) and for a putative receptor of the mating pheromone synthesized by cells of the **a** mating type (**a** factor). It is believed that α-specific genes are not expressed in the other two yeast cell types because of the absence of *MATα1* product in those cells.

This view is derived from genetic and physiological studies (2) coupled with experiments using cloned genes to measure RNA transcript levels. These studies have led to the following specific picture of the control of cell type by the mating-type locus alleles, *MAT*α and *MAT***a** (the α1–α2 hypothesis, Fig. 1; see ref. 2). *MAT*α encodes two regulatory functions. As discussed above, *MATα1* is a positive regulator and is required for RNA production both from the *STE3* locus (5), which may encode the **a**-factor receptor (unpublished observations), and from *MFα1* (R. Jensen, K. Wilson, and I. Herskowitz, personal communication), which is an α-factor structural gene (6). *MATα2* is a negative regulator that inhibits RNA production from **a**-specific genes including *STE6* (7), *BAR1* (V. MacKay, personal communication), and an **a**-factor structural gene (A. Brake, personal communication). *MAT***a** encodes a single regulator, **a1**, which has no known function in **a** cells, but in a/α cells it acts in concert with the *MATα2* product to inhibit expression of a variety of

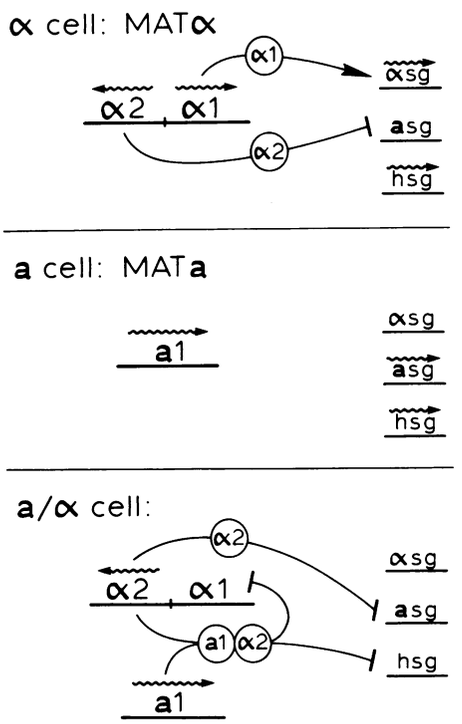

FIG. 1. Control of cell type by mating-type locus (the α1–α2 hypothesis). Expression of functions encoded by the *MAT* alleles and of unlinked genes that are controlled by *MAT* is shown for α, **a**, and a/α cells. Wavy line indicates gene expression; line with an arrowhead indicates stimulation of gene expression; line with a terminal bar indicates inhibition of gene expression. Circles represent regulatory products (presumably proteins) of the *MAT* genes. In an α cell, *MATα1* stimulates expression of α-specific genes (*αsg*) and *MATα2* product inhibits expression of **a**-specific genes (**a***sg*). In an **a** cell, **a***sg* are expressed because *MATα2* product is absent, and *αsg* are not expressed because *MATα1* product is absent. Haploid-specific genes (*hsg*) are expressed in both **a** and α cells. Inhibition of their expression in a/α cells requires the presence of both the *MAT***a***1* and *MATα2* products (referred to as **a1**–α2). Also in a/α cells, the *MATα2* product inhibits expression of **a***sg*, and **a1**–α2 inhibits expression of *MATα1*. The absence of *MATα1* product prevents expression of *αsg*. This report demonstrates that the absence of *MATα1* product in **a** cells is sufficient to account for the lack of expression of *αsg* in those cells, but that the absence of *MATα1* product in a/α cells is not sufficient to account for the failure to express *αsg*.

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Abbreviations: bp, base pair(s); kbp, kilobase pair(s).

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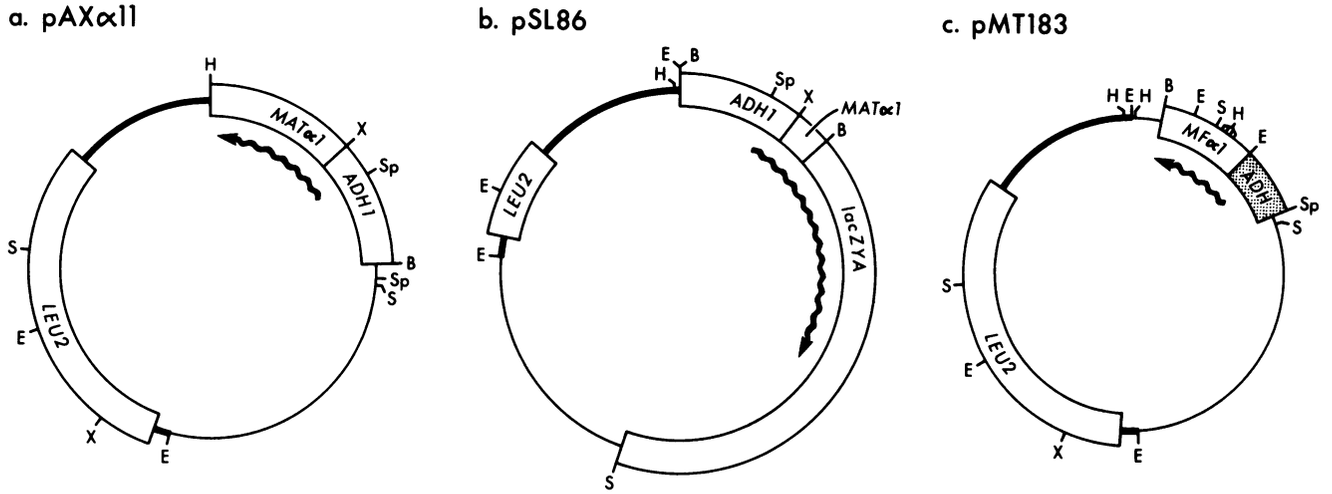

FIG. 2. Structures of plasmids pAXα11, pSL86, and pMT183. Positions of *Hin*dIII (H), *Bam*HI (B), *Sal* I (S), *Xho* I (X), *Sph* I (Sp), and *Eco*RI (E) restriction endonuclease sites are drawn to approximate physical scale. The fine lines in the vector diagrams represent DNA derived from plasmid pBR322 (13); broad lines represent DNA derived from 2-μm circle plasmid of yeast; boxes represent cloned fragments of DNA as labeled. Wavy lines represent transcripts originating from either the *S. cerevisiae ADH1* promoter or the *S. pombe ADH* promoter. (*a*) pAXα11. A 1.5-kbp *Bam*HI/*Xho* I fragment containing the *ADH1* promoter (19) was ligated to a 1.8-kbp *Hin*dIII/*Xho* I fragment derived from a *MATα1 Xho* I linker mutation, αx52 (20), and the gene fusion was inserted into vector YEp13 (21). (*b*) pSL86. The carboxyl terminus of *MATα1* coding sequences in the *ADH1–MATα1* gene fusion was replaced with sequences from the *E. coli lacZ* gene and the resulting 8.2-kbp *Bam*HI/*Sal* I fragment containing the *ADH1–MATα1–lacZ* trihybrid fusion was inserted into pSL57, a derivative of pJDB207 (22). The fragment labeled *lacZ* includes *E. coli lac* operon sequences from the ninth codon of *lacZ* through *lacA*; the promoter and first eight codons of *lacZ* are not present. The *lacZ* fragment was derived from plasmid pMC1403 (23). (*c*) pMT183. Approximately 800 bp of the 5′ flanking region of the *S. pombe ADH* gene was connected to coding sequences of the *MFα1* gene. The junction is within the untranslated leader of the *MFα1* gene, 70 bp from the initiator methionine. The fusion was subcloned as an *Sph* I/*Bam*HI fragment into the vector YEp13. The *S. pombe ADH* segment is stippled to highlight that it is of a different origin than the *S. cerevisiae ADH1* segment.

genes including *MATα1* (8, 9) and several genes unlinked to *MAT* [haploid-specific genes; e.g., *HO* (10) and *STE5* (V. MacKay, J. Thorner, and K. Nasmyth, personal communication)]. Thus, both **a** and **a**/α cells lack the *MATα1* product—**a** cells because they do not contain the *MATα1* gene, and **a**/α cells because *MATα1* expression is blocked by combination of the *MAT***a**1 and *MATα2* products (referred to below as **a**1–α2).

The presence of *MATα1* product in α cells and its absence from **a** and **a**/α cells is clearly sufficient to account for proper regulation of α-specific genes. However, additional control mechanisms may exist in **a** or **a**/α cells to ensure that α-specific genes are not expressed. We have therefore asked whether simply supplying *MATα1* product in **a** or **a**/α cells allows expression of two α-specific genes, *STE3* and *MFα1*.

MATERIALS AND METHODS

Strains and Media. *Saccharomyces cerevisiae* strains used were GM3C-2 (*MATα trp1 leu2 cyc1 cyc7*), GA2 (*MAT***a**/*MATα trp1/trp1 leu2/leu2*), PS23-6A (*MAT***a** *trp1 leu2*), RC629 (see ref. 11; *MAT***a** *sst1-2 ade2 ura1 his6 met1 can1 cyh1 gal2*), and HR125-5d (see ref. 12; *MAT***a** *leu2-3 leu2-112 trp1 ura3-52 his3 his4*). In addition, isogeneic *MATα* and *MAT***a**/*MATα* derivatives of HR125-5d were constructed by *HO*-promoted mating-type interconversion (see ref. 10; the isogeneic **a**, α, and **a**/α strains were kindly provided by Rob Jensen).

Escherichia coli strains used for plasmid propagation were RR1 (see ref. 13; *leuB pro thi hsdR hsdM supE22 ara-14 gal-2 xyl-5 mtl-1* EndoI$^-$ Str^R) and SB69 (14), a *pro*$^+$ *lac*Δ derivative of RR1.

YEPD and SD-leu media for yeast growth were as described (14, 15). The bacterial medium used was LBH (1% tryptone/0.5% yeast extract/0.5% NaCl/1 mM NaOH), supplemented with ampicillin at 100 μg/ml when needed for plasmid selection. The plate assay for α-factor pheromone was performed as described (16–18), with minor modifications.

Plasmids. Plasmid pAXα11 (Fig. 2) contains *MATα1* coding sequences linked to the constitutive alcohol dehydrogenase promoter (*ADH1*) in vector YEp13 (21). The *ADH1* promoter is located on a 1.5-kilobase-pair (kbp) *BamHI/Xho* I DNA fragment, with transcription oriented toward the *Xho* I site (19). This fragment contains ≈1.1 kbp of 5′ flanking DNA that is not required for promoter activity. Sequences necessary for promotion and transcription initiation are confined to the 410 bp proximal to the *Xho* I site and include sequences corresponding to the untranslated portion of the *ADH1* mRNA. The *Xho* I site is an artificial restriction endonuclease site (created by the use of an oligonucleotide linker) at position −10 with respect to the translation initiation codon (19). The 5′ ends of the mature *ADH1* RNA are at positions −37 and −27 relative to the translation initiation codon (24). This promoter fragment is joined to *MATα1* at an *Xho* I site located within sequences that encode the 5′ untranslated leader of *MATα1* mRNA; the *Xho* I site was created by *in vitro* mutagenesis (ref. 20; mutation αx52).

Plasmid pSL86 contains a derivative of the *ADH1–MATα1* fusion from pAXα11 in which the 150th codon of *MATα1* is joined to the 9th codon of the *E. coli lacZ* gene, creating a trihybrid gene fusion (Fig. 2; unpublished observations). The *ADH1–MATα1–lacZ* fusion is carried in vector pJDB207 (22), with the direction of transcription of the fusion with respect to vector sequences opposite that of the *ADH1–MATα1* fusion borne on plasmid pAXα11. In both cases, the gene fusions are buffered from potential effects due to vector sequences by 1 kbp or more of flanking DNA.

Plasmid pMT183 (kindly supplied by M. Hansen, Novo Industri A/S, Denmark) is a derivative of YEp13 that contains a fusion of the *Schizosaccharomyces pombe ADH* promoter with sequences coding for the *MFα1* gene (Fig. 2). The promoter, which is located on an 800-bp *Sph* I/*Eco*RI fragment, initiates transcription efficiently in *Saccharomyces*

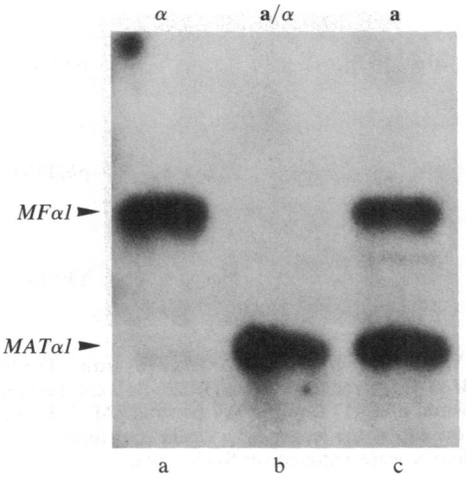

FIG. 3. Expression of the *MATα1* and *MFα1* transcripts in different yeast strains. Lane a, an α strain (GM3C-2) transformed with YEp13 (21); lane b, an **a**/α strain (GA2) transformed with pAXα11; lane c, an **a** strain (PS23-6A) also transformed with pAXα11. For each lane, 10 μg of poly(A) RNA (5, 28) was fractionated by agarose electrophoresis and transferred to nitrocellulose paper (29). The filter was hybridized to a ^{32}P-labeled DNA fragment (2×10^6 cpm) containing *MATα1* sequence, and the RNA·DNA hybrids were visualized by autoradiography. After the first DNA probe was removed in boiling water, the filter was rehybridized to a probe specific for *MFα1*. The independent autoradiograms are shown superimposed and aligned in a fashion that does not reflect true transcript sites. The transcripts corresponding to *MATα1* and *MFα1* are indicated.

cerevisiae (25). The coding sequences of *MFα1* reside on a 1.2-kbp *Eco*RI/*Bam*HI fragment. The *Bam*HI site in the 3′ flanking region is the result of a cloning artifact. The *Eco*RI site was obtained by replacing the *Hin*fI site at position −70 from the initiator ATG with a synthetic *Eco*RI linker.

Restriction endonuclease digestions, ligations, and other enzymatic manipulations of DNA were carried out as specified by the commercial supplier of the enzyme. Transformations of *E. coli* (26) and yeast (27) were as described.

RNA Preparation and Hybridization. Isolation, fractionation, and hybridization of yeast RNA samples were as described (14, 28, 29).

RESULTS AND DISCUSSION

To determine whether the presence of *MATα1* product in **a** and **a**/α cells is sufficient to allow production of *STE3* and *MFα1* RNAs, we used a hybrid gene (Fig. 2) in which the *MATα1* coding sequences were fused to the promoter of the yeast *ADH1* gene (alcohol dehydrogenase) with the expectation that expression of *MATα1* would thereby be divorced from control by the *MAT***a***1* and *MATα2* products (**a**1–α2). We first determined whether the *ADH1–MATα1* hybrid gene in fact permitted *MATα1* expression in all cell types. Plasmid pAXα11, which in addition to *ADH1–MATα1* contains yeast *LEU2* and 2μ sequences enabling selection and autonomous replication of the plasmid, was introduced by DNA transformation into **a** and **a**/α strains. The level of *MATα1* RNA was assayed by RNA blot analysis. As shown in Fig. 3, cells bearing pAXα11 contained *MATα1* transcripts regardless of the genotype at the mating-type locus. Moreover, the concentration of *MATα1* RNA in these cells was significantly greater than that found in α cells lacking the plasmid.

We have shown that the *MATα1* RNA transcribed in the *ADH1–MATα1* fusion is translationally active and functional by two tests. First, when plasmid pAXα11 was introduced into *matα1* mutant strains, mating competence was restored

Table 1. β-Galactosidase activities in isogeneic **a**, α, and **a**/α strains bearing pSL86

MAT genotype	Plasmid	β-galactosidase activity
MATα	None	<4
*MAT***a**	None	<4
*MAT***a**/*MATα*	None	<4
MATα	pSL86	140
*MAT***a**	pSL86	130
*MAT***a**/*MATα*	pSL86	190

Isogeneic *MAT***a**, *MATα*, and *MAT***a**/*MATα* strains were derived from strain HR125-5d by *HO*-promoted mating-type interconversion and were kindly provided by R. Jensen. When present, plasmid pSL86 was introduced into isogeneic strains by DNA transformation as described (27). β-Galactosidase activity was measured by a modification of the procedure of Miller (30) as described (14). Activities (Miller units) are the average of two independent determinations.

(data not shown). Second, we have replaced the carboxyl terminus of *MATα1* with *E. coli lacZ* coding sequences, creating a trihybrid gene in which the 150th codon of *MATα1* is joined to the 9th codon of *lacZ* (Fig. 2). The resultant *ADH1–MATα1–lacZ* trihybrid gene is borne on plasmid pSL86 (Fig. 2). When introduced into isogeneic **a**, α, and **a**/α strains, pSL86 caused production of essentially identical levels of β-galactosidase activity (Table 1).

Since the above assays indicate that the *ADH1–MATα1* hybrid gene present in plasmid pAXα11 is active and functional, we have used pAXα11 to determine whether **a** and **a**/α cells fail to express α-specific genes simply because *MATα1* product is lacking. *STE3* RNA levels were measured in isogeneic **a**, α, and **a**/α strains, with and without plasmid pAXα11 present. When the strains did not contain pAXα11, *STE3* RNA was detected only in α cells, as expected (Fig. 4, lanes a–c). When pAXα11 was present, *STE3* RNA was

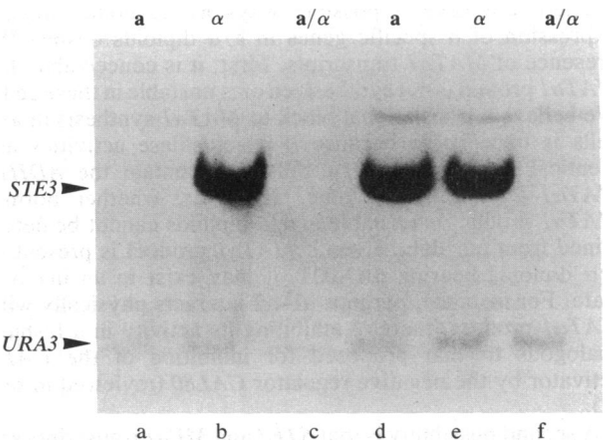

FIG. 4. Expression of *STE3* in isogeneic **a**, α, and **a**/α strains with and without plasmid pAXα11. RNA was isolated (5) from isogeneic **a**, α, and **a**/α strains that either lacked pAXα11 (lanes a–c) or contained pAXα11 (lanes d–f). For each lane, 20 μg of total RNA was fractionated by agarose electrophoresis and transferred to nitrocellulose paper (5, 29). The RNAs were hybridized with a mixture of DNA segments made radioactive by nick-translation in the presence of [α^{32}P]dCTP; one segment contained only *STE3* sequences and the other contained only *URA3* sequences. RNA·DNA hybrids were visualized by autoradiography. The positions of *STE3* and *URA3* transcripts are indicated. The high molecular weight transcript present in lanes d–f is unidentified. It appears to be encoded by vector sequences because it is present in cells bearing YEp13 or pJDB207 (data not shown). Apparently, the *STE3* probe cross-hybridizes with this transcript. See *Materials and Methods* for a description for the isogeneic strains.

produced in both **a** and α cells (lanes d and e). Comparable results were obtained when RNA production from a second α-specific gene, *MFα1*, was assayed (Fig. 3, lane c). These results indicate that the only function of *MATα* required for expression of α-specific genes is supplied by the *MATα1* product and further suggest that a negative regulator of α-specific genes is not present in **a** cells. It is worth noting that **a** cells bearing pAXα11 contain not only transcripts encoding α facor (*MFα1*) and the putative **a**-factor receptor (*STE3*), as shown here, but presumably also transcripts encoding **a** factor and the α-factor receptor, as expected for **a** cells. Since one consequence of binding of **a** or α factor to its cognate receptor is arrest of the cell division cycle (reviewed in refs. 3 and 4), it is puzzling that **a** cells with plasmid pAXα11 are viable. The same puzzle exists for *matα2* mutants, which also contain these four transcript species. As has been proposed for *matα2* mutants (2, 18), perhaps the simultaneous expression of **a**- and α-specific genes in **a** cells bearing pAXα11 leads to functional antagonisms between the gene products. Whatever the explanation, these cells are viable and healthy.

When we asked whether the provision of *MATα1* product is sufficient to permit transcription of α-specific genes in **a**/α diploids, the results were strikingly different from those described above for **a** cells. Specifically, neither *MFα1* (Fig. 3, lane b) nor *STE3* (Fig. 4, lane f) transcripts were detectable in **a**/α cells bearing plasmid pAXα11. This result indicates that a form of negative regulation exists in **a**/α cells that supersedes activation by the *MATα1* product. The second form of regulation is likely to be a consequence of the combined action of the *MAT***a***1* and *MATα2* products (**a**1–α2) because *STE3* RNA is present in *MAT***a**/*matα2* or *mat***a***1*/*MATα* diploids, even in the absence of pAXα11 (data not shown). The existence of this additional regulation of α-specific genes is also supported by data recently reported by Siliciano and Tatchell (31). They showed that when **a**/α cells contained *MATα1* transcript by virtue of a mutation in the regulatory region of *MATα1*, these cells nonetheless failed to express the α-factor structural gene, *MFα1*.

There are several possible ways **a**1–α2 could prevent expression of α-specific genes in **a**/α diploids despite the presence of *MATα1* transcripts. First, it is conceivable that *MATα1* protein is not synthesized or is unstable in these cells. We believe a translational block to *MATα1* synthesis in **a**/α cells is improbable because β-galactosidase activities are identical in **a**, α, and **a**/α cells that contain the *ADH1–MATα1–lacZ* trihybrid gene. However, whether normal *MATα1* product is unstable in **a**/α diploids cannot be determined from our data. Even if *MATα1* product is present in **a**/α diploids bearing pAXα11, it may exist in an inactive state. For instance, perhaps **a**1–α2 interacts physically with *MATα1* product, thereby inhibiting its activity in a fashion analogous to that proposed for inhibition of the *GAL4* activator by the negative regulator *GAL80* (reviewed in ref. 32).

A second possibility is that *STE3* and *MFα1* transcripts are unstable in **a**/α cells. To examine this possibility, we measured production of α-factor pheromone by **a**/α cells that contain a plasmid-borne fusion of the *MFα1* coding region to a constitutive promoter (plasmid pMT183). As shown in Fig. 5, **a**/α cells bearing this fusion secrete as much (or more) α factor as do wild-type α cells. Thus, it seems unlikely that *MFα1* (or *STE3*) transcript is inherently unstable in **a**/α cells. Production of α factor by pMT183-bearing **a**/α cells also provides information about expression of functions necessary for α-factor synthesis in plasmid-free **a**/α cells. Namely, all the enzymes and other machinery necessary for production of mature α factor from the α-factor precursor are present in normal **a**/α cells; it is only the lack of transcription of the wild-type *MFα1* gene that precludes production of α factor by **a**/α cells.

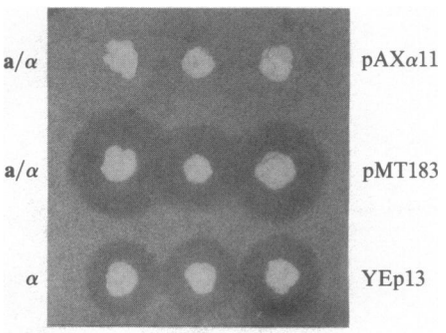

FIG. 5. Assay for α-factor expression. Diploid cells (*MAT***a**/*MATα*) derived from strain HR125-5d were transformed with either plasmid pAXα11 (top row) or plasmid pMT183 (middle row). Bottom row shows the isogeneic α strain transformed with YEp13. Transformants were patched on SD-leu plates, covered with 0.8% top agar containing 5×10^5 cells per ml of the tester strain RC629 (11). Strains producing α factor inhibit the growth of the tester strain, thereby creating a clear zone surrounding the patch (16–18).

Since *MAT***a***1 and MATα2* are already known to act in concert to inhibit RNA production from the haploid-specific genes *HO* and *STE5* and also from *MATα1* (see Fig. 1), perhaps the most likely possibility for the failure to detect *STE3* and *MFα1* transcripts in **a**/α cells containing *MATα1* mRNA is that **a**1–α2 also inhibits RNA production from α-specific genes. The negative regulatory activity of **a**1-α2 could be a direct consequence of action on α-specific genes (for example, as a repressor of their expression). Alternatively, regulation of α-specific genes by **a**1–α2 could be indirect. For example, **a**1–α2 may inhibit expression or function of an activator of α-specific gene expression (in addition to *MATα1*).

Our results demonstrate that there is redundant regulation of α-specific genes in **a**/α cells. Not only is expression of a known activator prevented, but an additional negative control mechanism is operating as well. These findings do not invalidate the basic tenets of the α1–α2 hypothesis, but rather show that some aspects of regulation of mating type are more complex than originally anticipated.

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