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N^{α} Acetylation Is Required for Normal Growth and Mating of *Saccharomyces cerevisiae*

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Acetylation is the most frequently occurring chemical modification of the α-NH_2 group of eucaryotic proteins and is catalyzed by N^{α}-acetyltransferase. The yeast enzyme is encoded by the *AAA1* (amino-terminal α-amino acetyltransferase) gene. A null mutation (*aaa1-1*) created by gene replacement, while not lethal, slows cell growth and results in heterogeneous colony morphology. In comparison with wild-type cells, *aaa1-1/aaa1-1* diploids cannot enter stationary phase, are sporulation defective, and are sensitive to heat shock. In addition, the *aaa1-1* mutation specifically reduces mating functions of *MAT*a cells. These results indicate that N^{α} acetylation plays a crucial role in yeast cell growth and mating.

ACKNOWLEDGMENTS

We thank members of the laboratory of Jack W. Szostak for providing strains and helping with genetic techniques, and we are especially thankful to Hong Sun for her support and advice. A plasmid containing the yeast β-tubulin gene was kindly provided by Joan Park. We also thank members of the laboratories of Gerald Fink, Nancy Kleckner, Malcolm Whiteway, and Duane Jenness for providing strains and plasmids and Thierry Vernet for providing several expression vectors. We also thank Ulrich Teichert for helpful suggestions during the preparation of the manuscript.

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ADDENDUM

After the review of the manuscript, a study describing the identification and characterization of a gene (*NAT1*) affecting N^{α} acetylation was published (J. R. Mullen, P. S. Kayne, M. Moerschell, S. Tsunasawa, M. Grunstein, F. Sherman, and R. Sternglanz, EMBO J. **8**:2067–2075, 1989). Our previously published cDNA cloning and sequence analysis (21) and the results presented here are in agreement with their data for the cloning and sequencing of an identical gene, for the phenotypes associated with disruption of that gene, and for the relationship of that gene to *ARD1*.

MATERIALS AND METHODS

Strains, plasmids, and media. Strains used are listed in Table 1. Yeast culture media were prepared as described by Sherman et al. (34). YPD contained 1% yeast extract, 2% Bacto-Peptone, and 2% glucose; YPG contained 1% yeast extract, 2% Bacto-Peptone, and 3% glycerol; SD contained 0.7% yeast nitrogen base without amino acids (Difco Laboratories) and 2% glucose; and nutrients essential for auxotrophic strains were supplied at specified concentrations (34). Sporulation was carried out as previously described (34). Presporulation plates contained 0.5% yeast, 0.5% Bacto-Peptone, 1% glucose, and 2% Bacto-Agar. Cells were grown on these plates for 1 day before being transferred to sporulation plates containing 1% potassium acetate, 0.1% yeast extract, 2% Bacto-Agar, and appropriate auxotrophic nutrients. Cells were grown at 30°C unless otherwise indicated. Yeast transformation was by the lithium acetate method (14). Standard techniques were used for diploid construction and tetrad dissection (34). Plasmids were constructed by standard protocols as described by Maniatis et al. (25).

Expression plasmids for the *AAA1* gene were constructed by inserting the *AAA1* coding region into the pVT-L100 (containing a *LEU2* marker) or pVT-U100 (containing a *URA3* marker) expression vectors at the *Xba*I site immediately following the *ADH1* promoter (46). These plasmids are identified as pLA1 and pUA1, respectively.

Disruption of the yeast *AAA1* gene. The yeast *URA3* gene was inserted at a single *Eco*RV site in the *AAA1* gene as follows (Fig. 1A). Plasmid pBNH9 was constructed by deleting the 3′ end of *AAA1* from the *Hin*dIII site in the *AAA1* insert to the *Hin*dIII site in the Bluescript (Stratagene) and then self-ligating. The 3.8-kilobase (kb) DNA fragment containing the yeast *URA3* gene and two *hisG* repeat sequences was excised from plasmid pNKY51 (1) by digestion with *Bgl*II and *Bam*HI, and its sticky ends were filled in by Klenow fragment. Plasmid pBNH9 was opened by cutting with *Eco*RV, and the 3.8-kb *hisG-URA3-hisG*-containing fragment was blunt-end ligated into pBNH9, resulting in pBNHU9.

Gene disruption mutants were constructed by a one-step gene disruption method (32). Basically, a 4.9-kb DNA fragment was released from pBNHU9 by digestion with *Xho*I, and this fragment was used to transform various strains. Uracil prototrophs were selected.

Elimination of the *URA3* gene and one *hisG* repeat was carried out by patching a Ura$^+$ *aaa1* mutant strain (AB18-a) onto 5-fluoro-orotic acid plates, which are selective for *ura3* strains (uracil plus 5-fluoro-orotic acid), as described previously (4). Thereby, AB18-ap (*aaa1*::*hisG ura3*), a 5-fluoro-orotic acid-resistant strain, was derived from AB18-a.

DNA blot analysis. All restriction enzymes were purchased from New England BioLabs, Inc. DNA markers were obtained from Bethesda Research Laboratories, Inc. GeneScreen Plus membrane was from New England Nuclear Corp. Yeast genomic DNA was isolated (34), digested with restriction enzymes, electrophoresed on 0.8% agarose in Tris-borate buffer, transferred onto a GeneScreen Plus membrane, hybridized with a random-primed, ^{32}P-labeled *Xho*I-*Bam*HI fragment *AAA1* (derived from pBN9) for 24 h, washed, and autoradiographed (36).

Phenotype tests. (i) Colony morphology was examined by growing the tested strains in YPD medium at 30°C for 3 days and then plating the cells on YPD plates. The sizes and morphologies of colonies were evaluated after 5 days of growth. (ii) Specific growth rates of tested strains were obtained by growing cells in YPD medium at 30°C and 200 rpm, and optical density values at 600 nm were determined at specific time intervals. (iii) Entry into stationary phase was determined as follows. The percentage of budded cells in 3-day-old cultures grown in YPD medium was determined by mixing a portion of the culture with an equal volume of 10% formaldehyde, sonicating briefly, and counting budded and unbudded cells with a hemacytometer (~1,000 cells per determination). A second method was to determine the survival percentage in stationary phase. Cells were maintained in SD medium at 30°C for 5 days, and after dilution, cells were plated on YPD plates. After 2 days the colonies were counted. A third method was to determine glycogen accumulation by inverting 5-day-old culture plates over iodine crystals in a closed container for 3 to 5 min and noting the appearance of dark brown colonies containing glycogen. (iv) Sporulation efficiency was tested as follows. Cells were grown on YPD plates, transferred to sporulation medium (1% potassium acetate, 0.1% yeast extract, 0.05% glucose) with appropriate auxotrophic nutrients, and incubated at 25°C and 250 rpm for 1 day. Cells were harvested by centrifugation (1,200 × *g* for 5 min at 20°C), suspended in minimal sporulation medium (aqueous 1% potassium acetate) with appropriate auxotrophic nutrients, and incubated for 2 days. The percentage of sporulated cells was determined by counting >500 cells. (v) Heat sensitivity was determined by growing the cells to late log phase in YPD medium at 30°C, diluting to ~10^5/ml in SD medium, and heat shocking at 54°C. Samples were removed at the indicated times and chilled in an ice bath. After dilution, cells were plated on a YPD plate. Colonies were counted and survival percentages were determined 3 days later.

Preparation of crude lysates for assay of N^{α}-acetyltransferase. Crude yeast lysates were prepared, and N^{α}-acetyltransferase activity was determined as previously described (20). Portions of the lysate were added to 1.5-ml Eppendorf tubes containing a reaction mixture of 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) (pH 7.4), 150 mM KCl, 1 mM dithiothreitol, 25 μM [^{3}H]acetyl coenzyme A (0.5 μCi), and 50 μM adrenocorticotropin (amino acid residues 1 to 24) with an adjusted final volume of 100 μl. The assay mixture was incubated at 30°C for 30 min. The reaction was stopped by adding 0.5 M acetic acid and chilling in an ice bath. The reaction samples were filtered through SP membrane disks (Cuno) and then washed with 0.5 M acetic acid on a model 1225 sampling manifold (Millipore Corp.). The partially dried membranes were placed in a scintillation cocktail and counted with an LS 3801 scintillation counter (Beckman Instruments, Inc.).

Quantitative mating tests and pheromone responses. Strains to be tested for mating were grown overnight in YPD medium. Equal numbers of cells from each mating type ($\sim 5 \times 10^6$) were mixed, incubated in YPD medium for 6 h at 30°C, and examined for agglutination. In addition, the cells were plated on SD plates containing nutrients essential for auxotrophic selection on which only diploids resulting from mating should grow. The individual mating-type cells were also plated singly on SD plates to assay for the reversion of auxotrophic markers, and no prototrophs were observed.

aaa1 mutants (MGD502.4a and AB18-a; both are *MAT***a**) were tested for α-factor response. Cells were grown overnight at 30°C in YPD, washed, suspended in 5 ml of YPD containing α factor (1 μM) at a cell density of 10^6 cells per ml, and incubated at 30°C. Samples (0.1 ml) were removed at various intervals and mixed with an equal volume of 10% formaldehyde, and the number of G1-phase arrested cells was determined by the ratio of budded to unbudded cells.

Assay for pheromone production and barrier activity. For the **a**-factor assay, about 10^4 cells of the tester strain 3268-1-3 (α *sst2-1*) were spread onto a YPD (pH 4.5) plate, and cells of the *MAT***a** strains to be tested were spotted on the plate. Zones of growth inhibition were clearly visible after 2 to 3 days of incubation at 30°C.

For the α-factor assay, an analogous test, involving inhibition of the tester strain F676 (*MAT***a** *sst1*), was performed as described for the **a**-factor test, except that about 10^5 cells per plate were used.

The quantitative measurement of pheromone production was carried out as follows. Cells were grown to late log phase at 30°C and 200 rpm in YPD medium. Cells were pelleted twice by centrifugation at 13,000 $\times$ *g* for 5 min before the supernatant was assayed for pheromone activity. Serial dilutions (two- to fourfold) of pheromone-containing supernatants in citrate buffer (pH 4.5) were spotted (10 μl) onto a lawn of cells that were supersensitive to pheromones and then incubated for 36 to 48 h at 23°C.

Barrier activity was detected by interference in α-factor-produced zones by a streak of *MAT***a** cells, as described by Sprague and Herskowitz (38), and by using F676 (*sst1*) as the tester strain, as described by Hicks and Herskowitz (13). Synthetic α factor was purchased from Bachem Bioscience Inc. and dissolved in 90% methanol (2 mg/ml).

Transcriptional regulation of *AAA1* gene. Cells from 500-ml cultures were harvested at mid-log phase in YPG medium and at different stages of growth in YPD medium. Heat shock was carried out as follows. When the optical density at 600 nm of a YPD culture reached ~2.0, two 75-ml portions were removed; one was heated at 37°C for 2 h, and the other was incubated at 30°C. Total RNA was extracted from each sample (34). Yeast RNA (10 μg) was electrophoresed on a 1.2% agarose-formaldehyde gel (22). The lane containing the RNA markers was sliced out, visualized by staining with ethidium bromide, and used for determining the molecular sizes of the RNAs. The RNA was transferred onto a Gene-Screen Plus membrane and hybridized with random-primed ^{32}P-labeled *AAA1* (derived from pBN9; Fig. 1A) and β-tubulin (30) DNA for 24 h, washed, and autoradiographed (42). The levels of the mRNAs for *AAA1* and β-tubulin were determined.

TABLE 1. Yeast strains

Strain	Genotype[a]	Source
MGD502	*MATa/MATα +/ade2 arg4/+ cyh^r/cyh^s his3/+ leu2/leu2 trp1/trp1 ura3/ura3*	J. Szostak
MGD502-a	*MATa/MATα +/ade2 arg4/+ cyh^r/cyh^s his3/+ leu2/leu2 trp1/trp1 ura3/ura3 aaa1-1/AAA1*	This work
MGD502-2a	*MATa/MATα +/ade2 arg4/+ cyh^r/cyh^s his3/+ leu2/leu2 trp1/trp1 ura3/ura3 aaa1-1/aaa1-1*	This work
MGD502.4b	*MATa arg4 cyh^r his3 leu2 trp1 ura3 AAA1*	J. Szostak
MGD502.4a	*MATa arg4 cyh^r his3 leu2 trp1 ura3 aaa1-1*	This work
MGD502.4c	*MATα ade2 cyh^s leu2 trp1 ura3 AAA1*	J. Szostak
MGD502.4d	*MATα ade2 cyh^s leu2 trp1 ura3 aaa1-1*	This work
AB18	*MATa ade2-1 his5 lys2 trp1 ura3 AAA1*	I. Huang
AB18-a	*MATa ade2-1 his5 lys2 trp1 ura3 aaa1-1*	This work
AB18-ap	*MATa ade2-1 his5 lys2 trp1 ura3 aaa1-2*	This work
T3A	*MATα his3 leu2 ura3 AAA1*	J. Szostak
T3A-a	*MATα his3 leu2 ura3 aaa1-1*	This work
MS	*MATa/MATα ade2-1/+ his5/his3 +/leu2 lys2/+ trp1/+ ura3/ura3*	This work[b]
MS-a	*MATa/MATα ade2-1/+ his5/his3 +/leu2 lys2/+ trp1/+ ura3/ura3 aaa1-1/AAA1*	This work[c]
MS-2a	*MATa/MATα ade2-1/+ his5/his3 +/leu2 lys2/+ trp1/+ ura3/ura3 aaa1-1/aaa1-1*	This work[d]
F676	*MATa ade2 his6 met1 sst1-3 ura1 rem1*	G. Fink
3268-1-3	*MATα ade2 cry1 his4 lys2 sst2-1 trp1 tyr1 SUP4-3^{ts}*	D. Jenness

[a] *aaa1-1*, *aaa1::hisG-URA-hisG*; *aaa1-2*, *aaa1::hisG*, as described in Materials and Methods.
[b] Diploid from a cross of AB18 and T3A.
[c] Diploid from a cross of AB18-a and T3A.
[d] Diploid from a cross of AB18-a and T3A-a.

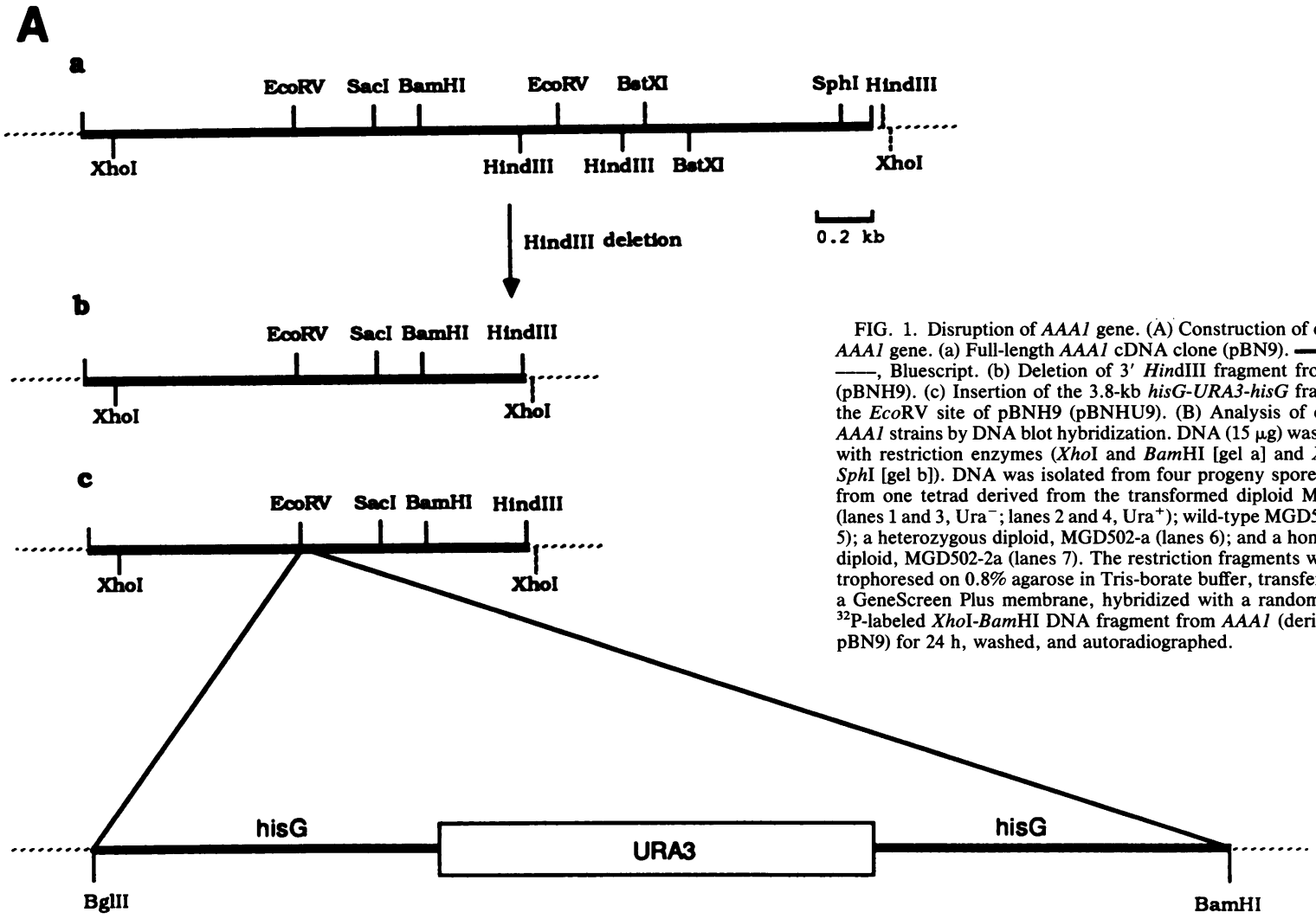
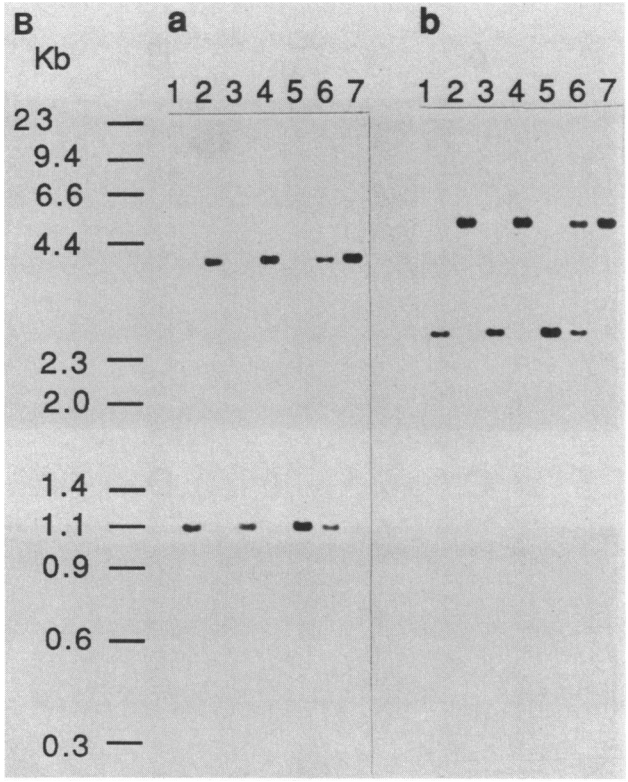


FIG. 1. Disruption of *AAA1* gene. (A) Construction of disrupted *AAA1* gene. (a) Full-length *AAA1* cDNA clone (pBN9). ▬, cDNA; ——, Bluescript. (b) Deletion of 3′ *Hin*dIII fragment from pBN9 (pBNH9). (c) Insertion of the 3.8-kb *hisG-URA3-hisG* fragment at the *Eco*RV site of pBNH9 (pBNHU9). (B) Analysis of disrupted *AAA1* strains by DNA blot hybridization. DNA (15 μg) was digested with restriction enzymes (*Xho*I and *Bam*HI [gel a] and *Xho*I and *Sph*I [gel b]). DNA was isolated from four progeny spore colonies from one tetrad derived from the transformed diploid MGD502-a (lanes 1 and 3, Ura^-; lanes 2 and 4, Ura^+); wild-type MGD502 (lanes 5); a heterozygous diploid, MGD502-a (lanes 6); and a homozygous diploid, MGD502-2a (lanes 7). The restriction fragments were electrophoresed on 0.8% agarose in Tris-borate buffer, transferred onto a GeneScreen Plus membrane, hybridized with a random-primed, ^{32}P-labeled *Xho*I-*Bam*HI DNA fragment from *AAA1* (derived from pBN9) for 24 h, washed, and autoradiographed.



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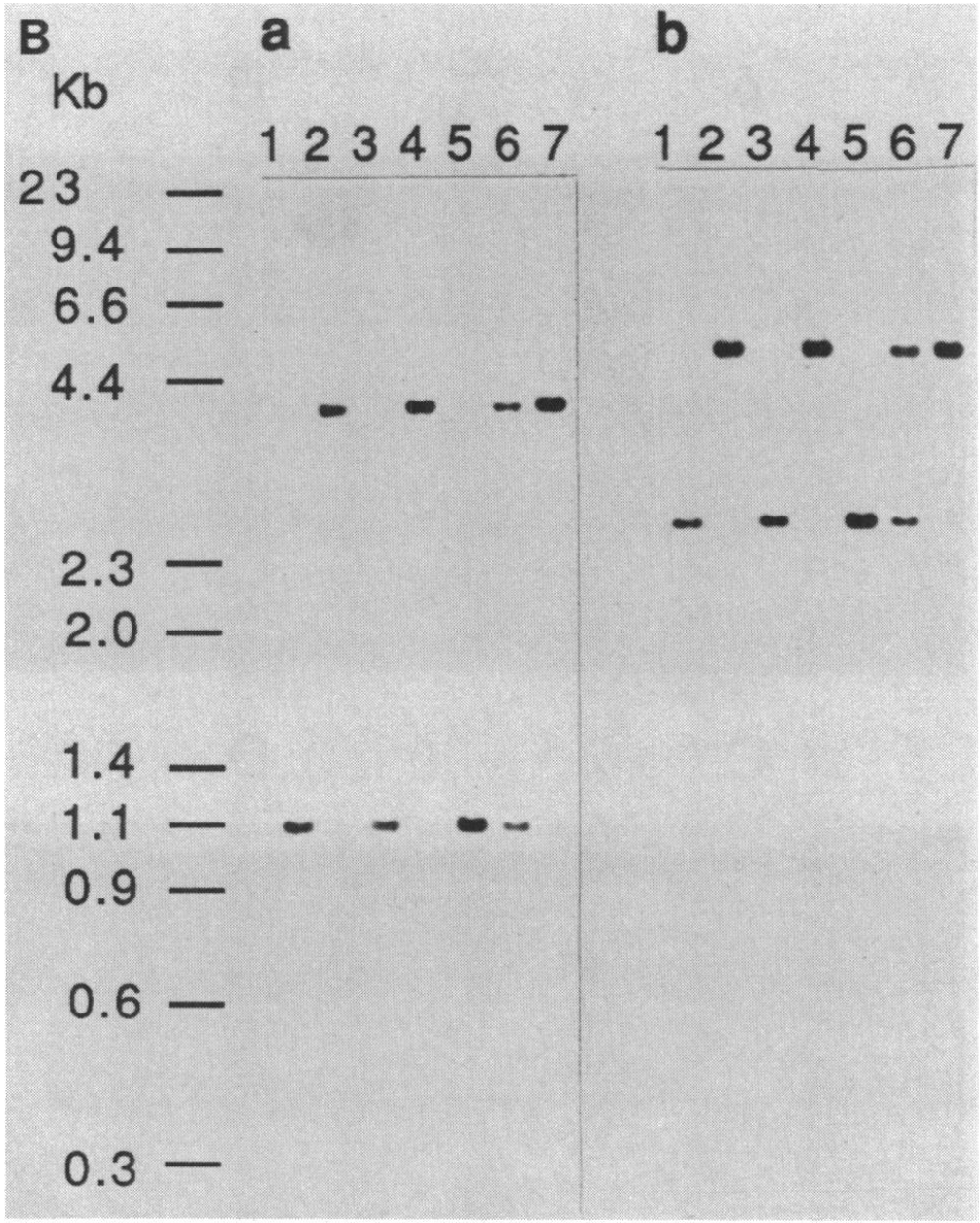
B
Kb
a
b
1 2 3 4 5 6 7
1 2 3 4 5 6 7
23
9.4
6.6
4.4
2.3
2.0
1.4
1.1
0.9
0.6
0.3

TABLE 2. Effect of *AAA1* on specific growth rate, entry into stationary phase, and enzymatic activity

Strain	Specific growth rate (per h)[a]	Budded cells (%)[b]	Survival in stationary phase (%)[c]	Glycogen accumulation	Enzyme-specific activity (U/mg)[d]
MGD502.4b (*AAA1*)	0.439	8	71	+	2.0
MGD502.4a (*aaa1-1*)	0.169	45	9	−	<0.02
MGD502.4c (*AAA1*)	0.435	10	62	+	1.9
MGD502.4d (*aaa1-1*)	0.179	48	6	−	<0.02
AB18 (*AAA1*)	0.421	7	56	+	1.7
AB18-a (*aaa1-1*)	0.172	46	6	−	<0.02
T3A (*AAA1*)	0.496	10	73	+	2.2
T3A-a (*aaa1-1*)	0.302	54	10	−	<0.02

[a] Cells were grown in YPD medium at 30°C and 200 rpm, and the optical density at 600 nm was determined at various time intervals.
[b] Cells were grown in YPD medium at 30°C for 3 days. After brief sonication, budded and unbudded cells were counted with a hemacytometer. Cells (>1,000) were counted for each determination.
[c] Strains were maintained in SD medium at 30°C for 5 days. Cells were plated on a YPD plate, and colonies were counted after 2 days.
[d] N^{α}-acetyltransferase activity was determined as described in reference 20.

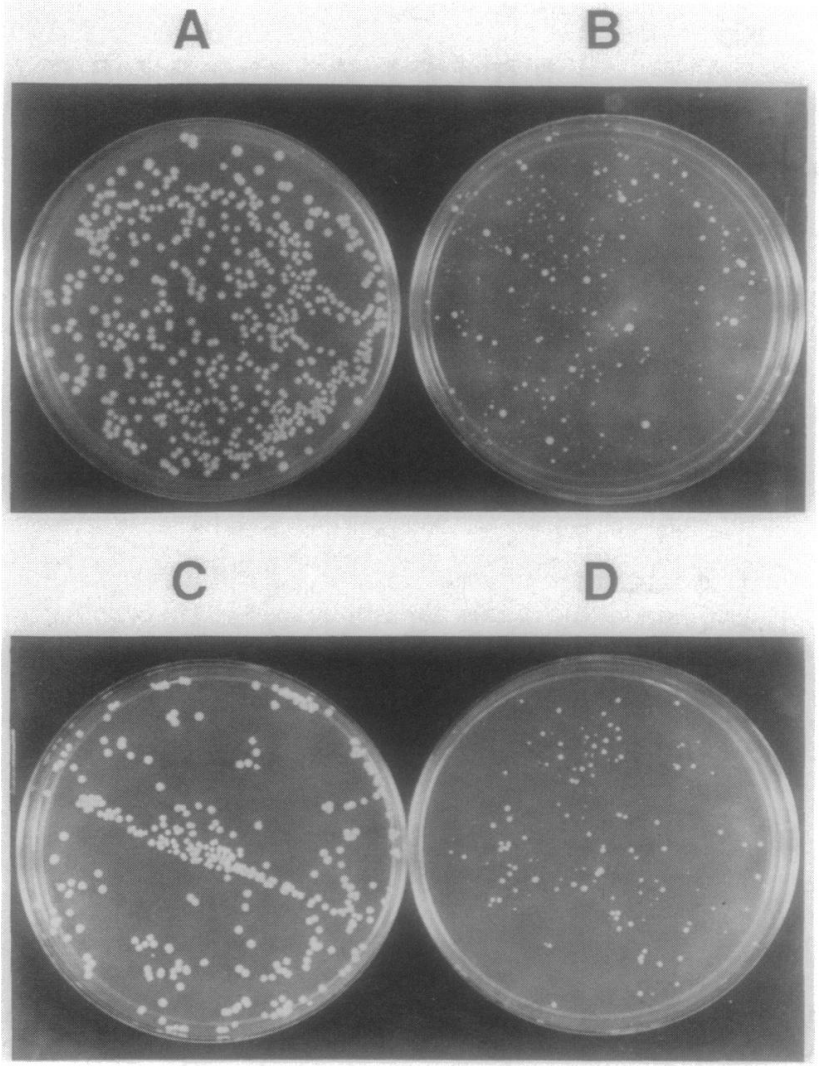

FIG. 2. Morphology of wild-type and *aaa1* strains. Wild-type strains MGD502.4b (A) and MGD502.4c (C) and *aaa1* strains MGD502.4a (B) and MGD502.4d (D) were grown on YPD plates at 30°C for 5 days. The genotypes of the strains are shown in Table 1.

TABLE 3. Effect of *AAA1* on sporulation efficiency[a]

Strain	*AAA1* locus	Sporulation efficiency (%)	Plasmid pLA1(*AAA1*$^+$)
MGD502	*AAA1/AAA1*	27	−
MGD502-a	*aaa1-1/AAA1*	23	−
MGD502-2a	*aaa1-1/aaa1-1*	<0.1	−
MGD502-2a/T[b]	*aaa1-1/aaa1-1*	21	+
MS	*AAA1/AAA1*	16	−
MS-a	*aaa1-1/AAA1*	12	−
MS-2a	*aaa1-1/aaa1-1*	<0.1	−

[a] Sporulation efficiency of each strain was determined after incubation in sporulation and minimal sporulation media at 25°C for 3 days, as described in Materials and Methods.

[b] MGD502-2a transformed with plasmid pLA1, which carries the *AAA1* gene.

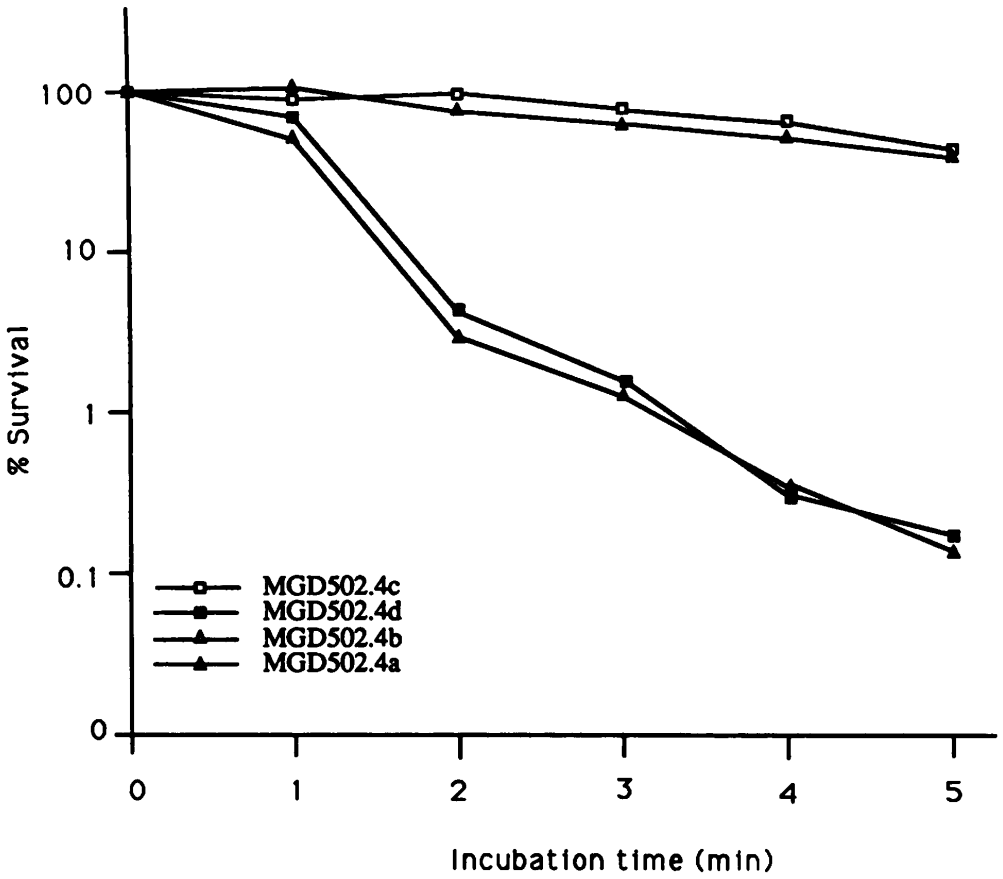

FIG. 3. Heat shock sensitivity of *aaa1* strains. Cells were grown to late log phase in YPD medium, diluted in SD medium, and incubated at 54°C. The survival percentage was determined at each indicated time. The genotypes of the strains are shown in Table 1.

TABLE 4. Mating efficiencies of wild-type and *aaa1* strains[a]

*MAT*α		*MAT***a**		Normalized mating efficiency (no. of diploids)
Strain	Genotype	Strain	Genotype	
MGD502.4c	α *AAA1*	MGD502.4b	**a** *AAA1*	2.1×10^5
MGD502.4c	α *AAA1*	MGD502.4a	**a** *aaa1-1*	97
MGD502.4d	α *aaa1-1*	MGD502.4b	**a** *AAA1*	8.2×10^4
MGD502.4d	α *aaa1-1*	MGD502.4a	**a** *aaa1-1*	89
T3A	α *AAA1*	AB18	**a** *AAA1*	1.4×10^5
T3A	α *AAA1*	AB18-a	**a** *aaa1-1*	94
T3A-a	α *aaa1-1*	AB18	**a** *AAA1*	7.5×10^4
T3A-a	α *aaa1-1*	AB18-a	**a** *aaa1-1*	85
MGD502.4c	α *AAA1*	MGD502.4a/T[b]	**a** *aaa1-1* pLA1(*AAA1*$^+$)	7.3×10^4
T3A	α *AAA1*	AB1-ap/T[c]	**a** *aaa1-2* pUA1(*AAA1*$^+$)	6.7×10^4

[a] Mating efficiency was determined at 30°C, as described in Materials and Methods.
[b] MGD502.4a transformed with pLA1 carrying the *AAA1* gene.
[c] AB18-ap transformed with pUA1 carrying the *AAA1* gene.

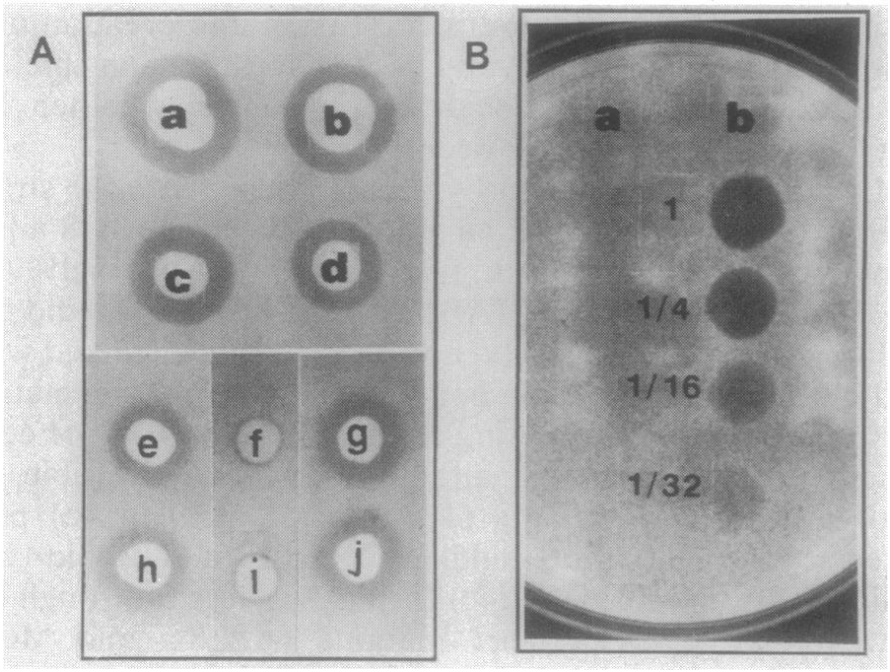

FIG. 4. Mating-type factor expression. (A) Production of mating factor in wild-type and *aaa1* strains. For the α-factor assay, late-log-phase MGD502.4c (*MAT*α *AAA1*) (a), MGD502.4d (*MAT*α *aaa1-1*) (b), T3A (*MAT*α *AAA1*) (c), and T3A-a (*MAT*α *aaa1-1*) (d) were spotted on a lawn of strain F676 (*MAT***a** *sst1*), a strain supersensitive to α factor. For the **a**-factor assay, late-log-phase MGD502.4b (*MAT***a** *AAA1*) (e), MGD.4a (*MAT***a** *aaa1-1*) (f), MGD.4a/T [*MAT***a** *aaa1-1*/*pUA1*(*AAA1*$^+$)] (g), AB18 (*MAT***a** *AAA1*) (h), AB18-a (*MAT***a** *aaa1-1*) (i), and AB18-ap/T [*MAT***a** *aaa1-1*/*pUA1*(*AAA1*$^+$)] (j) were spotted on a lawn of strain 3268-1-3 (*MAT*α *sst2-1*), a strain supersensitive to **a** factor. (B) Quantitation of **a**-mating-factor production. MGD.4a (*MAT***a** *aaa1-1*) (a) and MGD502.4b (*MAT***a** *AAA1*) (b) were grown to late log phase. Cells were removed by centrifugation, and the **a**-mating-factor activity was assayed by spotting 10 μl of the supernatant on a lawn of strain 3268-1-3 (*MAT*α *sst2-1*) and incubating it for 36 to 48 h at 23°C.

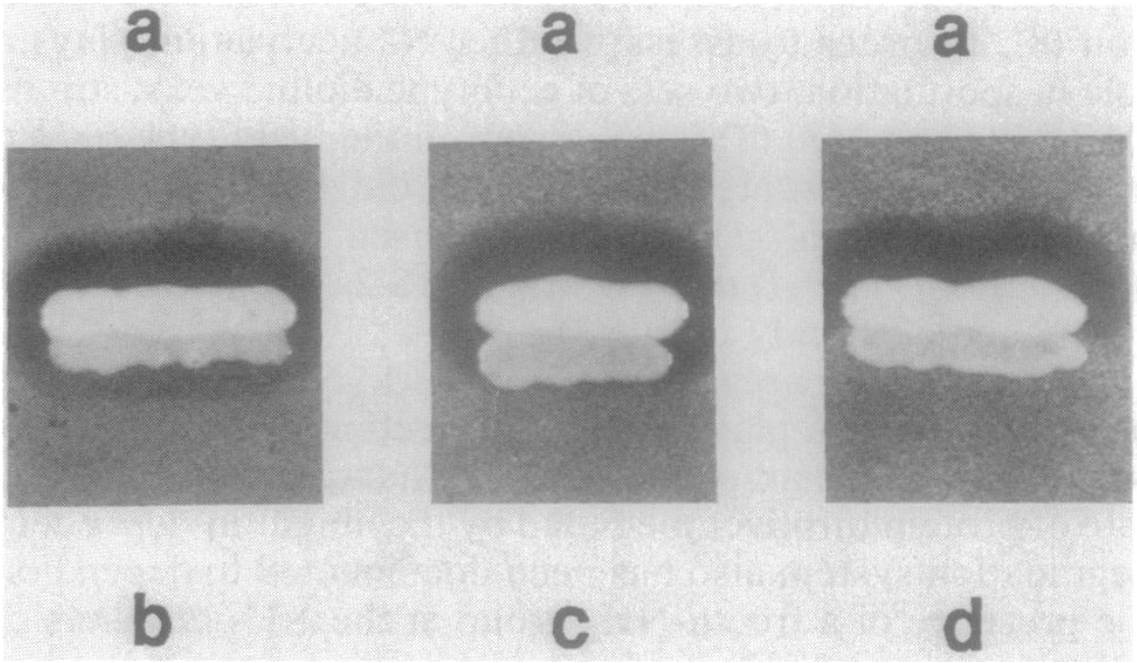

FIG. 5. Barrier activity of wild-type and *aaa1* strains. Cells of strain F676 (*MAT***a** *sst1*) were spread onto a YPD (pH 4.5) plate before the *MAT***a** strains to be tested were streaked. Streaks of barrier-producing cells disturbed the halo zone which was formed by inhibition of the growth of the tested strain F676 by the diffusing α factor. (a) T3A (*MAT*α), α-factor-producing strain; (b) F676 (*MAT***a** *sst1*); AB18-a (*MAT***a** *aaa1-1*); (d) AB18 (*MAT***a** *AAA1*).

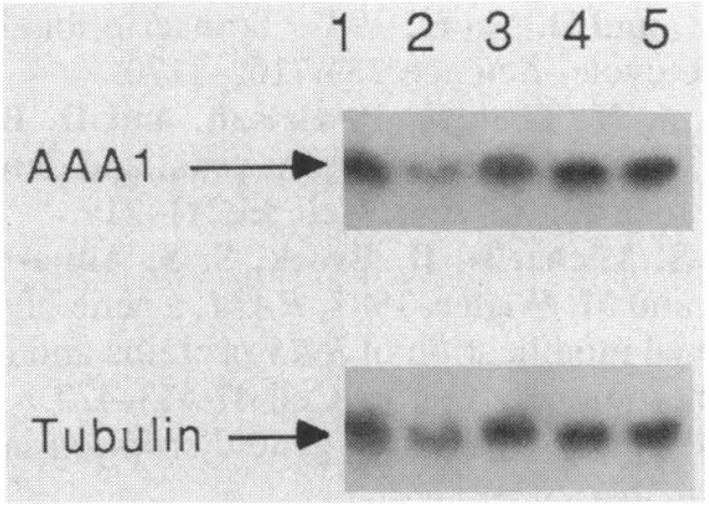

FIG. 6. RNA blot analysis of yeast cells at different growth conditions. Total RNA was prepared from cells grown in YPG medium at mid-log phase (lane 1) or in YPD medium at early log phase (lane 2), mid-log phase (lane 3), or stationary phase (lane 4) and after heat shock at 37°C for 2 h (lane 5). RNA (10 μg) was electrophoresed on a 1.2% agarose-formaldehyde gel (36). The RNA was transferred onto a GeneScreen Plus membrane, hybridized with random-primed, ^{32}P-radiolabeled *AAA1* (derived from pBN9; Fig. 1A) and yeast β-tubulin probes for 24 h, washed, and autoradiographed.