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Common signal transduction system shared by *STE2* and *STE3* in haploid cells of *Saccharomyces cerevisiae*: autocrine cell-cycle arrest results from forced expression of *STE2*

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Induction of *STE2* expression using the *GAL1* promoter both in a wild-type *MATα* strain and in a *MATα ste3* strain caused transient cell-cycle arrest and changes in morphology ('shmoo'-like phenotype) in a manner similar to *a* cells responding to α-factor. In addition, *STE2* expressed in a *MATα ste3* mutant allowed the cell to conjugate with *a* cells but at an efficiency lower than that of wild-type α cells. This result indicates that signal(s) generated by *a*-factor in α cells can be substituted by signal(s) generated by the interaction of α-factor with the expressed *STE2* product. When *STE2* or *STE3* was expressed in a *matα1* strain (insensitive to both α- and *a*-factors), the cell became sensitive to α- or *a*-factor, respectively, and resulted in morphological changes. These results suggest that *STE2* and *STE3* are the sole determinants for α-factor and *a*-factor sensitivity, respectively, in this strain. On the other hand, expression of *STE2* in an *a*/α diploid cell did not affect the α-factor insensitive phenotype. Haploid-specific components may be necessary to transduce the α-factor signal. These results are consistent with the idea that *STE2* encodes an α-factor receptor and *STE3* encodes an *a*-factor receptor, and suggest that both α- and *a*-factors may generate an exchangeable signal(s) within haploid cells.

Key words: mating pheromone/receptor/conjugation/sterile

Introduction

The yeast *Saccharomyces cerevisiae* has two haploid cell types, designated α cells and *a* cells, both of which undergo two different stages in their life cycles, vegetative growth and conjugation (mating). Commitment to the conjugation process is initiated by the reciprocal action of peptide mating-factors, α and *a*, secreted into the medium by α cells and *a* cells, respectively. The target for α-factor is strictly an *a* cell and that for *a*-factor is an α cell. Although the α- and *a*-factors have distinctly different amino acid sequences, both molecules evoke seemingly identical responses in their respective target cells. These include: (i) changes in gene expression (Manney, 1983; Strazdis and MacKay, 1983; Hagen and Sprague, 1984; Stetler and Thorner, 1984; Nakayama *et al.*, 1985); (ii) enhancement of adhesiveness to their mating partners (Sakai and Yanagishima, 1972; Fehrenbacher *et al.*, 1978); (iii) cell-cycle arrest at G1 phase (Bücking-Throm *et al.*, 1973; Wilkinson and Pringle, 1974); and (iv) 'shmoo' formation, a morphological change which is recognized as an intermediate stage of the conjugation process (Hartwell, 1973).

Both factors are thought to generate an intracellular signal(s) by interacting with their specific membrane receptors on the target cells to initiate the conjugation program. Genetic studies have suggested that the *a*-specific *STE2* gene may encode the receptor for α-factor and that the α-specific *STE3* gene may correspond to the receptor gene for *a*-factor (MacKay and Manney, 1974; Hartwell, 1980). This notion is supported by a number of observations. First, α-factor binds specifically to *a* cells and the ability of *a* cells to bind α-factor corresponds to the *STE2* function (Jenness *et al.*, 1983; Burkholder *et al.*, 1985). Second, nucleotide sequence analyses show that both genes may encode membrane proteins with multiple putative membrane-spanning regions similar to rhodopsin (Nathans and Hogness, 1983) and β-adrenergic receptor (Dixon *et al.*, 1986), although amino acid sequences of both gene products have no significant homologies (Nakayama *et al.*, 1985; Burkholder *et al.*, 1985; Hagen *et al.*, 1986). Third, a fusion protein composed of the NH_2-terminal region of the *STE3* product and *Escherichia coli* β-galactosidase localized predominantly in membrane fractions suggesting that the *STE3* product is a membrane protein (Hagen *et al.*, 1986).

If both *STE2* and *STE3* products are receptors, they may be capable of transducing a signal(s) by themselves or by interacting with other molecules. It is tempting to speculate that *a*- and α-factors share a common intracellular signal pathway since both mating pheromones affect their target cells in a similar manner. Experiments to study this problem were, first, to replace *STE3* with *STE2* in *MATα* strains (Figure 1B), and second, to express *STE2* and *STE3* separately in a strain of no-mating type (*matα1* strain) (Figure 1C), followed by examining whether they acquire sensitivity to α-factor or to *a*-factor based on their effect on the cell-division cycle, cell morphology ('shmoo' formation) and mating proficiency. In this report, we have expressed *STE2* and *STE3* under the control of the *GAL1* promoter in several mating-

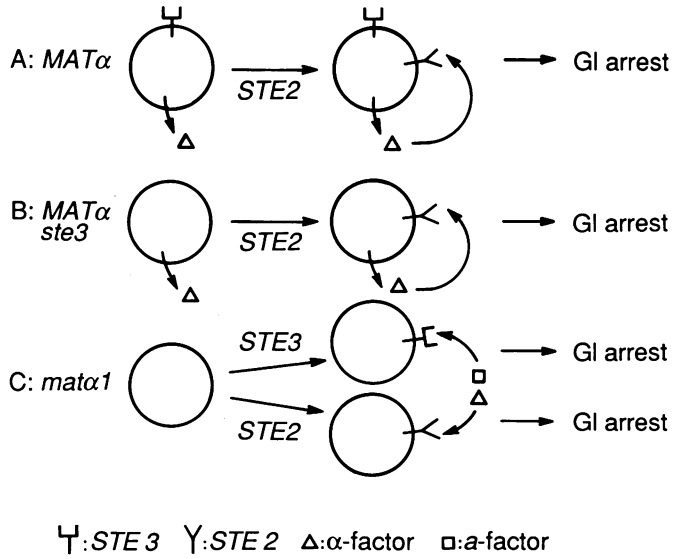

Fig. 1. Schematic representation of the experimental protocol and results. Left circles and right circles denote original yeast cells and cells expressing *STE2* or *STE3* as indicated, respectively. Secretion and action of mating factors are illustrated by arrows. (**A**) NNY10 (*MATα*); (**B**) NNY12 (*MATα Δste3::URA3*); (**C**) NNY13 or 15 (*Δmatα1::URA3*).

factor insensitive strains and found that *STE2* is the determinant for α-factor sensitivity not only in a *MATa* strain, but also in *MATα* and *matα1* strains, and that *STE3* renders a *matα1* strain sensitive to *a*-factor.

Results

Construction of YCpSTE215 and YCpSTE306

In order to express *STE2* and *STE3* in cells of either mating type, we constructed two plasmids, YCpSTE215 and YCpSTE306, in which the *GAL1* promoter sequence plus the entire 5′-untranslated region of the *GAL1* transcript was linked just upstream of the putative initiator ATG codons of *STE2* and *STE3* in place of the natural promoters and 5′-untranslated regions (Figure 2, Materials and methods). To demonstrate expression of genes so constructed, strains NNY10 (*MATα*), NNY12 (*MATα Δste3::URA3*) and NNY13 (*Δmatα1::URA3*) were transformed with YCpSTE215, grown on sucrose (SS medium) and induced with galactose (SSG medium). In Northern blot analysis of poly(A)$^+$ RNA from these transformants, the *STE2* probe specifically hybridized with a 1.6 kb and a longer RNA, both induced by galactose (Figure 3, lanes 2, 3, 5 and 7). The *STE2* gene is known to be expressed only in *a* cells. The *MATα2* product inhibits its transcription in both α cells and diploid cells (Hartig *et al.*, 1986). As expected, no *STE2* transcript was detected in uninduced cells (Figure 3, lanes 1, 4 and 6). The longer transcript which hybridized with the *STE2* probe probably arose due to incomplete transcriptional termination within the *STE2* fragment and efficient termination in the *TRP5* terminator (Miyajima *et al.*, 1984). These results indicate that *STE2* transcription is tightly regulated by the *GAL1* promoter and is induced within 2 h after addition of galactose.

Trans-complementation of ste3 by STE2

Complementation tests were performed to ensure the functional expression of *STE2 in vivo* (Materials and methods, Table I). The plasmid YCpSTE215 introduced into the *STE2*-deficient strain, NNY14 (*MATa Δste2::URA3*), restored its mating ability when the *STE2* expression was induced with galactose on SSG plates as did YCpSTE2B, the original *STE2*$^+$ genomic clone (Nakayama *et al.*, 1985), in the absence of galactose. Thus, the expressed *STE2* product is functional. Furthermore, induction of *STE2* expression in the *STE3* deficient strain, NNY12 (*MATα Δste3::URA3*), restored its mating ability, although the mating efficiency was much lower than that of the same strain transformed with YCpSTE3HpK (a *STE3*$^+$ plasmid, Nakayama *et al.*, 1985) or of a wild-type α cell (NNY10).

Autocrine cell-cycle arrest induced by expression of STE2

Judged by the size of colonies formed after 48 h of incubation at 30°C, NNY14 (*MATa Δste2::URA3*) carrying YCpSTE215 restored its sensitivity to exogenous α-factor (12 nM) under induced conditions (SSG plates), indicating that *STE2* transcribed from the *GAL1* promoter behaved similarly to naturally expressed *STE2* in *a* cells. We also found that a *MATα* strain (NNY10) transformed with YCpSTE215 grew slightly slower on SSG plates than when transformed with the vector plasmid YCpN1 (Nakayama *et al.*, 1985), and that a *MATα ste3* strain (NNY12) transformed with YCpSTE215 exhibited severe growth inhibition on SSG plates compared to NNY16 (*TRP1*$^+$ revertant of NNY12). This growth was inhibited on SSG plates but not on SS plates, indicating that the growth-inhibition is induced by galactose. Growth of *matα1* strains (NNY13 and NNY15) transformed with the same plasmid were not inhibited on SSG plates, but addition of exogenous α-factor (12 nM) caused growth inhibition to nearly the same extent as observed with transformed NNY12 on SSG plates. Addition of α-factor to SS plates had no effect on the growth rate of these strains, therefore, α-factor-induced growth inhibition of NNY13 or NNY15 is also dependent on *STE2*-induction.

To eliminate the possibility that these results were simply due to an effect of the expressed hydrophobic *STE2* product on cell growth, which is independent of cell-cycle, the growth rates of cells in liquid culture were analyzed (Materials and methods, Figure 4). NNY10 (*MATα*) transformed with YCpSTE215 showed transient cell-cycle arrest at an unbudded stage (G1 phase) for 2 h after a 4-h-incubation in SSG medium regardless of

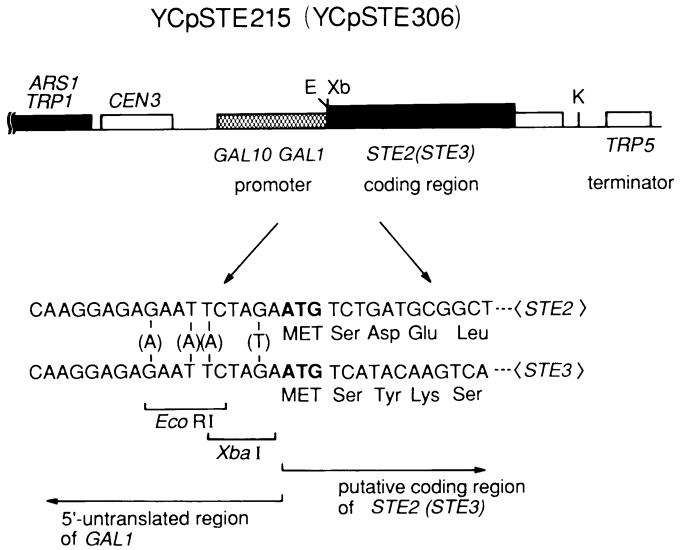

Fig. 2. Structure of the plasmids YCpSTE215 and YCpSTE306. *Eco*RI and *Xba*I sites were introduced just before the initiator ATG codon of the *GAL1* gene. Bases in parentheses are those which have been changed to generate the *Eco*RI and *Xba*I Sites. A *Xba*I site was also introduced just in front of the putative initatior ATG codons of both *STE2* and *STE3* and then fused with the *GAL1* promoter sequences at this site (Materials and methods). E: *Eco*RI; Xb: *Xba*I; K: *Kpn*I.

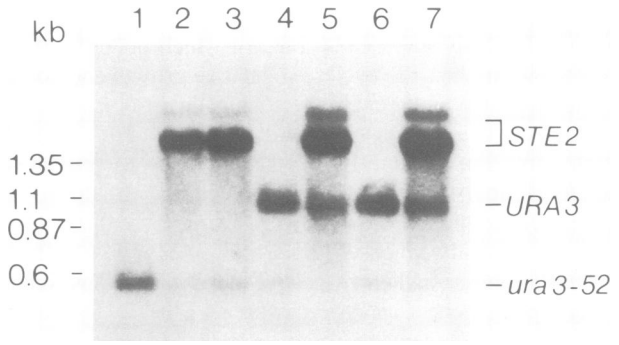

Fig. 3. Induction of *STE2* transcription by galactose. Poly(A)$^+$ RNA from strains NNY10 (*MATα*), NNY12 (*MATα Δste3::URA3*) and NNY13 (*Δmatα1::URA3*) transformed with YCpSTE215 were isolated and subjected to Northern blotting analysis (Materials and methods). The 2.3 kb *Hin*dIII fragment from pUSTE204 (Figure 6) containing the coding region of the *STE2* and *URA3* genes was used as a probe. **Lane 1**: 20 μg of poly(A)$^+$ RNA from NNY10 grown in SS medium; **lane 2**: 5 μg from NNY10 grown in SSG medium for 2 h after transfer from SS medium; **lane 3**: 5 μg from NNY10 grown in SSG medium for 4 h; **lane 4**: 10 μg from NNY12 grown in SS medium; **lane 5**: 5 μg from NNY12 grown in SSG medium for 2 h; **lane 6**: 10 μg from NNY13 grown in SS medium; **lane 7**: 5 μg from NNY13 grown in SSG medium for 2 h.

whether α-factor was present or not (Figure 4A, lower panel). 85% of the total cells were at G1 phase, some of which exhibited an aberrant cell morphology, similar to *a* cells arrested by α-factor. Under the uninduced condition (SS medium), the cells grew exponentially even with the same concentration of α-factor (Figure 4A, lower panel). Galactose itself had essentially no effect on growth rate of the cell since NNY10 carrying YCpN1 (Nakayama *et al.*, 1985) grew exponentially in SSG medium (Figure 4A, upper panel). NNY12 (*MATα Δste3::URA3*) transformed with YCpSTE215 displayed growth properties similar to NNY10 carrying YCpSTE215. After transfer to SSG medium, 90% of the total cell population accumulated in the unbudded stage (G1 phase). Subsequently, morphological change was observed with or without exogenous α-factor, and cells arrested for 4 h spontaneously recovered beyond this (Figure 4B, lower panel). These cell-cycle-arrest kinetics are similar to those of a *MATa sst1-2* strain (RC629) treated with the same concentration of α-factor at the same pH (5.5) (unpublished results; Chan and Otte, 1982). In the uninduced condition (SS medium), the cells grew exponentially (Figure 4B, lower panel) and α-factor had no effect on the growth rate and cell morphology. NNY15 (*Δmatα1::URA3*) transformed with the YCpSTE215 plasmid exhibited cell-cycle-arrest at the unbudded stage (Figure 4C, lower panel and Figure 5) and subsequent 'shmoo' formation only in the presence of exogenous α-factor under the induced condition (SSG medium). In SS medium, the same concentration of exogenous α-factor did not affect its growth rate (Figure 4C, upper panel) or its morphology. As with NNY12, 90% of the total cell population stopped dividing 2.5 h after α-factor addition in SSG medium, remained unbudded for 3–4 h, and then recovered from arrest (Figure 5). In summary, the results obtained from the liquid assay were consistent with those from the plate assay.

Table I. Effect of *STE2* expression on mating

Strain	Plasmid	Tester[a]	Plates	Diploid[b]
NNY11	–	α	SS, SSG	++
(*a*)	YCpSTE215	α	SS, SSG	++
NNY14	–	α	SS, SSG	–
(*a Δste2*)	YCpSTE215	α	SS	–
	YCpSTE215	α	SSG	++
	YCpSTE2B	α	SS, SSG	++
NNY10	–	*a*	SS, SSG	++
(α)	YCpSTE215	*a*	SS, SSG	++
NNY12	–	*a*	SS, SSG	–
(α *Δste3*)	YCpSTE215	*a*	SS	–
	YCpSTE215	*a*	SSG	+
	YCpSTE215	RC618(*a*)	SSG	+/–
	YCpSTE3HpK	*a*	SS, SSG	++

[a] *a*: AX47-4A (*MATa*); α: GT435-5B (*MATα*).
[b] Mating: (++) wild-type; (+) less than wild-type; (+/–) low level; (–) none.

STE3-dependent phenotypes

In order to examine whether *STE3* expression had analogous effects in a *MATa* strain, we introduced the plasmid YCpSTE306 into NNY14 (*MATa Δste2::URA3*) and NNY15 (*Δmatα1::URA3*) as well as into NNY10 (*MATα*) and NNY12 (*MATα Δste3::URA3*).

As shown in Table II, YCpSTE306 complemented the deficient mating of NNY12 on SSG plates to the same level as the wild-type *MATα* strain (NNY10), indicating that expressed *STE3* product is functional. NNY14 transformed with the plasmid displayed a weak but significantly different growth rate from the untransformed control as judged by colony size. However, this observation was not confirmed in liquid culture. When this strain was transferred from SS medium to galactose medium (SSG), the transformant grew exponentially for 9 h and addition of partially purified *a*-factor (2 U/ml, Betz *et al.*, 1977) did not ap-

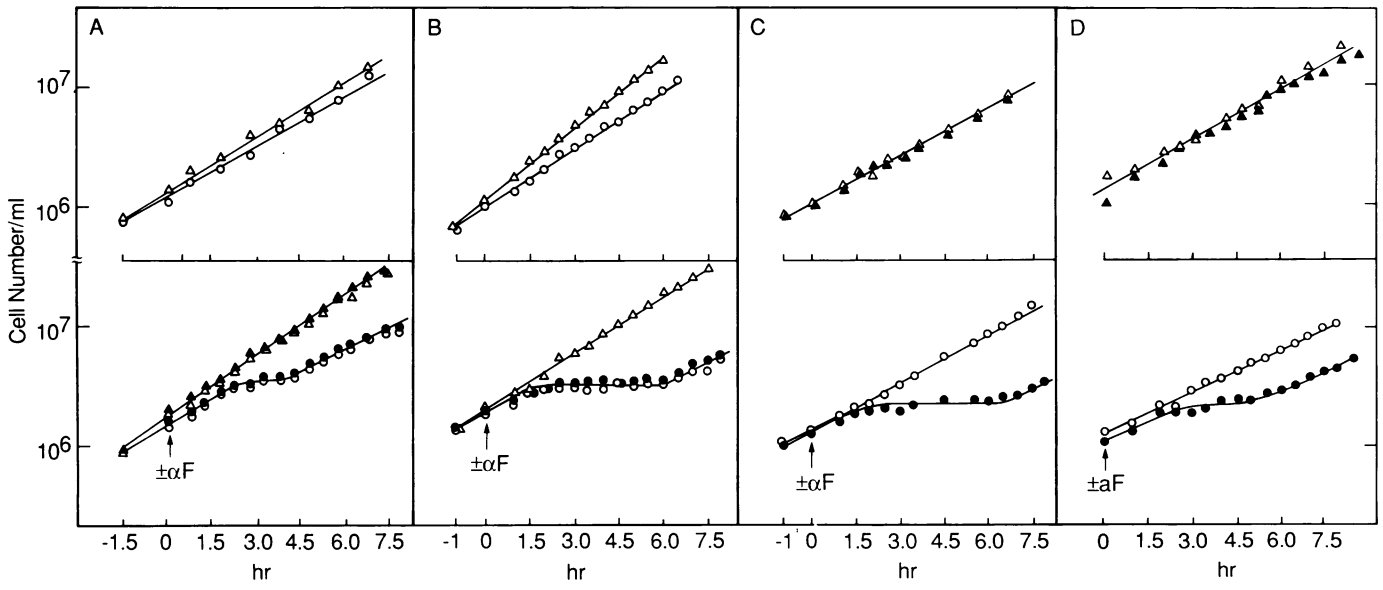

Fig. 4. Response to α-factor and *a*-factor of *MATα* derivative strains expressing *STE2* and *STE3*. (**A**) NNY10 (*MATα*) harboring YCpN1 (upper panel) or YCpSTE215 (lower panel), (**B**) NNY16: *TRP1* revertant of NNY12 (*MATα Δste3::URA3*) (upper panel), NNY12 carrying YCpSTE215 (lower panel), and (**C**) NNY15 (*Δmatα1::URA3*) transformed with YCpSTE215 (upper and lower panels) were subjected to a growth-rate analysis (Materials and methods). Cell numbers were plotted from the time when media were changed from SS to SSG for induction or to SS for control. The arrow indicates the time α-factor (αF) was added at 20 ng/ml (12 nM). (**D**) NNY15 transformed with YCpSTE306 was grown exponentially in both SS and SSG media. Each culture was then diluted to approximately 1×10^6 cells/ml with the same medium with or without crude *a*-factor preparation (1 U/ml, Betz *et al.*, 1977) (upper and lower panels). The arrow indicates the time when the *a*-factor (*a*F) preparation was added. △: uninduced conditions (SS medium); ▲: uninduced condition with factor indicated; ○: induced condition (SSG medium); ●: induced condition with factor indicated.

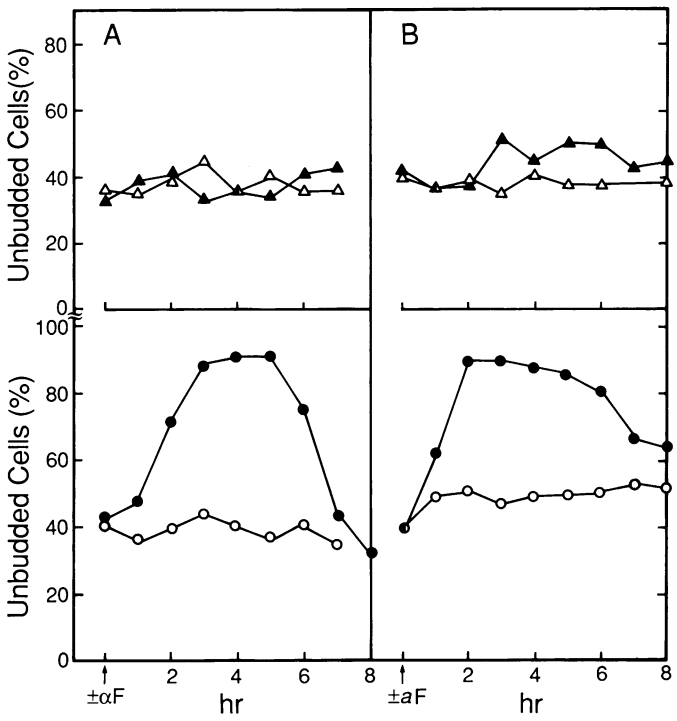

Fig. 5. Accumulation of unbudded cells in response to α-factor and *a*-factor. The results from the experiments described in Figures 4C and 4D using NNY15 (*Δmatα1::URA3*) transformed with YCpSTE215 (**A**) and YCpSTE306 (**B**) were analyzed to display kinetics of unbudded cell accumulation (per cent of total cell number) after α-factor (αF) or *a*-factor (*a*F) addition (arrow). Triangles denote uninduced (SS media) and circles, induced (SSG media) conditions in the absence (open) or presence (solid) of factors.

Table II. Effect of *STE3* expression on mating

Strain	Plasmid	Tester[a]	Plates	Diploid[b]
NNY10	–	*a*	SS, SSG	++
(α)	YCpSTE306	*a*	SS, SSG	++
NNY12	–	*a*	SS, SSG	–
(α *Δste3*)	YCpSTE306	*a*	SS	–
	YCpSTE306	*a*	SSG	++
NNY14	–	α	SS, SSG	–
(*a Δste2*)	YCpSTE306	α	SS	–
	YCpSTE306	α	SSG	P
	YCpSTE2B	α	SS, SSG	++

[a]*a*: AX47-4A (*MATa*); α: GT435-5B (*MATα*).
[b]Mating: (++) wild-type; (P) papillations – very low level; (–) none.

preciably affect the growth rate (data not shown). It is not clear yet whether the *STE3* product is not fully functional in a *MATa* strain until large amounts of the protein accumulate or it needs higher concentrations of *a*-factor to trigger the signal. Complementation of the mating deficiency of NNY14 (*MATa Δste2::URA3*) by induction of *STE3* expression was detected above the background (Table II). However, the efficiency was substantially lower than that of *ste3* cells (NNY12) expressing *STE2* of YCpSTE215 (Table I). This observation may be due to inability of the cell to exhibit efficient cell-cycle-arrest in an autocrine fashion or simply to the difference in genetic background of mating tester strains for *a* and α.

On the other hand, when the plasmid YCpSTE306 was introduced in a *matα1* strain (NNY15), increase in the cell number in SSG medium was greatly inhibited shortly after addition of

Table III. Strain list

Name	Genotype	Source
AX47-4A	*MATa ade6 GAL*	K.Matsumoto
GT435-5B	*MATα leu1 GAL*	K.Matsumoto
NNY10	*MATα trp1-289 ura3-52 lys2-801 ade2-101 GAL*	This work
NNY11	*MATa trp1-289 ura3-52 leu2-3 leu2-112 lys2-801 his3-Δ1 GAL*	This work
NNY12	*Δste3::URA3* in NNY10	This work
NNY13	*Δmatα1::URA3* in NNY10	This work
NNY14	*Δste2::URA3* in NNY11	This work
NNY15	*Δmatα1::URA3 MATα2* in NNY11	This work
NNY16	*TRP1*$^+$ revertant of NNY12	This work
RC618	*MATa ade2-1 ura1 his6 met1 can1 cyh2 rme GAL*	R.Chan
RC629	*sst1-2* in RC618	R.Chan
RC631	*sst2-1* in RC618	R.Chan

crude *a*-factor (1 U/ml) (Figure 4D, lower panel). As shown in Figure 5, 90% of the cells were arrested at G1 phase (unbudded stage) and showed an enlarged cell phenotype. In SS medium the addition of *a*-factor had no effect on the growth rate (Figure 4D, upper panel) and cell morphology. For this experiment, the transformant was grown either in SS medium or in SSG medium for 12 h prior to the addition of the crude *a*-factor.

Induction of STE2 expression in a diploid cell

In order to test the effect of *STE2* expression on the sensitivity of an *a*/α diploid cell to α-factor, we crossed the strains NNY14 (*MATa Δste2::URA3*) transformed with YCpSTE215 and NNY10 (*MATα*). As described previously, diploid cells were obtained only when mating was performed on an SSG plate, indicating that *STE2* expression was induced by galactose in NNY14. Since *STE2* was induced in NNY10 (Figure 3), we expected that galactose would also induce expression of *STE2* on the plasmid YCpSTE215 in the resulting diploid cell. The diploid strain carrying YCpSTE215, however, did not display any significant growth inhibition on addition of either 12 nM or 120 nM of exogenous α-factor on SSG plates.

Discussion

We have constructed four strains, NNY12 (*MATα Δste3::URA3*), NNY14 (*MATa Δste2::URA3*), and NNY13 and NNY15 (*Δmatα1::URA3*), by a one-step gene disruption method (see Materials and methods and Table III), so that the effect of *STE2* or *STE3* expression could be examined under different circumstances: (i) both *STE2* and *STE3* products present (NNY10); (ii) either *STE2* or *STE3* product present (NNY12 and NNY14); or (iii) *STE2* or *STE3* product present in a strain having no mating-type (NNY13 and NNY15).

As summarized in Figure 1, the results showed several points. (i) Artificial expression of *STE2* in a wild-type *MATα* strain caused a transient cell-cycle-arrest predominantly at the unbudded stage even without exogenous α-factor (autocrine-G1-arrest) (Figures 1A and 4A). Whether this *STE2*-dependent phenotype is triggered by intracellular α-factor, or by a secreted α-factor is not known. Expression of *STE2* in a *MATα ste3* mutant showed similar autocrine cell-cycle-arrest and morphology changes ('shmoo' formation) (Figures 1B and 4B). Partial complementation of the mating deficiency of *MATα ste3* mutant by expression of *STE2* suggests that the *STE2* product bypassed the function of *STE3 in vivo* (Table I). (ii) In a *matα1* mutant which lacks both α-specific and *a*-specific gene products, we have induced

either sensitivity to α-factor or sensitivity to *a*-factor by expressing *STE2* or *STE3*, respectively (Figure 1C). Expression of *STE2* or *STE3* alone did not affect the growth rate but addition of α-factor or *a*-factor, respectively, induced a transient cell-cycle-arrest at the unbudded stage and 'shmoo' formation with kinetics similar to those of a *MATα ste3* mutant exhibiting autocrine cell-cycle-arrest by expression of *STE2* (Figures 4 and 5). These results indicate that the *STE2*-dependent phenotypes can be triggered by exogenous α-factor and that a *matα1* mutant has components sufficient to transduce the cell-cycle-arrest signal of both α- and *a*-factors, except that this strain lacks both mating-factor receptors. (iii) The *STE2* product had no observable effect in *a*/α diploid cells. It is not clear whether this is due to an inability to produce functional *STE2* product or whether other components essential for transducing the α-factor signal are missing in an *a*/α diploid cell.

Therefore, we conclude that: (i) consistent with the idea that *STE2* and *STE3* encode receptors for α-factor and *a*-factor, respectively, the *STE2* product serves as a determinant of α-factor sensitivity in haploid cells tested, *MATa*, *MATα* and *matα1* strains, and the *STE3* product determines *a*-factor sensitivity in *MATα* and *matα1* strains; (ii) mating-factor α and *a*, through the function of *STE2* and *STE3*, respectively, may trigger signal(s) exchangeable with each other in an α cell. It is possible that the signal transduction system(s) exists only in haploid cells.

The signal(s) generated by the α-factor-*STE2* system and the *a*-factor-*STE3* system in *MATα* and *matα1* strains are likely to share a common intracellular target(s) or share signal transducing machinery itself since effects of α and *a*-factor on the target cells appear to be identical. It is noteworthy that the lag time to arrest the cell-cycle of the *MATα* strain (NNY10) after induction is longer than that of a *MATα ste3* strain (NNY12) or *matα1* strain (NNY15) and that the cells stayed arrested for a shorter period of time (Figures 4A–C). The *GAL1* product, galactokinase, increased more than 5-fold above basal level between 2 and 4 h after induction (data not shown). Therefore, the *MATα* strain may require more *STE2* product than the *MATα ste3* or *matα1* strains to respond to α-factor. Although the exact relationship between the amount of *STE2* and *STE3* products and the sensitivity of cells to α-factor or *a*-factor remains to be determined, it is tempting to speculate that the *STE3* product might interfere with signal transduction of α-factor by inhibiting the function of the *STE2* product, or by competing for a transducer molecule(s) common to both *STE2* and *STE3* products, so that larger amounts of *STE2* might be required to overcome the inhibitory effect of the *STE3* product in a *MATα* strain (NNY10). This possibility is supported by the observation that a *matα2* mutant, which expresses both α- and *a*-specific genes, is sterile as an α cell (partially sterile as an *a* cell), but that a double mutant, *matα2 ste3*, can conjugate with α cells at an efficiency comparable to wild-type *a* cells, suggesting that *STE3* antagonizes *a*-specific phenotype (Sprague *et al.*, 1981).

Expression of *STE2* in *MATα ste3* (NNY12) and *matα1* (NNY13 and NNY15) strains rendered these cells as sensitive to α-factor as a supersensitive strain (RC629) (Figures 4B and 4C). However, *STE2* complement only weakly the mating deficiency of NNY12 (Table I). The observation that production of α-factor is necessary for α cells to conjugate efficiently with *a* cells (Kurjan, 1985) indicates that reciprocal action and production of mating factors from the opposite type of cells may be necessary for efficient mating. If this is the case, mating efficiency of NNY12 would be low since α-factor secreted from the strain had to act both on itself and on target *a* cells so that the reciprocal action of mating factors was disturbed. In view of the observation that induction of *STE3* expression in the *MATa ste2* strain (NNY14) did not appreciably affect its growth rate even when exogenous *a*-factor was added to the media sufficient to arrest cell-cycle of *STE3*-expressing *matα1* strain (NNY15) (Figure 4D), *a* cells and α cells may not possess completely symmetrical mechanism for mating-factor signal transduction. Therefore, it is reasonable to consider another possibility that the signal(s) generated through the action of the *STE2* product, *a*-specific gene product, might not be utilized efficiently for the mating process in a *MATα* strain.

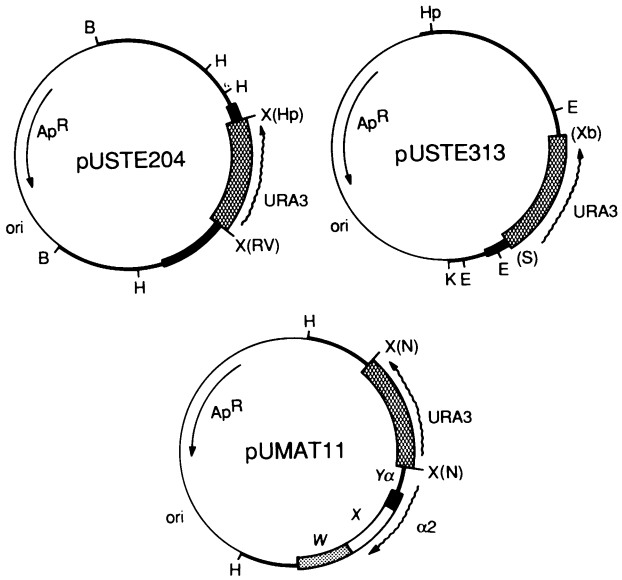

Fig. 6. Structure of plasmids for construction of disruption strains. pUSTE204: constructed for disruption of genomic *STE2* as described in Materials and methods. B: *Bam*HI; H: *Hin*dIII; Hp: *Hpa*I; RV: *Eco*RV; X, *Xho*I. pUSTE313: for deletion of *STE3*; E: *Eco*RI; K: *Kpn*I; S; *Sal*I; Xb: *Xba*I. pUMAT11: for deletion of *MATα*1. N: *Nde*I; *Yα*: *MATα* specific sequence (partial); boxes *X* and *W*: homologous sequences among the mating cassettes (Strathern *et al.*, 1980). Restriction sites in parentheses denote those which were altered by inserting the *URA3* fragment.

Materials and methods

Chemicals, media and enzymes

Oligonucleotides were synthesized by the 380A DNA synthesizer (Applied Biosystems). Synthetic α-factor was purchased from Sigma and from Peninsula Laboratories. Restriction enzymes and T4 ligase were from New England Biolabs and Boehringer Mannheim, respectively. Partially purified *a*-factor was prepared from the supernatant of a X2180-1A (Yeast Genetic Stock Centre) saturated culture by phosphocellulose P11 (Whatmann) (Betz *et al.*, 1977). Culture media and plates, 2% (w/v) agar, contain: SS, 0.67% (w/v) yeast nitrogen base, 0.5% (w/v) casamino acids, 50 μg/ml adenine, 50 μg/ml uracil and 2% (w/v) sucrose; SSG 0.67% (w/v) yeast nitrogen base, 0.5% (w/v) casamino acids, 50 μg/ml adenine, 50 μg/ml uracil, 0.2% (w/v) sucrose and 5% (w/v) galactose.

Yeast strains and plasmid vectors

NNY10 and NNY11 were obtained from tetrad segregants of diploid cells constructed by crossing YNN214 (*MATa GAL ura3-52 lys2-801 ade2-101*), M.Johnston, Washington University, with DBY746 (*MATα gal trp1-289 ura3-52 leu2-3 leu2-112 his3-Δ1*), Yeast Genetic Stock Centre. Expression vector pGT3, from I.Miyajima, DNAX Research Institute, contains the *GAL10–GAL1* promoter sequence with a *Xba*I site introduced just before the initiator ATG codon of *GAL1* so that transcripts of genes fused at the *Xba*I site include the same 5′-untranslated region as the *GAL*1 gene (Figure 2).

Construction of disruption strains of STE2, STE3 and MATα1

Three plasmids to perform one-step gene disruptions were constructed (Rothstein, 1983). For deletion and disruption of *STE2*, the 1 kb *Hin*dIII fragment con-

taining the entire *URA3* gene was introduced in the middle of the coding region of *STE2* in place of the *Hpa*I−*Eco*RV region of pUSTE2B (Nakayama *et al.*, 1985) to yield pUSTE204 (Figure 6). For deletion and disruption of *STE3*, the *URA3* fragment was inserted in place of the *Xba*I−*Sal*I region of pUSTE303 (see below). The resulting plasmid, pUSTE313, lacks nearly 70% of the coding region of *STE3* from the initiator ATG codon (Figure 6). For deletion of *MATα1*, first, a 4.3 kb *Hin*dIII fragment containing the entire *MATα* locus was subcloned from the plasmid pJA1-45TA provided by J.Anagnost, Schering Research, into a derivative of pUC8 which lacks the *Nde*I site. The *Nde*I fragment of the resulting plasmid, which includes the region from the TATA box to the 3′-untranslated sequence of *MATα1*, was replaced with the 1 kb *URA3* fragment to construct pUMAT11 (Figure 6). pUSTE204 was treated with *Bam*HI, pUSTE313 was digested with *Hpa*I and *Kpn*I and pUMAT11 was cut with *Hin*dIII to excise the inserts, which were then used to transform NNY10 and NNY11. Three Ura$^+$ transformants for each were subjected to genomic Southern blotting analyses to confirm the integration sites (data not shown).

Construction of the plasmids YCpSTE215 and YCpSTE306

The 1.6 kb *Hin*dIII fragment containing the entire coding region of STE2 was isolated from plasmid pUSTE2H-3 (Nakayama *et al.*, 1985) and subcloned into M13mp8 (Vierra and Messing, 1982). The 1.7 kb *Eco*RI−*Sal*I fragment containing the promoter and most of the coding region of *STE3* was isolated from YCpSTE3HS (Nakayama *et al.*, 1985) and subcloned into M13mp8. Oligonucleotides (31 mers) designed to insert a *Xba*I site just in front of the initiator ATG codon of *STE2* or *STE3* were synthesized, hybridized with *STE2* or *STE3*-containing M13mp8 single-stranded DNAs, elongated with DNA polymerase I Klenow fragment and transferred into *E. coli* JM101 [*Δlacpro, supE, thi, F′traD36, proAB, lacI^{q}ZΔM15*]. Mutated M13 clones were distinguished by colony-hybridization using the same oligonucleotides as probes (Zoller and Smith, 1982). Mutations introduced into the inserts were confirmed by restriction digestion of double-stranded M13 replicative form (RF) DNAs of each clone with *Xba*I. Inserts from the RFs were subcloned into pUC8 to yield pUSTE212 and pUSTE301 (data not shown). For *STE3*, the *Eco*RI−*Sal*I fragment from pUSTE301 was transferred into pUSTE3HpK (Nakayama *et al.*, 1985) to reconstitute an intact *STE3* coding region (pUSTE303, data not shown). The *Xba*I−*Kpn*I fragments from pUSTE212 and pUSTE303 which contain the entire coding sequence from the initiator ATG to the 3′-untranslated region of *STE2* and *STE3*, respectively, were then inserted in the *Xba*I−*Kpn*I region of pGT3 to fuse them with the *GAL1* promoter. *Sph*I−*Kpn*I fragments from the resulting plasmids containing the *GAL1* promoter fused to the *STE2* or *STE3* coding sequence were further subcloned in pTRP57 (Miyajima *et al.*, 1984) to produce YCpSTE215 and YCpSTE306 (Figure 2).

Isolation of poly(A)$^+$ RNA from yeast

NNY12 (*MATα Δste3::URA3*) and NNY13 (*Δmatα1::URA3*) each transformed with the plasmid YCpSTE215 were grown at 30°C in 1 l of SS medium. At OD_{600} 0.5 each culture was divided (500 ml each), the cells were collected, resuspended in an equal volume of SS or SSG medium, grown at 30°C for 2 h (OD_{600} 0.8−0.9) and harvested. NNY10(*MATα*) with YCpSTE215 was grown and collected as described above, resuspended in an equal volume of SS or in two volumes of SSG medium and grown at 30°C. After 2 h, the cells in SS medium and half of the cells in SSG medium were collected; after 4 h, the residual cells in SSG medium were harvested. Cells collected were washed once with ice-cold water, frozen in liquid nitrogen and stored at −80°C. Poly(A)$^+$ RNA was prepared as described previously (Miyajima *et al.*, 1984), dissolved in RNase-free water at 1 mg/ml and stored at −80°C.

Patch assay for mating

Each strain listed in Table I and Table II was grown in a patch on a SS plate at 30°C and replicated onto a lawn of mating tester cells on SS or SSG plates. After mating for 24 h at 30°C, all patch-cultures were replicated onto appropriate selection plates for growth of diploid cells in 24−48 h.

Cell-cycle arrest assay in liquid cultures

Samples (2 ml) of cultures grown in 25 ml of SS medium at 30°C to midlog phase (OD_{600} 1.0) were transferred into 20 ml of SS or SSG media and incubated at 30°C. After 1 h, synthetic α-factor was added to 12 nM (20 ng/ml). Every 30 min, 1 ml samples were treated with 0.1 ml of formaldehyde and the number of total cells and unbudded cells were counted microscopically with a hematocytometer.

Other methods

Transformation of yeast was by the lithium acetate method (Itoh *et al.*, 1983). Genomic and plasmid DNAs from yeast were prepared from saturated cultures (Davis *et al.*, 1980). Southern and Northern blottings were as described (Southern, 1975; Thomas, 1983). Standard techniques for cloning were based on Maniatis *et al.* (1982). *Escherichia coli* MC1061 [*araD139, Δ(ara, leu)7697, ΔlacX74, galU, galK, hsr, strA*] was routinely used for transformation and preparation of plasmids.

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