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Function of the Ste Signal Transduction Pathway for Mating Pheromones Sustains *MATα1* Transcription in *Saccharomyces cerevisiae*

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Sterile mutants of *Saccharomyces cerevisiae* were isolated from α* cells having the a/α *aar1-6* genotype (exhibiting α mating ability and weak a mating ability as a result of a defect in a1-α2 repression). Among these sterile mutants, we found two *ste5* mutants together with putative *ste7*, *ste11*, and *ste12* mutants of the signal transduction pathway of mating pheromones. The amino acid sequence of the Ste5p protein predicted from the nucleotide sequence of a cloned *STE5* DNA has a domain rich in acidic amino acids close to its C terminus, a cysteine-rich sequence, resembling part of a zinc finger structure, in its N-terminal half, and a possible target site of cyclic AMP-dependent protein kinase at its C terminus. Northern (RNA) blot analysis revealed that *STE5* transcription is under a1-α2-Aar1p repression. The *MATα1* cistron has a single copy of the pheromone response element in its 5′ upstream region, and its basal level of transcription was reduced in these *ste* mutant cells. However, expression of the *MATα1* cistron was not enhanced appreciably by pheromone signals. One of the *ste5* mutant alleles conferred a sterile phenotype to a/α *aar1-6* cells but a mating ability to *MAT*a cells.

Haploid cells of *Saccharomyces cerevisiae* differentiate into two mating types, **a** and α. These **a** and α haploid cells mate with each other and produce a third type of cells, **a**/α diploid cells, which do not mate but can undergo meiosis and sporulation. Several components involved in the mating process have been identified by isolation of sterile (*ste*) mutants defective in mating or insensitive to mating pheromones (for reviews, see references 16, 20, and 25). The *STE* genes are classified into three groups according to their mating-type specificity. One class is required in α cells for encoding a receptor protein that is responsive to **a**-factor (*STE3*) and an enzyme for processing the precursor of α-factor (*STE13*); the second class is specific to **a** cells, encoding a receptor protein responsive to α-factor (*STE2*) and enzymes for processing the **a**-factor precursor (*STE6*, *STE14*, and *STE16*). The third class of *STE* genes, the mating-type-nonspecific genes, are common to both **a** and α cells and function in transmission of pheromone signals from the receptor protein to the nucleus (*STE4*, *STE5*, *STE7*, *STE11*, *STE12*, and *STE18*) along with *GPA1*/*SCG1*/*CDC70*.

In a previous study, we identified *aar1* mutants defective in repression by **a**1-α2 and α2 in mating-type control of *S. cerevisiae* (28). Nucleotide sequence analysis revealed that the *AAR1* gene is identical to *TUP1*, which is allelic to the *AER2*, *AMM1*, *CYC9*, *FLK1*, *SFL2*, and *UMR7* genes. An *aar1-6* mutant allele of *AAR1* conferred upon haploid cells with the *MAT***a** *HML*α *HMR***a** *sir3* genotype a peculiar α* mating phenotype (exhibiting α mating ability and weak **a** mating ability) as a result of a defect in **a**1-α2 repression but functional for α2 repression, because the *sir3* mutation allowed expression of the *HML*α and *HMR***a** genes to confer both α and **a** information. The Ssn6p/Cyc8p protein (40), a protein with a tetratricopeptide repeat (11), is also directly involved in repression by **a**1-α2 and α2 and in other systems as a general repressor of transcription, showing close interaction with the Aar1p/Tup1p protein (18). (Proteins are named according to the new format for nomenclature for yeasts; e.g., the product of the *AAR1* gene is named Aar1p.) Mcm1p protein (33) is also involved in α2 repression, and a *gal11* mutation is also known to confer α-specific sterility (31).

For detection of other genes involved in cell type control, we isolated suppressors of *aar1-6*, a mutant allele of *AAR1*/*TUP1*. Among the mutants isolated as sterile mutants from haploid α* cells with a *MAT***a** *HML*α *HMR***a** *sir3* *aar1-6* genotype, we found two *ste5* mutants together with putative *ste7*, *ste11*, and *ste12* mutants. Transcription of the *α1* cistron is reduced in these *ste* mutants, and *STE5* transcription is under **a**1-α2 repression. The amino acid sequence of the Ste5p protein, deduced from the cloned nucleotide sequence, is rich in acidic amino acids close to its C terminus and has a cysteine-rich sequence in its N-terminal half and a possible target site for phosphorylation by a cyclic AMP (cAMP)-dependent protein kinase at its C terminus.

MATERIALS AND METHODS

Microorganisms and plasmids. The *S. cerevisiae* strains used are listed in Table 1. All strains have the *ho* genotype, and all were constructed in our laboratory except for two standard strains of the mating types (N248-1A and N248-1C) which were obtained from the Yeast Genetics Stock Center at the University of California, Berkeley, and a strain (KMG26-3C) from K. Matsumoto (29). Two *Escherichia coli* strains, DH5α and MV1184 (39), were used as hosts for propagation and manipulation of plasmids. The plasmid vectors used were YCp50, YEp24, YIp5, and YRp7 (32) and pUC118 and pUC119 (39). Plasmids pYMC2 and pYMC3, bearing the *MAT*α and *MAT***a** genes, respectively, on YCp50 as described previously (30) are designated YCp-MATα and YCp-MAT**a** in this report. Constructions of plasmids, YCp-AAR1 bearing a 3.3-kbp *Bam*HI-*Hin*dIII *AAR1*/*TUP1* DNA in YCp50, p69A bearing the *MFα1* gene (21), p334 carrying

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TABLE 1. *S. cerevisiae* strains used

Strain	Mating type[a]	Genotype[b]
HYP100	**a**	*MAT***a** *ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
HYP100-Δ*ste5*	Non	*ste5*::*URA3* disruptant of HYP100
HYP101	α	*MAT*α *ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
HYP101-Δ*ste5*	Non	*ste5*::*URA3* disruptant of HYP101
HYP101-Δ*ste12*	Non	*ste12*::*URA3* disruptant of HYP101
HYP140	Non	*MAT***a** *HML*α *HMR***a** *sir3*::*HIS3 ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
HYP140-Δ*ste5*	Non	*ste5*::*URA3* disruptant of HYP140
HYP150	**a**	*MAT***a** *aar1*::*HIS3 ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
HYP151	Non	*MAT*α *aar1*::*HIS3 ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
HYP160	Non	*MAT***a** *HML*α *HMR***a** *sir3*::*HIS3 aar1*::*HIS3 ura3-52 leu2-3,112 trp1Δ his3Δ ade2-101 lys2-801*
KMG26-3C	α[c]	*MAT*α *ste5*^{ts} *ura3 his3 leu2 lys2 trp1 tyr1*
KYC53	**a**	*MAT***a** *ura1 ura2 trp3 lys1 pho3 pho5*
KYC54	α	*MAT*α *ura1 ura2 trp3 lys1 pho3 pho5*
N248-1A[d]	**a**	*MAT***a** *ura3 leu1 trp1 his2 ade1 met14 gal1*
N248-1C[d]	α	*MAT*α *ura3 leu1 trp1 his2 ade1 met14 gal1*
SH2648	Non	*MAT***a** *HML*α *HMR***a** *sir3*::*LEU2 ura3 leu2 ade2-101 ade10 lys2-801 pho3 pho5*
YMH47	α	*MAT*α *ura3-52 leu2-3,112 trp1 pho3 pho5*
YMH48	**a**	*MAT***a** *ura3-52 leu2-3,112 trp1 pho3 pho5*
YMH153	α*	*MAT***a** *HML*α *HMR***a** *sir3*::*LEU2 aar1-6 ura3-52 leu2 his5 pho3-1 pho5-1 trp1*::[*TRP1 MAT*α1_p-*PHO5*][e]
YMH153-R101	Non	Putative *ste11* mutant of YMH153
YMH153-R102	Non	*ste5-101* mutant of YMH153
YMH153-R103	Non	*ste5-102* mutant of YMH153
YMH153-R104	Non	Putative *ste7* mutant of YMH153
YMH153-R105	Non	Putative *ste12* mutant of YMH153
YMH153-R106	Non	Putative *ste11* mutant of YMH153
YMH153-R107	Non	Unidentified *ste* mutant of YMH153
R102-Δ*aar1*	Non	*aar1*::*URA3* disruptant of YMH153-R102
R104-Δ*aar1*	Non	*aar1*::*URA3* disruptant of YMH153-R104
YMH153-Δ*aar1*	Non	*aar1*::*URA3* disruptant of YMH153
YMH154	α*	*MAT***a** *HML*α *HMR***a** *sir3*::*LEU2 aar1-6 ura3-52 leu2 lys2 pho3-1 pho5-1 trp1*::[*TRP1 MAT*α1_p-*PHO5*]
YMH169	Non	*MAT***a** *HML*α *HMR***a** *sir3*::*LEU2 aar1-6 ste5-101 ura3-52 leu2 lys2 pho3 pho5 trp1*::[*TRP1 MAT*α1_p-*PHO5*]
YMH217	Non	*MAT***a** *HML*α *HMR***a** *sir*::*LEU2 ura3 leu2 ade2-101 ade10 lys2-801 pho3 pho5 trp1*::[*TRP1 MAT*α1_p-*PHO5*]

[a] α* indicates a specific mating type able to mate with **a** cells and also weakly with α cells. Non indicates nonmating with the **a** and α testers.

[b] All strains have the *ho* genotype. Genetic symbols are as described by Mortimer et al. (27). The disrupted alleles of *SIR3*, *AAR1*, *STE5*, and *STE12* by insertion of the *LEU2*, *HIS3*, and *URA3* DNA (indicated as *sir3*::*LEU2*, *sir3*::*HIS3*, *aar1*::*HIS3*, *aar1*::*URA3*, *ste5*::*URA3*, and *ste12*::*URA3*, respectively) were confirmed by Southern blot analysis of the genomic DNAs.

[c] Showing mating type α at 30°C but sterile at 37°C.

[d] Obtained from the Yeast Genetics Stock Center of the University of California, Berkeley.

[e] *trp1*::[*TRP1 MAT*α1_p-*PHO5*] indicates the integration of a plasmid bearing the *MAT*α1_p-*PHO5* fusion gene into the *trp1* locus.

the *STE2* gene (14), and pYA301 bearing the *ACT1* gene (10) were described previously (28). Plasmid YCp-SIR3 was constructed by ligating a 6.9-kbp *Bam*HI fragment bearing the *SIR3* gene of plasmid pKAN63 (17) (obtained from J. B. Hicks) into the *Bam*HI site of YCp50. Plasmid YCp-STE11 was constructed by ligating a 4.6-kbp *Bam*HI-*Xho*I fragment bearing the *STE11* gene (35) (obtained from B. Errede) into the *Bam*HI-*Sal*I gap of YCp50. Plasmids bearing the *STE7* and *STE12* genes, respectively, were obtained previously in our laboratory by selecting plasmids complementing authentic *ste7* and *ste12* mutants from a gene library of *S. cerevisiae*, YCp50 "CEN BANK" A (37), obtained from the American Type Culture Collection (Rockville, Md.) (our unpublished results). The cloned fragments in these plasmids were confirmed to bear the *STE7* and *STE12* genes, respectively, by nucleotide sequence determination for at least 300 bp and comparison with data in the EMBL-GDB data base (release 13.0; December 1990). We did not investigate their detailed structures further but designated these plasmids YCp-STE7 and YCp-STE12, respectively. A 4.7-kbp *ste12*::*URA3* fragment was constructed by replacing a 1.1-kbp *Xba*I region of a 4.6-kbp *Hin*dIII fragment of *STE12* DNA of YCp-STE12 by a 1.2-kbp *Hin*dIII fragment bearing the *URA3* gene prepared from YEp24.

The *MAT*α1_p-*PHO5* fusion gene (the open reading frame [ORF] of *PHO5* encoding repressible acid phosphatase [APase; EC 3.1.3.2] connected to the downstream region of the *MAT*α*1* promoter DNA) was constructed with use of the polymerase chain reaction (PCR). A 1,592-bp fragment of the coding region of *PHO5* (1) from nucleotide positions −18 to +1574 (relative to the translation initiation codon) was amplified by PCR with the oligonucleotide 5′-CTCGGATC CGCAAATTCGAGATTACCAATG-3′ (corresponding to the sequence from −18 to +3 of the coding strand with an additional *Bam*HI restriction sequence and CTC at its 5′ end) as a forward primer, the oligonucleotide 5′-CTCAGAT CTAAATCTATTTCAGCAATATAG-3′ (corresponding to the sequence from +1574 to +1554 of the anticoding strand of *PHO5* with an additional *Bgl*II restriction sequence and CTC at the 5′ end) as a reverse primer, and plasmid pPHO5 (1) bearing the *PHO5* gene as a template. The amplified product was digested with *Bam*HI and *Bgl*II and inserted

into the *Bam*HI site of vector YIp5. We confirmed that the resultant plasmid, pSH39, has the inserted fragment in the same reading direction as the *tet* gene on the vector plasmid. Similarly, a 1,097-bp fragment from nucleotide positions 654 to 1750 (according to the numbering system of Astell et al. [2] for the *HMLα* gene) containing the intergenic promoter region of the *MATα1* and *MATα2* cistrons and also the *MATα2* ORF was amplified with an oligonucleotide with the sequence 5′-CTCAAGCTTATATGTATTTTGTTAACTCT-3′ (corresponding to the sequence from 654 to 673 of the coding strand of *MATα* with an additional *Hin*dIII restriction sequence and CTC at the 5′ end) as a forward primer, the oligonucleotide 5′-CTCGGATCCTATTAAGTTATTATAT ATGG-3′ (corresponding to the sequence from 1750 to 1731 of the anticoding strand of *MATα* with an additional *Bam*HI restriction sequence and CTC at the 5′ end) as a reverse primer, and plasmid YCp-MATα bearing the *MATα* gene as a template. The PCR product was digested with *Bam*HI and *Hin*dIII and ligated with a 7.3-kbp *Bam*HI-*Hin*dIII fragment of pSH39. A 2.5-kbp *Xba*I-*Sph*I fragment, containing the $MAT\alpha1_p$-*PHO5* fusion gene with deletion of the *MATα2* ORF prepared from the resultant plasmid, was ligated with a 4.8-kbp *Nhe*I-*Sph*I fragment from a derivative plasmid of pBR322 (which was disrupted at the unique *Hin*dIII site by restriction, filling in, and self-ligation and had an insert of a 0.8-kbp *Pst*I-*Eco*RI fragment containing the *TRP1* gene prepared from YRp7 in the *Pvu*II site of pBR322). To confirm that the $MAT\alpha1_p$-*PHO5* fusion gene on the resultant plasmid, pYMI20, functions as expected in vivo, we integrated pYMI20 into the *trp1* locus of the chromosomes of *S. cerevisiae* YMH47 (α), YMH48 (**a**), and SH2648 (**a**/α). Colonies of transformants of YMH47 and YMH48 showed APase activity, determined by the staining method described below, but those of SH2648 did not, indicating that the $MAT\alpha1_p$-*PHO5* fusion gene on pYMI20 is expressed in **a** and α cells but not in **a**/α cells, as expected from the current idea that *MATα1* is subject to **a**1-α2 repression (28).

Media and genetic and biochemical methods. The nutrient YPD media used for cultivation of *S. cerevisiae* (38) and LB broth used for *E. coli* cells (39) were as described previously. To construct a hybrid between two *S. cerevisiae* strains of the same mating type or between nonmating strains, we used the cell fusion technique with cell protoplasts (13). The mating types of *S. cerevisiae* cells with appropriate auxotrophic markers were determined by cross-streaking the cells with standard haploid strains having the **a** (N248-1A and KYC53) or α (N248-1C and KYC54) mating type on a plate as described previously (12). Quantitative assay of mating ability was performed as described by Hartwell (15) with the above four mating testers. For examination of APase productivity from the $MAT\alpha1_p$-*PHO5* fusion gene in response to **a**-factor, **a**-factor-conditioned YPD medium was prepared by cultivation of *MAT***a** cells of YMH48 in YPD medium overnight at 30°C and filtration of the culture medium through a nitrocellulose filter of 0.45-μm pore size (41). APase activity of yeast colonies was detected by a staining method based on a diazo-coupling reaction (43), and specific APase activity of cell suspensions (microunits per milliliter per unit of optical density at 660 nm) was determined by the method of Toh-e et al. (44). The methods used for preparation and manipulation of DNA and RNA, general methods for studies of yeast genetics, including sporulation conditions, mutagenesis with ethyl methanesulfonate, and transformation with lithium acetate, were as described by Rose et al. (38). The methods used for transformation of *E. coli* and for nucleotide sequence determination by the dideoxy-chain termination method were as described by Sambrook et al. (39).

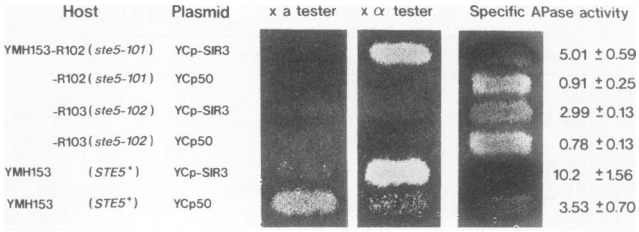

FIG. 1. Mating assays and APase activities of YCp-SIR3 transformants of *ste* mutants. Cells of mutants YMH153-R102 (*ste5-101*) and YMH153-R103 (*ste5-102*) and of YMH153 (*STE*$^+$) were streaked on a lawn of standard **a** (N248-1A) or α (N248-1C) cells on YPD agar plates, incubated at 30°C for 12 h, and replicated onto minimal plates to detect prototrophic hybrid cells. Numbers beside cell patches stained for APase activity show APase activities determined with cell suspensions as mean values for triplicate determinations ± standard deviations.

Nucleotide sequence accession number. The nucleotide sequence of *STE5* reported here has been deposited in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession number D12917.

RESULTS

Suppressor mutations to the *aar1/tup1* mutation are alleles of *ste* mutations. To obtain an extragenic suppressor of the *aar1/tup1* mutation, we constructed a haploid strain, YMH153 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 pho3 pho5 trp1*::[*TRP1* $MAT\alpha1_p$-*PHO5*] *his5*), which has α* mating ability, exhibiting α mating ability and weak **a** mating ability (Fig. 1; Tables 2 and 3) as a result of a defect by the *aar1-6* mutation in repression of the *α1* cistron and haploid-specific genes by the **a**1 and α2 proteins produced from the *MAT***a** allele and the *HMLα* and *HMR***a** genes derepressed by the *sir3* mutation (12, 28). The $MAT\alpha1_p$-*PHO5* fusion gene should facilitate screening of such mutants exhibiting mating-type disregulation; the APase encoded by *PHO5* of the fusion gene transcribed by the promoter of *MATα1* is expressed in α and **a** cells but not in **a**/α cells. Therefore, colonies of YMH153 showed APase activity (staining red). Cells of YMH153 were subjected to ethyl methanesulfonate mutagenesis and plated on YPD after appropriate dilution. The colonies that developed were screened for the APase$^-$ phenotype by staining (white) and subsequently for the

TABLE 2. APase activities from the $MAT\alpha1_p$-*PHO5* fusion gene in cells having various *ste* and *aar1* mutations

Strain	Relevant genotype[a]	APase sp act[b]
YMH153-R102	*ste5-101 aar1-6*	0.52 ± 0.04
YMH153-R104	*ste7 aar1-6*	0.44 ± 0.06
YMH153-R101	*ste11 aar1-6*	0.68 ± 0.06
YMH153-R105	*ste12 aar1-6*	0.99 ± 0.11
YMH153	*STE*$^+$ *aar1-6*	5.13 ± 0.13
R102-Δ*aar1*	*ste5-101 aar1*::*URA3*	4.36 ± 0.37
R104-Δ*aar1*	*ste7 aar1*::*URA3*	9.08 ± 1.26
YMH153-Δ*aar1*	*STE*$^+$ *aar1*::*URA3*	8.50 ± 1.85
YMH217	*STE*$^+$ *AAR1*$^+$	0.15 ± 0.01

[a] All strains have the *MAT***a** *HMLα HMR***a** *sir3*::*LEU2* genotype.
[b] Values (microunits per milliliter per unit of optical density at 660 nm) are means for triplicate determinations ± standard deviations.

TABLE 3. Mating efficiencies of *ste* mutants

Strain	Relevant genotype	Mating efficiency with[a]:	
		a tester	α tester
YMH153	*STE*$^+$ *sir3 aar1-6*	8.6×10^{-4}	5.4×10^{-5}
YMH153-R101	*ste11 sir3 aar1-6*	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
YMH153-R102	*ste5-101 sir3 aar1-6*	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
YMH153-R103	*ste5-102 sir3 aar1-6*	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
YMH153-R104	*ste7 sir3 aar1-6*	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
YMH153-R105	*ste12 sir3 aar1-6*	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
HYP100	*MAT***a** *STE5*$^+$ *SIR*$^+$ *AAR1*$^+$	3.2×10^{-5}	2.5×10^{-1}
HYP100-Δ*ste5*	*MAT***a** Δ*ste5 SIR*$^+$ *AAR1*$^+$	2.6×10^{-5}	8.8×10^{-6}
HYP101	*MATα STE5*$^+$ *SIR*$^+$ *AAR1*$^+$	1.4×10^{-1}	1.2×10^{-5}
HYP101-Δ*ste5*	*MATα* Δ*ste5 SIR*$^+$ *AAR1*$^+$	2.4×10^{-6}	$<9 \times 10^{-7}$
HYP140	*MAT***a** *HMLα HMR***a** *sir3 STE5*$^+$ *AAR1*$^+$	4.5×10^{-4}	5.8×10^{-5}
HYP140-Δ*ste5*	*MAT***a** *HMLα HMR***a** *sir3* Δ*ste5 AAR1*$^+$	1.3×10^{-4}	$<9 \times 10^{-7}$

[a] Mating testers were KYC53 (**a**) and KYC54 (α).

sterile phenotype. We presumed that these phenotypes resulted from restoration or modification of **a**1-α2 repression in the **a**/α *aar1-6* cells. Among 20,000 colonies screened, we obtained seven mutants showing the APase$^-$ phenotype (data for some of these mutants are listed in Table 2) and sterile phenotype (some of the data are listed in Table 3).

To test whether the mutations were dominant or recessive, we fused each mutant with strain YMH154 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 pho3 pho5 trp1*::[*TRP1 MATα1*$_p$-*PHO5*] *lys2*). Colonies showing the His$^+$ Lys$^+$ phenotype stained red on APase staining and showed the α* mating type. Thus, all seven mutations are recessive to the wild-type counterpart. This recessiveness also suggested that these mutations were not due to reversion of *aar1-6* to *AAR1*$^+$. The resultant fusants sporulated well, and the asci were subjected to tetrad dissection. We found that at least eight asci of each fusant showed 2 α*:2 non segregation (non indicates nonmater phenotype), indicating that each of these seven mutants has a single mutation.

We confirmed that the mutations were not alleles of *MAT***a**, *HMLα*, and *HMR***a** by demonstrating that plasmids YCp-MATα and YCp-MAT**a** could not complement the mutations. Similarly, we confirmed that the mutations were not in the *AAR1* locus, as suggested above, by the findings that colonies of the Ura$^+$ transformants with plasmid YCp-AAR1 were white on staining for APase activity and the cells were sterile. For further characterization, these mutants were transformed to the Ura$^+$ phenotype with plasmid YCp-SIR3. The resultant transformants should express only *MAT***a**, because the active Sir3p protein silences the *HMLα* and *HMR***a** genes. The transformant of one of the mutants, R102, showed the **a** mating type, like the original YMH153 cells harboring YCp-SIR3 (Fig. 1 and Table 4; only data for mutants R102 and R103 and the original YMH153 strain are shown), while the transformants of the other six mutants were nonmaters. Thus, all the mutants except R102 showed the sterile phenotype in *MAT***a** cells.

A mutation in the *STE4*, *STE5*, *STE7*, *STE11*, *STE12*, or *STE18* gene is known to confer sterility on cells with the *MAT***a** or *MATα* genotype (25). Therefore, we examined whether the seven Ste$^-$ mutants isolated here are allelic to these *ste* mutations by introducing plasmids YCp-STE7, YCp-STE11, and YCp-STE12 into them. We found that the R101 and R106 mutants were complemented with YCp-STE11, R104 was complemented with YCp-STE7, and R105 was complemented with YCp-STE12, whereas the other three mutants, R102, R103, and R107, were not complemented with any of these plasmids (data not shown). Although another possibility, that each of these YCp-STE plasmids acts as a suppressor in complementation of the mutants, was not negated, the mutations in R101 and R106 are, most probably, *ste11*, that in R104 is *ste7*, and that in R105 is *ste12*, as they were complemented with the relevant YCp-STE plasmid.

To investigate the R102 mutation, whose transformant with YCp-SIR3 showed the **a** mating type (Fig. 1 and Table 4), we selected a plasmid complementing the mutation from the gene library of *S. cerevisiae*, YCp50 "CEN BANK" A. We isolated one such plasmid clone by screening about 11,000 Ura$^+$ transformants. The plasmid, designated pR102, recovered from this transformant after its propagation in *E. coli* cells had a 7.7-kbp insert in the *Bam*HI site of YCp50 (Fig. 2). We introduced plasmid pR102 into the other six mutants and found that a transformant from one mutant, R103, showed the APase$^+$ phenotype and α* mating type. Thus, mutants R102 and R103 should have a mutation in the same locus.

To confirm that pR102 bears a copy of the gene mutated in R102 (and R103), the 8.9-kbp *Pvu*I-*Sph*I fragment containing the 7.7-kbp insert (Fig. 2) was ligated into a *Pvu*I-*Sph*I gap of YIp5. The resultant plasmid, pYMI23, was integrated into a chromosome of mutants R102 and R103 after linearization of pYMI23 by restriction at the unique *Xho*I site in the 7.7-kbp fragment. Several Ura$^+$ transformants of R102 and R103 were fused with strain YMH154 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6*). The diploids from the transformant of R102, how-

TABLE 4. Mating efficiencies of the *ste* mutants harboring plasmid YCp-SIR3

Strain	Relevant genotype[a]	Plasmid	Mating efficiency with[b]:	
			a tester	α tester
YMH153	*STE5*$^+$	YCpp-SIR3	4.2×10^{-5}	1.7×10^{-1}
		YCp50	2.9×10^{-3}	6.1×10^{-5}
YMH153-R102	*ste5-101*	YCp-SIR3	5.8×10^{-6}	2.7×10^{-2}
		YCp50	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
YMH153-R103	*ste5-102*	YCp-SIR3	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$
		YCp50	$<4 \times 10^{-7}$	$<4 \times 10^{-7}$

[a] All strains have the *MAT***a** *HMLα HMR***a** *sir3*::*LEU2* genotype.
[b] Mating testers were N248-1A (**a**) and N248-1C (α).

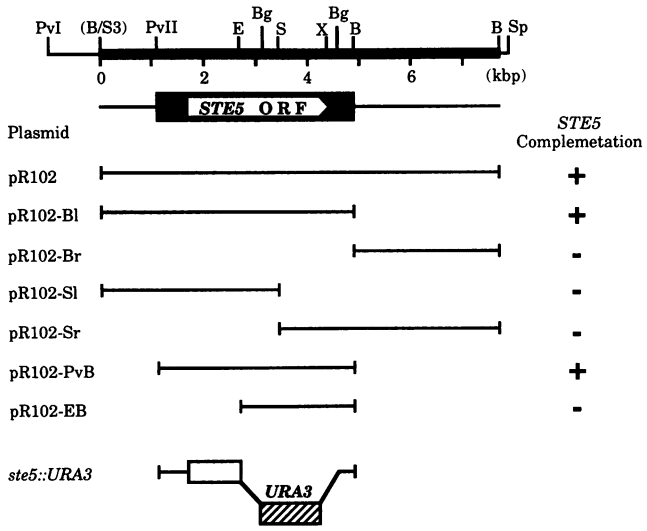

FIG. 2. Restriction map of the 7.7-kbp insert of pR102 and delimitation of its functional region for complementation of the *ste5* mutation. The inserted DNA fragment in pR102 (bold line) and its deletion derivatives (thin lines) are shown. Complementation of the *ste5* mutation of R102 was detected by testing for the ability to restore the sterile phenotype to the α* mating type and the APase$^-$ phenotype to APase$^+$ by introducing plasmids bearing various subfragments of the 7.7-kbp region. The nucleotide sequence of the 3.8-kbp *Pvu*II-*Bam*HI region (closed box with an open arrow) was determined. The open arrow indicates the approximate position and direction of the *STE5* ORF. The hatched box labeled *URA3* indicates the 1.2-kbp *URA3* DNA, prepared from YEp24, substituted for the 2.0-kbp *Eco*RI-*Bgl*II region of the *STE5* DNA. + and − represent ability and inability, respectively, to restore the sterile phenotype to the α* phenotype. Abbreviations for restriction sites: B, *Bam*HI; Bg, *Bgl*II; E, *Eco*RI; PvI, *Pvu*I; PvII, *Pvu*II; S, *Sal*I; Sp, *Sph*I; X, *Xho*I. B/S3 is the junction site of *Bam*HI and *Sau*3AI.

ever, could not sporulate for some unknown reason, while those of R103 could sporulate. When we dissected asci from one of the diploids of R103 transformants, all tetrad segregants from 19 asci dissected showed the α* mating type. Thus, the 7.7-kbp fragment contains a copy of the gene mutated in R103. Then we fused the R103 transformant inserted with pYMI23 with strain YMH169 (non), which is a tetrad segregant of a fusant between R102 and YMH154 and has the same sterile mutation of R102 in the *MAT***a** *HML*α *HMR***a** *sir3 aar1-6* background. The resultant diploid was sporulated and dissected. The diploid showed 2α*:2 non segregation in all 10 asci dissected, and all of the α* clones showed the Ura$^+$ phenotype, indicating that the mutation in R102 is allelic with that in R103.

The R102 (and also R103) mutation should be on chromosome IV, because the ^{32}P-labeled cloned DNA fragment showed hybridization signals on this chromosome on Southern blot hybridization of chromosomes after contour-clamped homogeneous electric field electrophoresis (data not shown). This observation, together with that of the sterile phenotype of R103 transformed with YCp-SIR3 as well as the other *ste* mutants isolated here, strongly suggested that the R102 and R103 mutations are alleles of *ste5*. We confirmed this possibility by showing that pR102 could complement the temperature-sensitive sterile phenotype of the *ste5* mutant KMG26-3C (i.e., having the α mating type at 30°C but sterile at 37°C), because a Ura$^+$ transformant of KMG26-3C with plasmid pR102 could mate as α at both 30 and 37°C. For further confirmation, a *ste5*::*URA3* disruptant, HYP100-Δ*ste5* (described below) (Table 3), was fused with strain KMG26-3C, and the resultant diploid was sporulated and dissected. All of the tetrad segregants in 10 asci dissected showed the nonmating phenotype at 37°C, indicating that the cloned DNA in pR102 encodes the *STE5* gene. Thus, the mutations in R102 and R103 are allelic with *ste5*. We designated the mutations of R102 and R103 as *ste5-101* and *ste5-102*, respectively. Although we did not test other *STE* genes, including *STE4* and *STE18*, the genotype of the other mutant, R107, is not known.

Nucleotide sequence of the *STE5* gene. To locate the *STE5* gene within the cloned DNA, we ligated various subclones of the 7.7-kbp insert (Fig. 2) into the YCp50 vector and tested their ability to complement the *ste5* mutation by transformation of R102 (*MAT***a** *HML*α *HMR***a** *sir3 aar1-6 ste5-101 trp1*::[*TRP1 MAT*α*1*$_p$*-PHO5*]). The results indicated that the *STE5* gene is in a 3.8-kbp *Pvu*II-*Bam*HI fragment (pR102-PvB; Fig. 2).

The nucleotide sequence of the 3.8-kbp *Pvu*II-*Bam*HI fragment was determined (Fig. 3). We found only one ORF and no intron splicing site (5′-GTATGT-3′) in the fragment (Fig. 2 and 3). The Ste5p protein predicted from the ORF is composed of 917 amino acid residues with a calculated molecular mass of 103 kDa. The predicted amino acid sequence was compared with those in the SWISS-PROT data base (release 19.0; August 1991). No significant homology with registered proteins was found. However, we found that the predicted Ste5p protein has a domain rich in acidic amino acids close to its C terminus (summarized in Fig. 4). There are 11 putative glycosylation sites (Asn-X-Ser or Asn-X-Thr) and one possible site for phosphorylation by cAMP-dependent protein kinase (X-Arg-Arg-X-Ser-X) in the region from amino acid residues 894 to 899 but none of the other potential phosphorylation sites for protein kinases described by Kemp and Pearson (19). No obvious transmembrane domain or N-terminal signal sequence was identified, and the N and C termini of the protein are hydrophilic, as determined by Kyte-Doolittle (22) hydropathy analysis (data not shown). A cysteine-rich sequence resembling part of a zinc finger structure (3) was found in the N-terminal half. A similar cysteine-rich sequence was found in the Far1p protein of *S. cerevisiae* (6, 30a). The *FAR1* gene is inducible by mating pheromones, and Far1p inhibits the function of a species of G1 cyclin, Cln2p. In addition to the cysteine and histidine residues, many other residues are conserved in Ste5p and Far1p in the cysteine-rich and flanking sequences (Fig. 5). The cysteine-rich sequences of Ste5p and Far1p also have similarity with two other cysteine-rich motifs: the LIM motif found in a number of homeodomain proteins (9) and an unnamed motif found in a number of genes, including yeast *RAD18* (4). The sequences of Ste5p and Far1p are more similar to each other than to these two motifs. The 5′ upstream region contained two partially overlapping sequences homologous to that proposed as the protein binding site for repression by **a**1-α2 (26).

Disruption of *STE5* reduces transcription of the a- and α-specific genes. The *ste5* mutations so far identified confer sterility upon both *MAT***a** and *MAT*α cells (15, 24). To determine whether this was also true for the *ste5* null mutation, we disrupted the chromosomal *STE5* gene. A 2.9-kbp *Pvu*II-*Bam*HI *ste5*::*URA3* fragment (Fig. 2) was used to replace the genomic wild-type *STE5* gene of strains HYP100 (**a**), HYP101 (α), and HYP140 (a haploid **a**/α strain with a disrupted *sir3*). The Ura$^+$ transformants of HYP100 and HYP101 were sterile like that of HYP140 (Table 3).

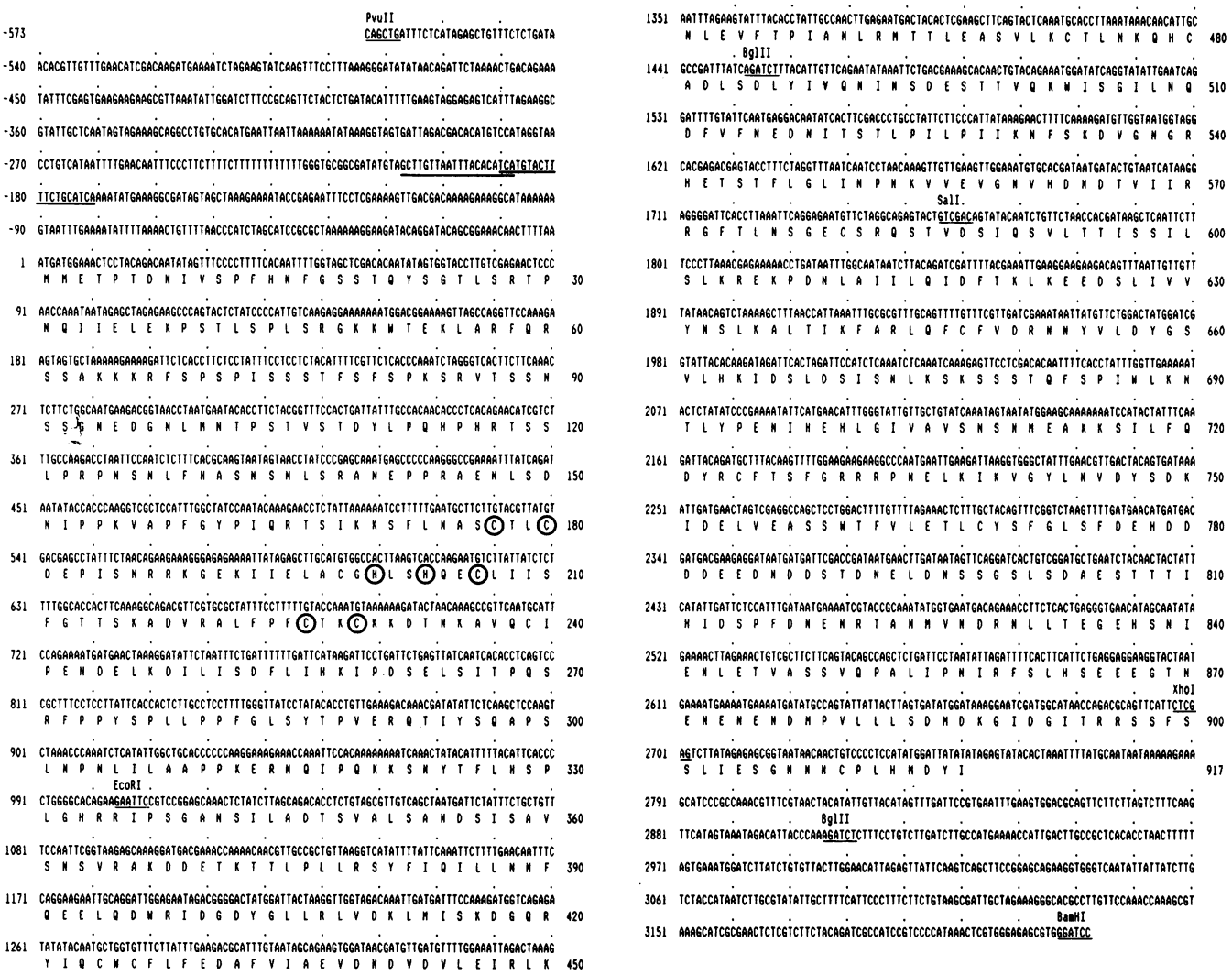

FIG. 3. Nucleotide sequence and deduced ORF of the *STE5* gene. Numbers on the left represent nucleotide positions, and those on the right indicate amino acid positions. Nucleotide +1 is the A of the ATG translational start codon. Two underlined partially overlapping sequences in the 5′ upstream region are homologous to that proposed as the protein(s) binding site for the a1-α2 repression (26). The encircled amino acid residues represent cysteine and histidine residues analogous to a part of the zinc finger structure (3).

These results indicate that disruption of the *STE5* gene results in a sterile phenotype independent of the mating-type gene.

It is known that Ste7p, Ste11p, and Ste12p are components of the signal transduction pathway of mating pheromones and that defects in these proteins reduce the transcription of pheromone-inducible genes, including the **a**- and α-specific genes (7). As the Ste5p protein is also claimed to be involved in this mechanism (20, 25), we examined the effects of the *ste5* disruptant on transcription of the *MFα1* (an α-specific gene) and *STE2* (an **a**-specific gene) genes, using RNA samples from cells of various genotypes for mating-type information and for the *STE5* gene. We observed significantly lower levels of transcription of the *MFα1* gene in the α *ste5*::*URA3* disruptant (Fig. 6, lane 3) and of *STE2* in the **a** *ste5*::*URA3* disruptant (lane 5) compared with levels in *STE5*$^+$ α cells (lane 4) and **a** cells (lane 6). No *MFα1* and *STE2* transcripts were observed in **a**/α *ste5*::*URA3* cells, as in wild-type **a**/α cells (lanes 1 and 2). These results indicate that the Ste5p protein, like Ste7p, Ste11p, and

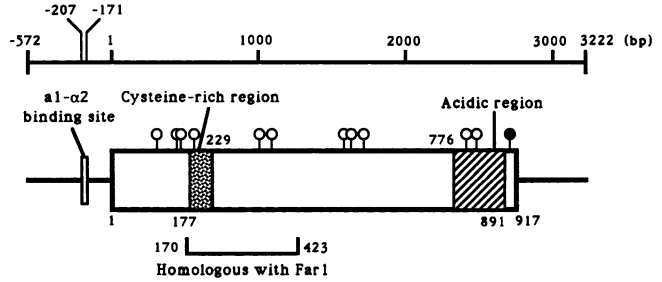

FIG. 4. Primary structure of the *STE5* gene and the Ste5p protein deduced from the nucleotide sequence of the gene. The bold lines indicate DNA sequences of the 5′ upstream and 3′ downstream regions of the *STE5* ORF, which is boxed. Lines with open circles above the ORF represent approximate sites of nucleotides encoding possible potential N-linked glycosylation sites in the Ste5$_p$ protein, and the line with a closed circle indicates a site encoding a possible potential site for phosphorylation by cAMP-dependent protein kinase. Numbers above the *STE5* ORF represent codon numbers for initiation and termination of the indicated region.

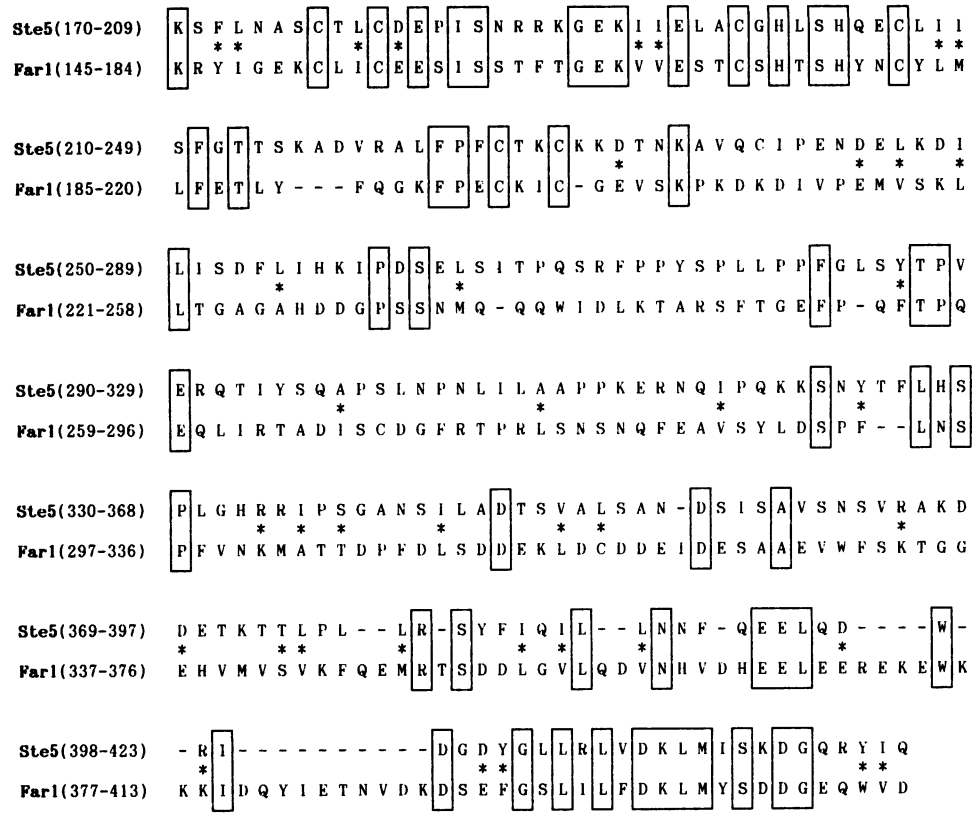

FIG. 5. Homology of the cysteine-rich regions of Ste5p and Far1p proteins. Identical amino acids in these two proteins are boxed. Asterisks indicate conserved substitutions between, respectively, serine and threonine; alanine, cysteine, isoleucine, leucine, methionine, and valine; phenylalanine, tryptophan, and tyrosine; arginine and lysine; aspartic acid and glutamic acid; and asparagine and glutamine.

Ste12p, is required for the transcription of mating-type specific genes, at least the *MFα1* and *STE2* genes.

The *ste* mutations reduce transcription of the *α1* cistron. Transcription of the *α1* cistron seemed to be reduced in these *ste* mutants, because all seven *ste* mutants were isolated as

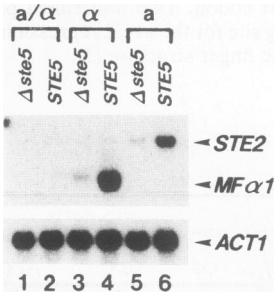

FIG. 6. Effect of *ste5* disruption on *STE2* and *MFα1* transcription. Total RNA samples were prepared from cells of HYP140-Δ*ste5* (*MAT***a** *HMLα HMR***a** *sir3 ste5*::*URA3*) (lane 1), HYP140 (*MAT***a** *HMLα HMR***a** *sir3 STE5*$^+$) (lane 2), HYP101-Δ*ste5* (*MATα ste5*::*URA3*) (lane 3), HYP101 (*MATα STE5*$^+$) (lane 4), HYP100-Δ*ste5* (*MAT***a** *ste5*::*URA3*) (lane 5), and HYP100 (*MAT***a** *STE5*$^+$) (lane 6). Samples of 2 μg were subjected to electrophoresis in agarose gel (1.5%) in the presence of formaldehyde and transferred to a nylon filter. The RNA blots were hybridized with a mixture of a ^{32}P-labeled 1.4-kbp *Eco*RI-*Sal*I fragment bearing the *MFα1* gene prepared from plasmid p69A and a 1.3-kbp *Hin*dIII-*Pst*I fragment bearing the *STE2* gene from plasmid p334 as probes and rehybridized with a ^{32}P-labeled 1.0-kbp *Hin*dIII-*Xho*I fragment carrying the *ACT1* gene from plasmid pYA301 as an internal marker. The radioactivities of the probes were adjusted to 10^8 cpm/μg of DNA.

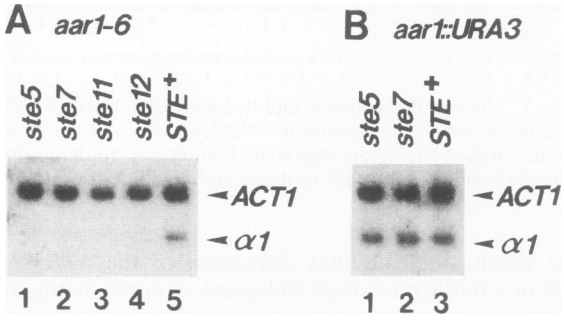

FIG. 7. Transcription of the *α1* cistron in *ste aar1* mutants. (A) *α1* transcription (from *HMLα* with the *sir3* mutation) in various *ste* mutants with the *aar1-6* mutation. Total RNA was prepared from mutant YMH153-R102 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste5-101*) (lane 1), YMH153-R104 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste7*) (lane 2), YMH153-R101 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste11*) (lane 3), YMH153-R105 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste12*) (lane 4), and YMH153 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 STE*$^+$) (lane 5). (B) *α1* transcription in *ste5* and *ste7* mutants with an *aar1* disrupted allele. Total RNA was prepared from mutant R102-Δ*aar1* (*MAT***a** *HMLα HMR***a** *sir3 aar1*::*URA3 ste5-101*) (lane 1), R104-Δ*aar1* (*MAT***a** *HMLα HMR***a** *sir3 aar1*::*URA3 ste7*) (lane 2), and YMH153-Δ*aar1* (*MAT***a** *HMLα HMR***a** *sir3 aar1*::*URA3 STE*$^+$) (lane 3). Samples of 2 μg of total RNA were applied to slots in an agarose gel (1.5%) containing formaldehyde and separated by electrophoresis. The gel was blotted onto a nylon filter and hybridized with the ^{32}P-labeled 0.7-kbp *Nde*I fragment bearing the *MATα1* cistron, prepared from plasmid YCp-MATα, and with the ^{32}P-labeled *ACT1* DNA, as described in the legend to Fig. 6, as probes.

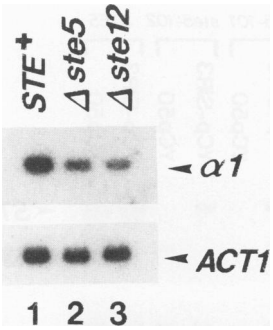

FIG. 8. Effects of *ste5* and *ste12* disruptions on transcription of the *MATα1* cistron. Total RNAs were prepared from cells of HYP101 (*MATα STE*$^+$) (lane 1), HYP101-Δ*ste5* (*MATα ste5*::*URA3*) (lane 2), and HYP101-Δ*ste12* (*MATα ste12*::*URA3*) (lane 3), separated on an agarose gel (1.5%) by electrophoresis, blotted, and hybridized with a ^{32}P-labeled 0.7-kbp *Nde*I fragment bearing the *MATα1* cistron and with a 1.0-kbp fragment of *ACT1* as described in the legend to Fig. 6.

those showing decreased APase activity from the *MATα1*$_p$-*PHO5* fusion gene in an **a**/α *aar1-6* background (Table 2). To confirm this prediction, we examined transcription of the *α1* cistron of the *HMLα* gene (derepressed by the *sir3* mutation) of these mutants. Total RNAs were prepared from the *ste5-101* mutant (R102), the putative *ste7*, *ste11*, and *ste12* mutants (R104, R101, and R105, respectively), and the original *STE*$^+$ strain (YMH153). The RNA samples were subjected to Northern (RNA) blot hybridization with a ^{32}P-labeled 0.7-kbp *Nde*I fragment of the *MATα1* DNA as a probe. From this blot, we estimated that the *α1* transcripts from *HMLα* in these *ste* mutants were significantly reduced (Fig. 7A, lanes 1 to 4) and that the reductions were roughly parallel with the APase activities (Table 2). Thus, the reduced APase activities of the mutants might be due to reduced transcription of the *MATα1*$_p$-*PHO5* fused gene.

Fields and Herskowitz (8) reported that *MATα1* transcription in haploid α cells is not affected by the *ste12* mutation. This fact suggests some difference from the above observation for **a**/α *aar1-6* cells. To determine whether the *MATα1* cistron in haploid α cells is affected by *ste* mutations, we prepared total RNA from cells of the *MATα ste5* disruptant (HYP101-Δ*ste5*) and *MATα ste12* disruptant (HYP101-Δ*ste12*) and subjected RNA samples to Northern blot hybridization with ^{32}P-labeled *MATα1* DNA as a probe. We found that *MATα* cells with a disrupted allele of *ste5* or *ste12* contained two to three times less *MATα1* transcript than did wild-type cells (Fig. 8). Thus, transcription of the *α1* cistron is affected by the functions of Ste5p and Ste12p (and probably also Ste7p and Ste11p) proteins. Our result was supported by the finding of one copy of the 5′-TGAAACA-3′ sequence, exactly the same sequence as that of the pheromone response element (PRE; the binding site of Ste12p [25]), in the upstream region of the *α1* cistron from nucleotide positions −95 to −89 (relative to the initiation codon of the *MATα1* ORF). Other genes in which transcription is induced by mating pheromones are known, however, to have two or more copies of the PRE motif (25). We cannot explain the discrepancy between the present results and those of Fields and Herskowitz (8); possibly the *ste12* mutation that they used is a leaky one or there is some difference in strain lineage.

The level of *α1* transcription (from *HMLα*) was reduced markedly in the **a**/α *aar1-6 ste* mutants (Fig. 7A) but only slightly in haploid *MATα AAR1*$^+$ *ste* disruptant cells (Fig. 8). To determine whether *ste* mutations affect **a**1-α2 repression of *α1* transcription, we disrupted the *aar1-6* mutant allele in the original *ste5* and *ste7* mutants by its replacement with the *aar1*::*URA3* fragment. In the resultant *sir3 aar1*::*URA3* disruptants with a *ste5* or *ste7* mutation, the APase activities from the *MATα1*$_p$-*PHO5* fused gene were the same as or similar to that in the *STE*$^+$ *aar1*::*URA3* strain (Table 2). Similar results were obtained for *α1* transcription of *HMLα* in these strains (Fig. 7B). These observations indicate that the *aar1-6* allele is leaky and retains some of its function for **a**1-α2-Aar1p repression. This leakiness of the *aar1-6* mutant allele was favorable for selection of *ste* mutations.

Genes with the PRE motif in their upstream regions are known to be expressed at a basal level without pheromone signals and to be induced by the signals (25). Therefore, we investigated whether transcription of the *MATα1* cistron was enhanced by mating pheromones. Since the signal transduction pathway of the mating pheromone is common to *MAT***a** and *MATα* cells, except for the receptor proteins, *MAT***a** strain YMH48, with a *MATα1*$_p$-*PHO5* fusion gene integrated into the *trp1* locus, was used for examination of the effect of the pheromone dosage on induction of the *MATα1* cistron. The *MATα1*$_p$-*PHO5* transformant cells were inoculated into YPD medium, and the culture was shaken at 30°C for 4 to 5 h until the optical density at 660 nm reached 0.4. Then the culture was supplemented with α-factor (2 μM, final concentration; Difco Laboratories, Detroit, Mich.) and shaken for 2 h. The cells were then collected and suspended in water, and their APase activities were measured. The specific APase activities of the cell suspension were 6.81 ± 0.71 μU with addition of α-factor and 4.4 ± 0.61 μU without α-factor. Similarly, *MATα* cells (YMH47 with the *MATα1*$_p$-*PHO5* fusion gene inserted into the *trp1* locus) were shaken in YPD medium conditioned with **a**-factor as described in Materials and Methods for 2 h at 30°C. The specific APase activities of the cell suspension were 2.26 ± 0.13 μU with addition of **a**-factor and 2.11 ± 0.06 μU without **a**-factor. These results indicate that mating pheromones did not stimulate expression of the *MATα1* cistron appreciably. However, *MATα1*$_p$-*PHO5* expression was reduced 5- to 12-fold in the *ste* mutants (Table 2). Thus, we conclude that the signal transduction system is important for maintaining the basal level of *MATα1* transcription but that pheromone signals do not enhance expression of this cistron.

a1-α2-Aar1p represses *STE5* transcription. Transcription of the *STE5* gene is suggested to be repressed in **a**/α cells (24). Since the Aar1p/Tup1p protein is a component of **a**1-α2 repression (28), we examined whether the *aar1* mutation affects *STE5* transcription in **a**/α cells. Total RNAs were prepared from cells of various genotypes of the mating-type, *AAR1*/*TUP1*, and *STE5* genes, and RNA samples were subjected to Northern blot hybridization with a ^{32}P-labeled 1.5-kbp *Bgl*II fragment of the *STE5* DNA (Fig. 2) as a probe. A single hybridization band of about 2.8 kb was observed in both α and **a** cells (Fig. 9, lanes 6 and 8) but not in **a**/α cells with the *AAR1*$^+$ genotype (lane 4). These results accord well with a previous report (24) that transcription of the *STE5* gene is under **a**1-α2 repression. The results are also consistent with the existence of two **a**1-α2 elements in the 5′ upstream region of the *STE5* ORF (nucleotide positions −207 to −188 and −190 to −171; Fig. 3 and 4), as described by Miller et al. (26). In contrast, **a**/α cells with the *aar1-6* allele or the *aar1*::*HIS3* disrupted allele transcribed *STE5* mRNA (Fig. 9, lanes 1 to 3) as well as *MAT***a** and *MATα* cells, in which transcription of *STE5* occurred irrespective of

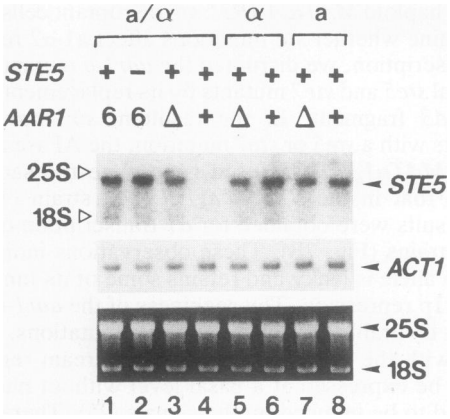

FIG. 9. Transcription of the *STE5* gene under various conditions of mating-type information. Total RNAs were prepared from strain YMH153 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6*) (lane 1), YMH153-R102 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste5-101*) (lane 2), HYP160 (*MAT***a** *HMLα HMR***a** *sir3 aar1*::*HIS3 STE5*$^+$) (lane 3), HYP140 (*MAT***a** *HMLα HMR***a** *sir3 AAR1*$^+$ *STE5*$^+$) *(lane 4), HYP151 (MATα aar1*::*HIS3 STE5*$^+$) (lane 5), HYP101 (*MATα AAR1*$^+$ *STE5*$^+$) (lane 6), HYP150 (*MAT***a** *aar1*::*HIS3 STE*$^+$) (lane 7), and HYP100 (*MAT***a** *AAR1*$^+$ *STE5*$^+$) (lane 8). Total RNAs (2 μg per lane) were subjected to electrophoresis on an agarose gel (1.5%) and blotted onto a nylon filter as described in the legend to Fig. 6. The RNA blots were hybridized with a mixture of the ^{32}P-labeled 1.5-kbp *Bgl*II fragment bearing the *STE5* gene (Fig. 2) and with the ^{32}P-labeled *ACT1* DNA as probes. 25S and 18S indicate the migration positions of the respective rRNAs of *S. cerevisiae* used as size markers (34). Ethidium bromide staining of the total RNA samples is shown below.

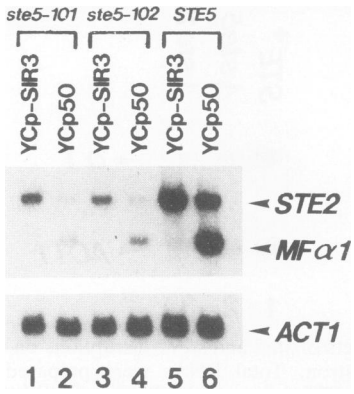

FIG. 10. Effects of two different *ste5* mutant alleles on the transcriptions of *MFα1* and *STE2*. Total RNA samples were prepared from transformant cells of YMH153-R102 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste5-101*) harboring YCp-SIR3 (lane 1) or YCp50 (lane 2), of YMH153-R103 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 ste5-102*) harboring YCp-SIR3 (lane 3) or YCp50 (lane 4), or of YMH153 (*MAT***a** *HMLα HMR***a** *sir3 aar1-6 STE5*$^+$) harboring YCp-SIR3 (lane 5) or YCp50 (lane 6). The RNA samples were separated on an agarose gel, blotted, and hybridized with the same probes as for Fig. 6.

whether the *AAR1* gene was disrupted (lanes 5 to 8). These results indicate that the *STE5* gene is repressed by the **a**1-α2-Aar1p complex, like the *MATα1* cistron and the *RME1* gene (12, 28). The size of the *STE5* transcript, 2.8 kb, is consistent with the predicted size of the *STE5* ORF of 2,751 bp.

Characterization of the α-specific sterility of the *ste5* mutants. As described above, the original *ste5-101* mutant cells with an introduced YCp-SIR3 plasmid had some potency of **a** mating type, whereas the *ste5-102* mutant was nonmating (Fig. 1 and Table 4). To determine whether this phenotypic difference was due to differences in transcriptions of the α- and **a**-specific genes, we examined the amounts of *MFα1* and *STE2* transcripts in these mutants transformed with plasmid YCp-SIR3 by Northern blot hybridization. In these RNA samples, we could detect the *STE2* transcript (Fig. 10, lanes 1 and 3) but not *MFα1*. However, we found that the *ste5-102* transformant harboring YCp-SIR3 transcribes somewhat less *STE2* mRNA (lane 3) than does the *ste5-101* transformant (lane 1). This subtle difference in the transcriptional levels of *STE2* might result in the difference in mating types of *ste5-101* (**a** mater) and *ste5-102* (nonmater) mutants harboring YCp-SIR3, as described earlier (Fig. 1 and Table 4). The levels of transcription of the *MFα1* and *STE2* genes were significantly reduced in the *ste5-101* (lane 2) and *ste5-102* (lane 4) mutants, as in the *ste5*::*URA3* disruptants (Fig. 6), but high in the original *STE*$^+$ cells (Fig. 10, lane 6). The finding that the level of APase activity encoded by the *MATα1*$_p$*-PHO5* fusion gene in the *ste5-101* mutant is substantially higher than that in the *ste5-102* mutant (Fig. 1) indicates that the *ste5-101* allele still retains some activity of the *STE5* gene, whereas its loss by *ste5-102* is greater. Both of these *ste5* mutant alleles, however, are leaky, because mutants harboring YCp-SIR3 showed much higher *STE2* transcription (Fig. 10, lanes 1 and 3) than did the *ste5*::*URA3* disruptant (Fig. 6, lane 5).

DISCUSSION

During isolation of suppressor mutations conferring a sterile phenotype to **a**/α *aar1-6* cells showing the α* mating type, we obtained seven *ste* mutants, including two *ste5* mutants. One of these two *ste5* mutants, *ste5-101*, exhibited the sterile phenotype in **a**/α *aar1-6* cells but **a** mating ability in *MAT***a** cells, whereas the others conferred sterility on both *MAT***a** and **a**/α *aar1-6* cells (Fig. 1). Using these *ste5* mutants, we cloned *STE5* DNA. With the cloned *STE5* fragment, we demonstrated that transcription of the *STE5* gene is under **a**1-α2 repression (Fig. 9) but that expression of the *STE5* gene is indispensable for transcription of **a**- and α-specific genes (Fig. 6).

Fields and Herskowitz (8) reported that transcription of the *MATα1* cistron is not affected by *ste12* mutation, but we observed appreciable reduction of *MATα1* transcription in *MATα* cells with a disrupted allele of *ste5* or *ste12* (Fig. 8). We do not know the reason for this difference. Possibly their *ste12* mutant specifically transcribed the α*1* cistron but reduced transcription of the α- and **a**-specific genes. Therefore, we believe that the PRE upstream of the *MATα1* cistron is functional, at least for the basal level of transcription. The *ste* mutants with the **a**/α *aar1-6* genotype showed markedly reduced transcription of the α*1* cistron (Table 2 and Fig. 7A). However, when the *AAR1* gene in these cells was disrupted, the α*1* cistron was transcribed at the same level as in the **a**/α *STE*$^+$ *aar1* disruptant cells (Fig. 7B). Thus, transcription of the α*1* cistron is repressed by **a**1-α2-Aar1p and sustained by the *STE* pheromone response pathway, and the derepression of α*1* transcription by the *aar1* disruption is epistatic over the *ste* mutation.

With regard to these cross-regulations of the α*1* cistron by the Aar1p and Ste12p proteins, it is noteworthy that the PRE motif, 5′-TGAAACA-3′, at nucleotide positions −95 to −89

upstream of the *MATα1* cistron is located beside a sequence, 5′-CAATGTAGAAAAGTACATCA-3′, at positions −117 to −98 responsible to a1-α2-Aar1p repression (26). These facts suggest that the signals from the *MAT* genes and from the mating pheromones may interact with each other directly in the 5′ upstream region of *MATα1* and that binding of the a1-α2-Aar1p complex to the a1-α2 element may block the binding of Ste12p protein to the PRE motif in the promoter of *MATα1*. The absence or a defect of the a1-α2-Aar1p complex in a/α cells allows Ste12p entry into the PRE motif, because drastic reduction of *α1* transcription was observed in a/α *ste* mutant cells with the *aar1-6* mutant allele (Fig. 7A), whereas *aar1* disruptant cells showed substantial transcription of the *α1* cistron (Fig. 7B). Thus, we conclude that the *aar1-6* mutation is leaky and that the Aar1-6p protein retains residual repressor function.

The PRE motif is known to be required for induction of transcription of the gene in response to signals of mating pheromone and also to maintain the basal level of transcription of the gene (25). Maintenance of the basal levels of transcription of pheromone-inducible genes has been proposed to be due to the presence of some β-γ complex dissociated from the α subunit of the G protein even in the absence of mating pheromones that keeps the signal transduction pathways in a semiactivated state (45). This idea is in good accord with the observation that a *ste* mutation reduced the basal level of *α1* transcription. There are reports that the upstream regions of genes inducible with pheromone generally contain multiple PRE sequences (25) and that a single copy of PRE is not effective for induction (41). This is also the case for *MATα1*, but a single copy of PRE is effective for maintaining the basal transcription level.

Recent epistasis tests with dominant mutant alleles of the *STE11* protein kinase gene established the following order of action of components in the pheromone response pathway: Ste2p (or Ste3p) → Gpa1p (α subunit of the G protein) → (Ste4p [β subunit], Ste18p [γ subunit]) → Ste5p → Ste11p → Ste7p → Ste12p (5, 20, 25, 29, 42). A new *STE* gene, *STE20*, which encodes a putative protein kinase was reported recently by Leberer et al. (23) and suggested to act upstream of Ste5p and possibly to be the direct target of Ste4p and Ste18p. Therefore, the Ste5p protein is considered to mediate pheromone signals, presumably acting between Ste20p and Ste11p proteins. The Ste5p protein is hydrophilic, especially at its N and C termini, suggesting that it is localized in the cytoplasm, although it may bind to a membrane-bound protein via zinc, because it has the partial structure of a zinc finger in its N-terminal half that may facilitate dimerization or complex formation with some other protein(s) (36).

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