Overproduction of the yeast STE12 protein leads to constitutive transcriptional induction

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Haploid *a* and α cells of the yeast *Saccharomyces cerevisiae* respond to the pheromones α - and *a*-factor, respectively, by increasing transcription of inducible genes, arresting cell division, and forming cell-surface projections. These responses are dependent on the activity of several genes, including *STE12*, whose product binds to the pheromone response element located within the regulatory DNA sequences of inducible genes. We assayed the effects of overproducing the STE12 protein in both *STE*⁺ cells, as well as *ste2*, *ste7*, and *ste11* mutant cells. We find that overproduction leads to increased transcription of pheromone-inducible genes and is able to suppress the mating defect of the *ste* mutants. These results suggest that one effect of pheromone treatment may be to increase the ability of the STE12 protein to activate transcription. In addition, we observed that cells cannot tolerate very high levels of the STE12 protein, and many arrest in G₁ with a large size and morphological changes. Thus, constitutively high-level transcription of pheromone-inducible genes causes cells to display some features similar to treatment with pheromone.

[Key Words: Yeast; STE12 protein; pheromone response; transcription; cell-type-specific genes]

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The yeast *Saccharomyces cerevisiae* exhibits two haploid cell types, designated *a* and α . The *a* and α cell (or mating) types differ from one another in that each transcribes a unique set of genes, the *a*- and the α -specific genes, respectively (for review, see Sprague et al. 1983; Nasmyth and Shore 1987; Herskowitz 1988). These gene sets encode such products as the mating pheromones and the receptors to these pheromones. The response of *a* and α cells to the appropriate pheromone includes increased transcription of responsive genes, increased agglutinability, arrest of cell division in the G₁ phase of the cell cycle, and formation of projections (shmoo) (for review, see Cross et al. 1988). The result of mating between an *a* and α cell is the third cell type, the *a*/ α diploid. In the diploid, the *a*- and α -specific sets of genes are repressed, as is the set of haploid-specific genes, which is expressed in both *a* and α cell types.

The transcriptional regulation of these gene sets is controlled by the products of several genes. The primary level of regulation is conferred by the products of the mating type locus (*MAT*) (Strathern et al. 1981). In addition, the product of the *MCM1* gene, also designated as PRTF (pheromone/receptor transcription factor) or GRM (general regulator of mating type), cooperates with the *MAT* products in both positive and negative control (Bender and Sprague 1987; Hayes et al. 1988; Keleher et al. 1988; Tan et al. 1988; Jarvis et al. 1989; Passmore et al. 1989). Furthermore, the products of the *STE4*, *STE5*, *STE7*, *STE11*, and *STE12* genes are necessary for

cell-type-specific transcription (Hartwell 1980; Fields and Herskowitz 1985; Hartig et al. 1986; Jenness et al. 1987; McCaffrey et al. 1987; Fields et al. 1988). *STE4* encodes a subunit of the G protein involved in the signal response pathway (Whiteway et al. 1989), *STE7* and *STE11* appear to encode protein kinases (Teague et al. 1986; L. Connell and B. Errede, pers. comm.), *STE12* encodes a protein that binds to the DNA sequence mediating pheromone response (Dolan et al. 1989; Errede and Ammerer 1989), and the function of *STE5* is currently unknown.

The *STE* genes are required for wild-type levels of expression from the *a*- and α -specific genes, as well as certain haploid-specific genes, although a low level of transcripts is observed even in deletion mutants (Fields et al. 1988). These *STE* genes are also required for the induction of cell-type-specific expression in response to treatment with pheromone (Hartwell 1980). The level of this induction in response to pheromone varies. Some genes, such as *FUS1*, are expressed at significant levels only after treatment with pheromone (McCaffrey et al. 1987; Trueheart et al. 1987), whereas others, such as those encoding the pheromones and receptors, are expressed at an enhanced level following treatment (Hagen and Sprague 1984; Nakayama et al. 1985; Hartig et al. 1986). Pheromone induction of transcription might be defective in *ste* mutants because the *STE* genes are required for the synthesis of the pheromone response pathway or because they, themselves, are part of the pathway, or

both (e.g., see Jenness et al. 1987; Fields et al. 1988). Point mutations in the *STE* genes, some of which lead to only slight reduction in cell-type-specific expression, still have severe effects on mating ability (Hartwell 1980). Thus, the activities of these products are consistent with a role in the transduction of the signal produced by pheromone treatment and not simply in the synthesis of the pathway.

The pheromone response is transmitted via a G protein composed of the products of the *GPA1*, *STE4*, and *STE18* genes (Dietzel and Kurjan 1987; Miyajima et al. 1987; Jahng et al. 1988; Whiteway et al. 1989). The *GPA1* product (the α -subunit) negatively regulates response in the absence of pheromone; the *STE4* and *STE18* products (β - and γ -subunits, respectively) act positively to trigger response after binding of pheromone. Free β γ -subunits produce an unidentified signal that results in the many changes observed in the pheromone-treated cell. It is still to be determined, however, how these changes are coordinated and where branches in the pathway may occur. Null mutations in the *GPA1* gene are recessive haploid lethals because they lead to constitutive response and, hence, cell-cycle arrest (Dietzel and Kurjan 1987; Miyajima et al. 1987), whereas dominant haploid-lethal mutations in *STE4* represent constitutive alleles (Blinder et al. 1989). Both of these classes of lethal mutations are suppressed by *ste5*, *ste7*, *ste11*, and *ste12* mutations (Nakayama et al. 1988; Blinder et al. 1989), suggesting that these four *STE* genes act after the G protein.

We have demonstrated previously that the *STE12* product (designated here as STE12) plays a critical role in pheromone response by binding to the pheromone response element (PRE) located within the regulatory region of inducible genes (Dolan et al. 1989). The PRE sequence was identified by Kronstad et al. (1987) and Van Arsdell and Thorner (1987) as being sufficient to mediate pheromone induction of transcription. The requirement for STE12 in both uninduced and pheromone-induced transcription suggests that the pheromone response pathway is constitutively active at a low level, perhaps maintaining STE12 in a partially modified state capable of some transcriptional activation. Binding of pheromone may then lead to increased STE12 modification and, thus, increased transcription. In this report, we examine the effects of STE12 overproduction. We find that overproduction leads to increased transcription of the cell-type-specific genes and suppression of the mating defect of other *ste* mutants. These results suggest that pheromone induction of transcription may be mediated through changes in the activity of STE12.

Results

Overproduction of STE12 results in increased transcription from pheromone-inducible genes

The STE12 protein, as the factor that binds to the PRE, may be an ultimate target of the signal response pathway triggered by pheromone binding. We reasoned that increased levels of STE12 may lead to transcrip-

tional induction in the absence of pheromone, because either the absolute amount of STE12 is normally limiting or the amount of STE12 activated by the constitutive signal through the response pathway is normally limiting. In the latter case, overproduction may lead to increased levels of activated STE12 as a result of the constitutive signal or through the fortuitous activity of proteins (e.g., protein kinases) that normally act on other substrates.

We constructed two kinds of plasmids to overproduce STE12. The first is a multicopy plasmid that carries the *STE12* gene expressed from its own promoter, either pSY1 (Dolan et al. 1989) or pSY2, which was derived from pSY1 by deleting a short region of vector sequences. These plasmids will be designated 2 μ -STE12. The second kind of plasmid, designated P_{GAL}STE12, is centromere-containing and carries the *STE12* gene expressed from the galactose-inducible *GAL1,10* promoter. We introduced these plasmids into *STE*⁺ strains, isogenic *ste7* and *ste11* single mutants, and a *ste7 ste11* double mutant (EG123 and 246-1-1 background; Table 1). These strains are Gal⁻ (presumably because of a *gal2* mutation) but could be grown on media containing 5% galactose, albeit much more slowly than GAL⁺ strains. Therefore, in addition, we transformed P_{GAL}STE12 into a GAL⁺STE⁺ background (W303-1A; Table 1).

The presence of 2 μ -STE12 led to a fourfold overproduction of STE12 (see below) and an increased expression of several pheromone-inducible genes. A *FUS1-lacZ* gene was used to measure *FUS1* transcription, which has been shown to be strictly dependent on the presence of wild-type *STE7*, *STE11*, and *STE12* products and to be highly inducible by pheromone (McCaffrey et al. 1987; Trueheart et al. 1987). The *FUS1-lacZ* gene was introduced on plasmid pSB231; this plasmid will be designated FUS1-lacZ. A *STE*⁺ cells carrying FUS1-lacZ produced ~0.1 units of β -galactosidase activity in the absence of pheromone and 25–30 units in the presence of α -factor (Table 2). Mutant *ste* strains carrying this plasmid showed no detectable β -galactosidase activity in the absence or presence of α -factor (Table 2).

A *STE*⁺ cells carrying both FUS1-lacZ and 2 μ -STE12 produced an average of 5–10 units of β -galactosidase activity, an increase >50-fold relative to a *STE*⁺ cells not carrying 2 μ -STE12. However, on treatment with α -factor, these double transformants produced essentially the same activity as that seen in α -factor-treated cells lacking 2 μ -STE12 (Table 2). Mutant strains carrying both FUS1-lacZ and 2 μ -STE12 showed an increase in uninduced expression comparable to that seen in the *STE*⁺ strain (0.7–7 units). However, unlike the *STE*⁺ strain, the mutant strains failed to induce higher levels of FUS1- β -galactosidase in response to α -factor (Table 2). These results indicate that the amount of STE12 produced from a multicopy plasmid is capable of increasing uninduced transcription, regardless of the presence of *ste7* or *ste11* mutations. However, it is not sufficient to overcome the lack of responsiveness of these mutants to pheromone. Furthermore, in the *STE*⁺ background, this level of STE12 does not lead to an increased α -factor-in-

Table 1. Yeast strains

Strain	Genotype	Source
EG123	<i>MATa STE⁺ can1 his4 leu2 trp1 ura3</i>	Siliciano and Tatchell (1984)
SF167-5a	<i>EG123 ste12 :: LEU2</i>	Fields and Herskowitz (1987)
DC129	<i>EG123 ste7Δ1</i>	Fields et al. (1988)
DC40	<i>EG123 ste11Δ1</i>	Chaleff and Tatchell (1985)
YY709	<i>EG123 ste7Δ ste11Δ</i>	Fields et al. (1988)
438	<i>EG123 ste2 :: TRP1</i>	B. Hoopes
246-1-1	<i>MATa STE⁺ can1 his4 leu2 trp1 ura3</i>	Siliciano and Tatchell (1984)
SF167-1c	<i>246-1-1 ste12 :: LEU2</i>	Fields and Herskowitz (1987)
DC130	<i>246-1-1 ste7Δ1</i>	Fields et al. (1988)
DC39	<i>246-1-1 ste11Δ1</i>	Chaleff and Tatchell (1985)
YY408	<i>246-1-1 ste7Δ ste11Δ</i>	Fields et al. (1988)
EMPY11	<i>MATa GAL⁺ STE⁺ ade2-101 his3Δ200 lys2-801 suc2 ura3-52</i>	E. Phizicky
W303-1A	<i>MATa GAL⁺ STE⁺ ade2-1 can1-101 his3-11,15 leu2-3,112 trp1-1 ura3</i>	R. Rothstein
227a	<i>MATa cry1 lys1</i>	I. Herskowitz
AM227α	<i>MATa lys1</i>	A. Mitchell
RC631	<i>MATa sst2-1 ade2 can1 cyh2 his6 met1 rme1 ura3</i>	Chan and Otte (1982)
RC757	<i>MATa sst2-1 can1 cyh2 his6 met1</i>	Chan and Otte (1982)

duced level of transcription, indicating that the level of *STE12* is not limiting after pheromone induction.

We assayed the effect of *STE12* overproduction on expression of the *a*-factor genes by means of a halo assay (Wilson and Herskowitz 1984). The amount of *a*-factor produced is visualized by a zone of growth inhibition in a lawn of α cells carrying an *sst2* mutation (Chan and Otte 1982), which makes them supersensitive to *a*-factor. As seen in Figure 1a, a *STE⁺* cells produced a large, well-defined *a*-factor halo, whereas the *ste7*, *ste11*, and *ste7 ste11* mutant cells produced a halo that was barely detectable. The presence of 2 μ -*STE12* in the *STE⁺* cells increased the size of the halo, consistent with an increased level of transcription of *MFA1* and/or *MFA2*.

Table 2. β -Galactosidase activity of *a* cells containing plasmids that carry the *FUS1-lacZ* gene and the *STE12* gene

Strain ^a	Plasmid ^b	- α -Factor ^c	+ α -Factor ^c
<i>STE⁺</i>	<i>FUS1-lacZ</i>	0.1	30.1
<i>ste7</i>	<i>FUS1-lacZ</i>	0	0
<i>ste11</i>	<i>FUS1-lacZ</i>	0	0
<i>ste7,ste11</i>	<i>FUS1-lacZ</i>	0	0
<i>STE⁺</i>	<i>FUS1-lacZ</i>	6.8	23.3
	2 μ - <i>STE12</i>		
<i>ste7</i>	<i>FUS1-lacZ</i>	0.7	0.8
	2 μ - <i>STE12</i>		
<i>ste11</i>	<i>FUS1-lacZ</i>	6.6	7.6
	2 μ - <i>STE12</i>		
<i>ste7,ste11</i>	<i>FUS1-lacZ</i>	3.8	4.2
	2 μ - <i>STE12</i>		

^a*STE⁺* strain is EG123 and *ste* strains are EG123 derivatives.

^b*FUS1-lacZ* is plasmid pSB231 and 2 μ -*STE12* is plasmid pSY2.

^c β -Galactosidase assays were performed on whole cells as described in Experimental procedures. Numbers represent Miller units and are the average of at least three independent assays.

The presence of this plasmid also resulted in a substantial increase in the size of the halo produced by the *ste* cells, to a level almost as large as that produced by the *STE⁺* strain with a control plasmid. As observed with *FUS1* transcription, the effect of *STE12* overproduction on expression of the *a*-factor genes is least dramatic in the *ste7* mutant. However, the *a ste7* mutant in this assay contained the plasmid pSY1, from which pSY2 was derived. pSY1 contains a duplication of vector sequences that appears to stimulate plasmid instability and may partially account for the smaller halo associated with this strain. α Cells carrying 2 μ -*STE12* were assayed for α -factor production by use of a lawn of a *sst2* cells. As with *a* cells, the presence of 2 μ -*STE12* restored the ability of the αste cells to produce pheromone at a level comparable to wild type (Fig. 1b).

The level of *BAR1* expression was also measured in cells containing and lacking 2 μ -*STE12*. The barrier function, encoded by *BAR1*, is secreted by *a* cells to degrade α -factor (Sprague and Herskowitz 1981). Barrier activity was very low or absent in the *ste* mutants, but on introduction of 2 μ -*STE12*, these mutants secreted barrier at a level approaching that seen with *STE⁺* cells (data not shown).

Overproduction of *STE12* suppresses *ste2*, *ste7*, and *ste11* mating defects

Although the presence of 2 μ -*STE12* was insufficient to allow α -factor induction in *ste7* and *ste11* mutants, it was capable of partially restoring mating ability to these strains. We demonstrated this suppression in two assays of mating ability. As shown in Figure 2, the mutants transformed with the vector YEpl3 produced no diploids when replica-plated to a lawn of *STE⁺* cells of the opposite mating type. However, the presence of plasmid 2 μ -

Figure 1. Halo assays for *a*-factor and α -factor. (a) The patches are strains being tested for *a*-factor production, with the lawn being an α *ste2* mutant. (Left) Strains carrying the vector YEp13; (right) strains carrying the STE12 overproducing plasmid pSY2. The α *STE*⁺ strain carries YEp13. Secretion of *a*-factor prevents growth of the lawn and produces a clear zone around the source of *a*-factor. (b) Strains being tested for α -factor; the lawn is an *a* *ste2* mutant. The vector and 2μ-STE12 plasmids are as in a; the *a* *STE*⁺ strain carries YEp13.

STE12 restored the ability of the mutants to mate, albeit at a very low level relative to *STE*⁺ cells. This overproduction of STE12 was able to suppress the mating defect caused by the *ste* mutations in both *a* and α backgrounds (Fig. 2). Double mutants containing both *ste* mutations

appeared to be suppressed as well as either single mutant (Fig. 2).

A quantitative assay was also used to determine the mating ability of these strains, as well as that of an *a* *ste2* mutant. The *STE2* gene encodes the α -factor re-

Figure 2. Mating assays of strains overproducing STE12. (a) A plate assay testing patches of strains for their ability to mate as *a* cells, with strain and plasmid designations indicated below it. The test strains have been replica-plated from an SD-leucine plate onto an SD-minimal plate spread with a lawn of an α *STE*⁺ strain. (b) A similar experiment testing the ability of strains to mate as α cells with an *a* *STE*⁺ lawn. The 2μ-STE12 in these transformants is pSY1.

ceptor (Burkholder and Hartwell 1985; Nakayama et al. 1985; Blumer et al. 1988), so that *ste2* mutants are also completely unresponsive to pheromone. However, the *ste2* mutation, unlike *ste7*, *ste11*, or *ste12* mutations, does not affect the level of uninduced cell-type-specific expression (Hartwell 1980). The *ste* mutants without a plasmid produced no diploids ($<10^{-7}$), whereas the *ste7* and *ste11* mutants carrying 2 μ -STE12 (plasmid pSY2) mated at an average frequency of 5×10^{-5} , an increase of at least 500-fold (Table 3). The *ste2* mutant was suppressed at a lower level (Table 3). The mating results of the *ste* mutants carrying 2 μ -STE12, combined with the inability of the *ste7* and *ste11* strains with this plasmid to induce FUS1-lacZ expression in response to α -factor, indicate that yeast can mate, although at much reduced efficiency, apparently without the pheromone response pathway operating. Presumably, the increased level of transcription from pheromone-inducible genes, mediated by the overproduced STE12, is capable of allowing a small percentage of the cells in the population to carry out the mating process.

We also tested the ability of the *a ste* mutants carrying P_{GAL}STE12 to mate. Gel mobility-shift assays indicated that this plasmid caused an approximate eightfold overproduction of STE12 in these strains (data not shown). Cells were grown in raffinose medium, shifted to 5% galactose for 8 hr, mixed with an α STE⁺ strain in YEP galactose for 4 hr, and then plated for diploids. Efficiency of mating for the *ste7* and *ste11* strains averaged 8×10^{-4} , and for the *ste2* strain, 1.5×10^{-5} (Table 3). These frequencies were ~ 10 -fold greater than those of strains containing 2 μ -STE12, even though STE⁺ cells subjected to this same galactose regimen mated 10-fold less efficiently than STE⁺ cells in glucose.

We assayed whether overproduction of other STE products, the STE7 and STE11 proteins, could suppress

mating defects of the appropriate *ste* mutants. Transformation with plasmid pJD7, a multicopy plasmid carrying the STE7 gene, could complement a *ste7* mutation, but it did not lead to suppression of either *ste11* or *ste12* mutants (data not shown). Similarly, plasmid pJD11, carrying the STE11 gene on a multicopy plasmid, could complement a *ste11* mutation but did not lead to diploids when present in *ste7* or *ste12* strains (data not shown). These results suggest, first, that the STE7 and STE11 products are acting before the STE12 product in the response pathway: Overproduction of the two presumed protein kinases cannot suppress the requirement for the transcription factor bound to the PRE. Second, the inability of a high copy plasmid carrying STE7 to suppress a *ste11* mutation, and vice versa, suggests that these two proteins may not have overlapping functions or specificity. (The failure of a STE7 plasmid to complement a *ste11* mutation and a STE11 plasmid to complement a *ste7* mutation had been noted previously by Chaleff and Tatchell 1985.)

STE12 expression is relatively unaffected by the *ste7* and *ste11* mutations

One possible explanation for the incomplete suppression of *ste7* and *ste11* mutants carrying 2 μ -STE12 is that the expression of the STE12 gene is absolutely dependent on the products of the STE7 and STE11 genes. In this case, the level of STE12 protein in the transformed mutants would still be lower than in the STE⁺ strain. To investigate this possibility, we prepared extracts from STE⁺ and *ste7* and *ste11* strains, both containing and lacking 2 μ -STE12. These extracts were used in gel mobility assays with a fragment from the MFA2 gene as probe (Fig. 3, lane 1). This probe can produce the two STE12-bound shifted bands, indicated by arrows (cf. lanes 2 and 7). In strains not carrying 2 μ -STE12 (lanes 3–6), the level of STE12 DNA binding activity was not dramatically different between STE⁺ and *ste7* or *ste11* backgrounds. In general, this activity was reduced two- to threefold in the *ste7* and *ste11* strains relative to wild type; this reduction varied somewhat from extract to extract.

The presence of 2 μ -STE12 resulted in an approximate fourfold increase in STE12 DNA binding activity in both STE⁺ and mutant strains (Fig. 3, lanes 7–10). The level of activity in the mutants was in excess of the level seen in the STE⁺ strain lacking the plasmid (cf. lane 3 with lanes 8–10). Therefore, the inability of *ste7* and *ste11* strains carrying either 2 μ -STE12 or P_{GAL}STE12 to mate with wild-type efficiency is not due to an insufficient amount of STE12 being produced.

STE12 produced from a galactose-inducible promoter causes lethality

Although cells are able to tolerate the continuous expression of STE12 from a multicopy plasmid, they show a more extreme phenotype when expressing this protein from the galactose-inducible promoter. The level of

Table 3. Quantitative mating efficiency of strains that overproduce STE12

Strain ^a	Plasmid	Carbon source	Mating efficiency
STE ⁺	2 μ -STE12	glucose	2.3×10^{-1}
<i>ste2</i>	2 μ -STE12	glucose	2.9×10^{-6}
	no plasmid		$<1.0 \times 10^{-7}$
<i>ste7</i>	2 μ -STE12	glucose	3.9×10^{-5}
	no plasmid		$<1.0 \times 10^{-7}$
<i>ste11</i>	2 μ -STE12	glucose	4.7×10^{-5}
	no plasmid		$<1.0 \times 10^{-7}$
<i>ste7,ste11</i>	2 μ -STE12	glucose	4.2×10^{-5}
	no plasmid		$<1.0 \times 10^{-7}$
STE ⁺	P _{GAL} STE12	galactose	2.3×10^{-2}
<i>ste2</i>	P _{GAL} STE12	raffinose	$<1.0 \times 10^{-7}$
		galactose	1.5×10^{-5}
<i>ste7</i>	P _{GAL} STE12	raffinose	$<1.0 \times 10^{-7}$
		galactose	4.8×10^{-4}
<i>ste11</i>	P _{GAL} STE12	raffinose	$<1.0 \times 10^{-7}$
		galactose	1.5×10^{-3}
<i>ste7,ste11</i>	P _{GAL} STE12	raffinose	$<1.0 \times 10^{-7}$
		galactose	4.0×10^{-4}

^aStrains as in Table 2.

Figure 3. Gel mobility-shift assay to detect STE12 DNA-binding activity. Extracts are from the following strains: (1) No extract; (2) a *ste12*; (3) a *STE*⁺; (4) a *ste7*; (5) a *ste11*; (6) a *ste7 ste11*; (7) a *STE*⁺ 2 μ -STE12; (8) a *ste7* 2 μ -STE12; (9) a *ste11* 2 μ -STE12; (10) a *ste7 ste11* 2 μ -STE12. The probe was a fragment of the *MFA2* gene containing two PRE sequences. Arrows indicate the position of the STE12-dependent DNA-protein complexes. The 2 μ -STE12 was plasmid pSY2.

STE12 in the *STE*⁺ *GAL*⁺ (W303-1A) cells containing the galactose-inducible *STE12* gene on plasmid P_{GAL}STE12 was ~20-fold higher in galactose as compared to raffinose, which does not induce the *GAL1,10* promoter (data not shown). W303-1A containing either P_{GAL}STE12 or the parental vector was grown in raffinose-containing medium and then shifted to galactose. Cells containing the vector pBM150 continued to grow normally in galactose (Fig. 4a), as did cells containing P_{GAL}STE12 maintained in raffinose (Fig. 4b). However, after 8 hr in galactose for the culture containing P_{GAL}STE12, most of the cells in the population exhibited some morphological changes, and only 20% of the cells showed the appearance of normal buds (Fig. 4c,d). Many cells were larger than control cells, had elongated regions or, in some cases, had two large buds. In addition, some of the cells contained long, thin projections.

The morphological changes associated with galactose induction suggested that many cells carrying P_{GAL}STE12 were arresting in the G₁ phase of the cell cycle. To assay directly for G₁ arrest, we analyzed cultures for their percentage of unbudded cells (Fig. 5a). Cultures containing the parental vector pBM150, grown in raffinose or shifted to galactose, showed an average of ~40% unbudded cells, as did cultures containing P_{GAL}STE12 grown in raffinose. When shifted to galactose, the culture containing P_{GAL}STE12 began to increase its percentage of unbudded cells, reaching a maximum of 70–80% after 6 hr in galactose, an indication that some G₁ arrest was occurring. This percentage did not reach 100%, as it does for α -factor treatment, nor did the mor-

phology of the cells overproducing STE12 resemble classical shmoos. Thus, although cells constitutively producing high levels of pheromone-inducible products showed some similarities to those treated with pheromone, they did not perfectly mimic all aspects of pheromone response.

When cells carrying P_{GAL}STE12 were inoculated onto a galactose plate that required maintenance of the plasmid marker (*URA3*), they failed to produce colonies (Fig. 5b), indicating that a very high level of STE12 is lethal. These cells did double a number of times, as evidenced by growth in the thickest part of the inoculum, presumably reflecting the need to build up sufficient levels of STE12 to cause a lethal phenotype. This lethality may be a consequence of cell-cycle arrest or may be due to the toxic buildup of other pheromone-induced proteins.

Discussion

The yeast STE12 protein binds to the PRE located in the regulatory region of inducible genes whose products are involved in the mating process. STE12 has been overexpressed by means of a multicopy plasmid and a galactose-inducible promoter, and the effects of this overproduction have been assayed in both *STE*⁺ and *ste2*, *ste7*, or *ste11* backgrounds. We find that overproduction of STE12 results in greater than wild-type levels of cell-type-specific products in a *STE*⁺ strain and increases the low level of these products found in *ste7* and *ste11* strains to nearly wild-type levels. These results suggest

Figure 4. Morphology of cells overproducing STE12 from a galactose-inducible promoter. The strain in all panels is W303-1a, a *GAL*⁺ *STE*⁺. (a) P_{GAL}STE12 transformant grown in raffinose; (b) vector pBM150 transformant grown in galactose; (c and d) two independent cultures of a P_{GAL}STE12 transformant after shift to galactose for 8 hr.

Figure 5. (a) STE12 overproduced from a galactose-inducible promoter causes the accumulation of unbudded cells. Cultures of an $a^+ GAL^+ STE^+$ strain containing the vector pBM150 (○) or P_{GAL}STE12 (●) were grown in raffinose, and at 2 hr, galactose was added. Aliquots were removed at the times indicated, sonicated, and examined microscopically for the fraction of unbudded cells. (b) P_{GAL}STE12 causes lethality on galactose. The strains, as in a, were inoculated onto an S-galactose–uracil plate (top) or onto an S-raffinose–uracil plate (bottom). (Left) Strains carried P_{GAL}STE12; (right) strains carried the vector pBM150.

that the activity of STE12 is normally limiting for cell-type-specific transcription and that pheromone treatment leads to transcriptional induction by increasing the activity of STE12. The effect of STE12 overproduction in *ste2*, *ste7*, and *ste11* strains is that the mating defect is partially suppressed, even though these strains are unable to respond to pheromone. However, the mating efficiency is dramatically lower than that of *STE*⁺ strains, indicating that the pheromone-induced signal has additional effects that can only be partially compensated for by increased transcription of pheromone-inducible genes.

Model of STE12 function

STE12 is a DNA-binding transcriptional activator that mediates pheromone induction by binding to the PRE present in inducible genes. The bound protein coop-

erates with other transcription factors to produce the observed levels of expression. In the absence of STE12 or STE12-binding sites, inducible genes are transcribed at a lower level. This level varies from one gene to another and is presumably dependent on the particular binding sites present for other transcription factors, such as the *MCM1* product. In addition, this lower level of transcription is insensitive to pheromone treatment, as observed by deletion of the PRE sequences from the *BAR1* gene (Kronstad et al. 1987).

Figure 6 illustrates the changes that we envision STE12 may undergo as a result of overproduction, pheromone treatment, and *ste* mutations. In uninduced *STE*⁺ cells, STE12 is present in limiting amounts in a partially modified state and is bound to at least some PREs. That STE12 is present in limiting quantities is supported by the fact that overproduction of STE12 results in increased levels of uninduced transcription. Partial modification is suggested by the effect of mutations on the level of STE12-dependent transcription in uninduced cells. Mutations that eliminate components of the pheromone response pathway, such as *ste4*, *ste5*, *ste7*, or *ste11*, affect transcription of responsive genes to the same extent as mutations in *STE12* (Hartig et al. 1986; Fields et al. 1988), which eliminate the transcription factor. The effect of these mutations also resembles that of mutations that eliminate PREs in responsive genes (Kronstad et al. 1987). The fact that pathway mutations can affect the uninduced activity of STE12 supports the idea of partial modification by a low-level constitutive signal through the response pathway. Partial modification may take either of two forms: All molecules of STE12 are partially modified or a fraction of the molecules are fully modified. The nature of the modification dependent on the pheromone response pathway is unknown; it could include post-translational modification of the STE12 protein itself (e.g., phosphorylation), changes in the stability of STE12, binding of STE12 to another activator, or dissociation of STE12 from a repressor. For simplicity in this discussion, we assume that STE12, itself, is the target for a post-translational modification.

In response to pheromone, the α - and α -specific and certain haploid-specific genes are transcribed at higher levels. We suggest that this transcriptional induction is mediated by more complete modification of STE12, based on the results reported here and the following reasons. First, the induction is rapid and occurs in the presence of cycloheximide (Hagen and Sprague 1984). Second, we have found that a hybrid protein composed of the GAL4 DNA-binding domain and all of STE12 is able to drive transcription from GAL4-binding sites but only after treatment of the cells with pheromone (O. Song and S. Fields, unpubl.). In addition to this rapid response, pheromone treatment also leads to increased *STE12* transcription (J.W. Dolan and S. Fields, unpubl.). Thus, maximum transcriptional activation occurs because sufficient STE12 is synthesized and is modified to its fully active form. In *ste7* and *ste11* cells, pheromone treatment has no effect on the ability of STE12 to acti-

Figure 6. Model of STE12 function. Shaded ovals represent STE12 proteins, X represents a modification dependent on the pheromone response pathway, and the number of arrows indicates the qualitative level of transcription of a pheromone-responsive gene.

vate transcription because the response pathway is not functional.

Overproduction of STE12 in *STE*⁺ or *ste* cells results in higher levels of transcription from inducible genes. Thus, the level of STE12 provided by the single chromosomal copy of the *STE12* gene in the *ste7* and *ste11* mutants is not sufficient for wild-type, uninduced expression of responsive genes, probably due to the loss of STE12 modification provided by the pheromone response pathway. However, uninduced levels of expression comparable to those seen in *STE*⁺ strains can be attained in the *ste7* and *ste11* mutants by overproduction of STE12; the overproduced STE12 may not be an effective transcriptional activator due to the lack of modification but can activate transcription by mass-action effects. A small percentage of the overproduced STE12 may also be fortuitously modified, and thus partially activated, by proteins that are not dependent on the pheromone response pathway.

Overproduction of STE12 in *STE*⁺ cells does not result in increased α -factor-induced transcription of *FUS1*. The failure to observe such an enhancement suggests either that another transcription component becomes limiting under inducing conditions or that an activity that modifies STE12 in response to pheromone becomes limiting. In the second case, only the wild-type level of STE12 becomes modified, whereas the excess that results from overproduction does not. Treatment of *ste7* or *ste11* cells overproducing STE12 with pheromone does not result in induced transcription: Only the enhanced level of uninduced transcription due to overproduction is seen. The addition of pheromone to these mutants overproducing STE12 has no effect on transcription from *FUS1* because the *ste* mutations block the signal generated by pheromone.

Pheromone response pathway

Overproduction of STE12 caused by galactose-inducible *STE12* transcription leads to additional phenotypes.

Many cells arrest in G₁, as evidenced by an increasing percentage of unbudded cells and, with time, form cell-surface projections. Although these cells do not exactly mimic pheromone-treated cells, their large size, arrested state, and projections bear certain similarities to cells responding to pheromone. These results suggest that some pheromone-inducible proteins may be interacting with constitutively present *CDC* (cell-division cycle) or *CLN* (cyclin) products as part of the G₁ arrest response. A candidate protein that is induced by pheromone and that may interact with cell-cycle components is the *FAR1* product (F. Chang and I. Herskowitz, pers. comm.); *far1* mutants induce transcription and shmoo but continue to divide in the presence of α -factor. The involvement of cyclins is indicated by the isolation of mutations in the *CLN3* gene (*DAF1-1* of Cross 1988 and *WHI1-1* of Nash et al. 1988) that cause α -factor resistance and a reduced G₁ phase of the cell cycle. Other pheromone-induced proteins may interact with constitutive proteins necessary for nuclear modification and projection formation. In addition, overproduction of STE12 may lead to a higher level of other pheromone-inducible components of the signal response pathway, itself, and these pathway components may contribute to certain aspects of pheromone response.

Any model of the response pathway must also account for the relatively inefficient mating of the *ste* mutants when overproducing STE12. It is clear that pheromone binding leads to responses that overproduction of STE12 does not. For example, an α cell apparently senses the nearby presence of an α cell and forms a mating projection directed toward the potential mating partner, whereas α cells suspended in medium containing α -factor form projections in random directions (Cross et al. 1988). Thus, cells that are forming projections that result from high levels of STE12 would not follow any directional constraints, resulting in reduced mating efficiency. In addition, pheromone treatment also leads to events initiating recovery from cell-cycle arrest. Cells overproducing STE12 and continuing to produce high

levels of pheromone-inducible products may be incapable of recovering properly from their effects.

The ability of STE12 overproduction to cause constitutively high levels of cell-type-specific products suggests that this protein, itself, is a target of the pheromone-induced signal. It will therefore be of interest to determine the biochemical mechanism by which STE12 becomes activated.

Experimental procedures

Yeast strains

The strains of *S. cerevisiae* used are listed in Table 1.

Plasmids

The STE12 gene was cloned into the vector YEpl3 to yield the plasmid pSY1 (Dolan et al. 1989). An *Sph*I fragment that contained duplicated vector sequences was deleted to yield pSY2. The STE12 gene was also cloned into the *Eco*RI site of pBM150 (Johnston and Davis 1984) as an *Eco*RI fragment beginning at the initiator ATG of the STE12 gene to yield P_{GAL}STE12. The *FUS1-lacZ* fusion carried on pSB231 was obtained from J. Trueheart; the *lacZ* fusion is described in Trueheart et al. (1987) and pSB231 is a *CEN4, URA3* plasmid. Plasmid pJD7 was constructed by moving the STE7 gene as a *Hind*III fragment from pSTE7 (Chaleff and Tatchell 1985) into the *Hind*III site of pBluescript II (Stratagene). The STE7 gene was then moved as a *Bam*HI–*Sal*I fragment into YEpl24 (Botstein et al. 1979). Plasmid pJD11 was constructed by moving the STE11 gene as a *Bam*HI–*Sal*I fragment from pSTE11 (Chaleff and Tatchell 1985) directly into YEpl24.

Media and yeast transformation

Media used were YEP and synthetic (S) (Sherman et al. 1986). Carbon sources used were glucose (SD and YEPD), raffinose (SRaf and YEP-Raf), and galactose (SGal and YEP-Gal). Synthetic media were used without supplements (e.g., SD-minimal) or supplemented with amino acids and nucleosides (Sherman et al. 1986). To maintain plasmids with selectable markers, the media were supplemented with all but the indicated component(s) (e.g., SD-uracil). Yeast transformations were done using the glucuronase procedure (Beggs 1978) or the lithium chloride procedure (Ito et al. 1983).

Mating assays

A patch mating test was used as a qualitative measure of mating competence. Strains carrying either 2 μ -STE12 or YEpl3 were inoculated onto SD-leucine plates and allowed to grow into a heavy patch, replica-plated onto a lawn of tester cells of the opposite mating type, either 227a or AM227 α , on SD-minimal medium, and incubated for 2–3 days to allow the growth of diploid colonies.

For quantitative mating tests, strains carrying 2 μ -STE12 or no plasmid were grown on SD-leucine or SD-complete medium, respectively, to a density of 1×10^7 to 5×10^7 cells/ml. Aliquots (300 μ l) were mixed with a 5- to 10-fold excess of EMPY11 grown in YEPD. The mixtures were incubated at 30°C for 2 hr and then plated on SD-minimal plates that had been supplemented with uracil. Strains carrying P_{GAL}STE12 were grown on SRaf-uracil to a density of $\sim 5 \times 10^6$ to 1×10^7 and then split into two aliquots. Galactose was added to 5% to one aliquot, and the cultures were incubated at 30°C for 8 hr to

induce STE12 expression. A 100- μ l aliquot was then mixed with an aliquot of EMPY11 grown in either YEP-Raf or YEP-Gal, as appropriate. The mixtures were incubated for 4 hr and then plated on SD plates containing the required supplements.

Total numbers of cells per milliliter were determined by plating appropriately diluted aliquots of each parental strain on YEPD plates and counting colonies. The mating frequency is the number of diploids produced divided by the number of haploid a cells in the mating reaction.

Extracts and gel mobility-shift assays

Yeast extracts and gel mobility-shift assays were prepared as described previously (Dolan et al. 1989). For the galactose induction of P_{GAL}STE12-containing strains, the cells were grown in SRaf-uracil to an OD₆₀₀ of 0.3 and galactose was added to a final concentration of 5%. The cells were allowed to induce for 8 hr before harvesting. The probe for the gel shift assay was the 209-bp *Eco*RI–*Hind*III fragment from pRR6 (Dolan et al. 1989) that contains upstream regulatory sequences from the MFA2 gene. This fragment was gel-purified and end-labeled using standard methods (Maniatis et al. 1982). Each gel mobility-shift reaction contained 20 μ g of protein, 0.5 ng of probe, and 2 μ g of poly[d(I-C)]/poly[d(I-C)] in a total of 20 μ l.

β -Galactosidase assays

Assays were performed as described (Miller 1972), except that cells were permeabilized by vortexing in 800 μ l of buffer Z with 50 μ l of chloroform and 20 μ l of 0.1% SDS. Following incubation with ONPG and quenching by sodium carbonate, the samples were centrifuged for 10 min to pellet cell debris. Activity was calculated as $[\text{OD}_{420} \times 1000] / [\text{OD}_{600} \times \text{time (min)} \times V \text{ (ml)}]$, where V is the volume of culture used in the assay. Pheromone induction was measured by adding α -factor (Sigma Chemicals) to a final concentration of 0.7 μ M, after which the cultures were incubated for 90 min at 30°C prior to assay.

Halo assays

A patch of wild-type a cells will inhibit the growth of a lawn of α *sst2-1* cells, producing a "halo" around the patch. The same effect is seen with patches of α cells on a lawn of a *sst2-1* cells. Strains to be tested for pheromone production were patched onto a master plate containing the appropriate selective medium. After the patches had grown, they were replicated onto a lawn of *sst2-1* cells on a YEPD plate. The plates were incubated overnight at 30°C and photographed.

Budding assay

Strains used were W303-1A, containing either P_{GAL}STE12 or the vector (pBM150). Cells from an overnight culture grown in SRaf-uracil were used to inoculate fresh SRaf-uracil, and the cells were grown for 2 hr. At that time, galactose was added to a final concentration of 2%. At 1-hr intervals, aliquots were removed, sonicated for 20 sec, and the number of budded and unbudded cells were counted. Total cell counts for each time point numbered ~ 200 .

Microscopy

Micrographs were taken with a Nikon Labophot using phase contrast. All micrographs were taken at 400 $\times$ magnification; the galactose-induced P_{GAL}STE12 cells were sonicated briefly before spreading for photography.

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References

- Beggs, J.D. 1978. Transformation of yeast by replicating hybrid plasmid. *Nature* **275**: 104–109.
- Bender, A., and G.F. Sprague, Jr. 1987. MAT α 1 protein, a yeast transcription activator, binds synergistically with a second protein to a set of cell-type-specific genes. *Cell* **50**: 681–691.
- Blinder, D., S. Bouvier, and D.D. Jenness. 1989. Constitutive mutants in the yeast pheromone response: Ordered function of the gene products. *Cell* **56**: 479–486.
- Blumer, K.J., J.E. Reneke, and J. Thorner. 1988. The STE2 gene product is the ligand binding component of the α -factor receptor of *Saccharomyces cerevisiae*. *J. Biol. Chem.* **263**: 10836–10842.
- Botstein, D., S.C. Falco, S.E. Stewart, M. Brennan, S. Scherer, D.T. Stinchcomb, K. Struhl, and R.W. Davis. 1979. Sterile host yeasts (SHY): A eukaryotic system of biological containment for recombinant DNA experiments. *Gene* **8**: 17–24.
- Burkholder, A.C. and L.H. Hartwell. 1985. The yeast α -factor receptor: Structural properties deduced from the sequence of the STE2 gene. *Nucleic Acids Res.* **13**: 8463–8475.
- Chaleff, D.T. and K. Tatchell. 1985. Molecular cloning and characterization of the STE7 and STE11 genes of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **5**: 1878–1886.
- Chan, R.K. and C.A. Otte. 1982. Isolation and genetic analysis of *Saccharomyces cerevisiae* mutants super-sensitive to G1 arrest by α -factor and α -factor pheromones. *Mol. Cell Biol.* **2**: 11–20.
- Cross, F. 1988. DAF1, a mutant gene affecting size control, pheromone arrest, and cell cycle kinetics of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **8**: 4675–4684.
- Cross, F., L.H. Hartwell, C. Jackson, and J.B. Konopka. 1988. Conjugation in *Saccharomyces cerevisiae*. *Annu. Rev. Cell Biol.* **4**: 429–457.
- Dietzel, D. and J. Kurjan. 1987. The yeast SCG1 gene: a G $_a$ -like protein implicated in the α - and α -factor response pathway. *Cell* **50**: 1001–1010.
- Dolan, J.W., C. Kirkman, and S. Fields. 1989. The yeast STE12 protein binds to the DNA sequence mediating pheromone induction. *Proc. Natl. Acad. Sci.* **86**: 5703–5707.
- Errede, B. and G. Ammerer. 1989. STE12, a protein involved in cell-type-specific transcription and signal transduction in yeast is part of protein–DNA complexes. *Genes Dev.* **3**: 1349–1361.
- Fields, S. and I. Herskowitz. 1985. The yeast STE12 product is required for expression of two sets of cell-type-specific genes. *Cell* **42**: 923–930.
- . 1987. Regulation by the yeast mating type locus of STE12, a gene required for cell-type-specific expression. *Mol. Cell Biol.* **7**: 3818–3821.
- Fields, S., D.T. Chaleff, and G.F. Sprague, Jr. 1988. Yeast STE7, STE11, and STE12 genes are required for expression of cell-type-specific genes. *Mol. Cell Biol.* **8**: 551–556.
- Hagen, D.C. and G.F. Sprague, Jr. 1984. Induction of the yeast α -specific STE3 gene by the peptide pheromone α -factor. *J. Mol. Biol.* **178**: 835–852.
- Hartig, A., J. Holly, G. Saari, and V.L. MacKay. 1986. Multiple regulation of STE2, a mating-type-specific gene of *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **6**: 2106–2114.
- Hartwell, L. 1980. Mutants of *Saccharomyces cerevisiae* unresponsive to cell division control by polypeptide mating hormone. *J. Cell Biol.* **85**: 811–822.
- Hayes, T.E., P. Sengupta, and B.H. Cochran. 1988. The human c-fos serum response factor and the yeast factors GRM/PRTF have related DNA-binding specificities. *Genes Dev.* **2**: 1713–1722.
- Herskowitz, I. 1988. Life cycle of the budding yeast *Saccharomyces cerevisiae*. *Microbiol. Rev.* **52**: 536–553.
- Ito, H., Y. Fukuda, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells treated with alkali cations. *J. Bacteriol.* **153**: 163–168.
- Jahng, K.-Y., J. Ferguson, and S.I. Reed. 1988. Mutations in a gene encoding the α subunit of a *Saccharomyces cerevisiae* G protein indicate a role in mating pheromone signaling. *Mol. Cell Biol.* **8**: 2484–2493.
- Jarvis, E.E., K.L. Clark, and G.F. Sprague, Jr. 1989. The yeast transcription activator PRTF, a homolog of the mammalian serum response factor, is encoded by the MCM1 gene. *Genes Dev.* **3**: 936–945.
- Jenness, D.D., B.S. Goldman, and L.H. Hartwell. 1987. *Saccharomyces cerevisiae* mutants unresponsive to α -factor pheromone: α -Factor binding and extragenic suppression. *Mol. Cell Biol.* **7**: 1311–1319.
- Johnston, M. and R.W. Davis. 1984. Sequences that regulate the divergent GAL1–GAL10 promoter in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **4**: 1440–1448.
- Keleher, C.A., C. Goutte, and A.D. Johnson. 1988. The yeast cell-type-specific repressor α 2 acts cooperatively with a non-cell-type-specific protein. *Cell* **53**: 927–936.
- Kronstad, J.W., J.A. Holly, and V.L. MacKay. 1987. A yeast operator overlaps an upstream activation site. *Cell* **50**: 369–377.
- McCaffrey, G., F.J. Clay, K. Kelsay, and G.F. Sprague, Jr. 1987. Identification and regulation of a gene required for cell fusion during mating of the yeast *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **7**: 2680–2690.
- Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Miller, J.H. 1972. *Experiments in molecular genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Miyajima, I., M. Nakafuku, N. Nakayama, C. Brenner, A. Miyajima, K. Kaibuchi, K. Arai, Y. Kaziro, and K. Matsumoto. 1987. GPA1, a haploid-specific essential gene, encodes a yeast homolog of mammalian G protein which may be involved in mating factor signal transduction. *Cell* **50**: 1011–1019.
- Nakayama, N., A. Miyajima, and K. Arai. 1985. Nucleotide sequences of STE2 and STE3, cell-type-specific sterile genes from *Saccharomyces cerevisiae*. *EMBO J.* **4**: 2643–2648.
- Nakayama, N., Y. Kaziro, K.-I. Arai, and K. Matsumoto. 1988. Role of STE genes in the mating factor signaling pathway mediated by GPA1 in *Saccharomyces cerevisiae*. *Mol. Cell Biol.* **8**: 3777–3783.
- Nash, R. G. Tokiwa, S. Anand, K. Erickson, and A.B. Futcher. 1988. The WH1 $^{+}$ gene of *Saccharomyces cerevisiae* tethers cell division to cell size and is a cyclin homolog. *EMBO J.* **7**: 4335–4346.
- Nasmyth, K. and D. Shore. 1987. Transcriptional regulation in the yeast life cycle. *Science* **237**: 1162–1170.
- Passmore, S., R. Elble, and B.-K. Tye. 1989. A protein involved in minichromosome maintenance in yeast binds a transcriptional enhancer conserved in eukaryotes. *Genes Dev.*

3: 921–935.

- Sherman, F., G.R. Fink, and J.B. Hicks. 1986. *Methods in yeast genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Siliciano, P.G. and K. Tatchell. 1984. Transcription and regulatory signals at the mating type locus in yeast. *Cell* 37: 969–978.
- Sprague, G.F. Jr. and I. Herskowitz. 1981. Control of yeast cell type by the mating type locus. I. Identification and control of expression of the α -specific gene, *BAR1*. *J. Mol. Biol.* 153: 305–321.
- Sprague, G.F., Jr., L.C. Blair, and J. Thorner. 1983. Cell interactions and regulation of cell type in yeast *Saccharomyces cerevisiae*. *Annu. Rev. Microbiol.* 37: 623–660.
- Strathern, J., J. Hicks, and I. Herskowitz. 1981. Control of cell type in yeast by the mating type locus: The $\alpha 1$ - $\alpha 2$ hypothesis. *J. Mol. Biol.* 147: 357–372.
- Tan, S., G. Ammerer, and T.J. Richmond. 1988. Interactions of purified transcription factors: Binding of yeast MAT $\alpha 1$ and PRTF to cell-type-specific, upstream activating sequences. *EMBO J.* 7: 4255–4264.
- Teague, M.A., D.T. Chaleff, and B. Errede. 1986. Nucleotide sequence of the yeast regulatory gene *STE7* predicts a protein homologous to protein kinases. *Proc. Natl. Acad. Sci.* 83: 7371–7375.
- Trueheart, J., J.D. Boeke, and G.R. Fink. 1987. Two genes required for cell fusion during yeast conjugation: Evidence for a pheromone-induced surface protein. *Mol. Cell Biol.* 7: 2316–2328.
- Van Arsdell, S.W. and J. Thorner. 1987. Hormonal regulation of gene expression in yeast. In *Transcriptional control mechanisms* (ed. D. Granner, M.G. Rosenfeld, and S. Chang), pp. 325–332. Alan R. Liss, New York.
- Whiteway, M., L. Hougan, L. Dignard, D.Y. Thomas, L. Bell, G.C. Saari, F.J. Grant, P. O'Hara, and V.L. MacKay. 1989. The *STE4* and *STE18* genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell* 56: 467–477.
- Wilson, K.L. and I. Herskowitz. 1984. Negative regulation of *STE6* gene expression by the $\alpha 2$ product of yeast. *Mol. Cell Biol.* 4: 2420–2427.