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# $N^{\alpha}$ 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 $N^{\alpha}$-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 $N^{\alpha}$ acetylation plays a crucial role in yeast cell growth and mating.**

α-Amino acetylation is an important cotranslational and posttranslational modification of proteins in procaryotic and eucaryotic cells, and $N^{\alpha}$ acetylation is the most common chemical modification of the α-$NH_2$ group of eucaryotic proteins (reviewed in references 7 and 45). $N^{\alpha}$ acetylation is mediated by at least one $N^{\alpha}$-acetyltransferase, which catalyzes the transfer of an acetyl group from acetyl coenzyme A to the α-$NH_2$ group of proteins and peptides, and a large number of proteins from various organisms have been shown to possess $N^{\alpha}$-acetylated $NH_2$-terminal residues (5, 6). $N^{\alpha}$ acetylation plays a role in normal eucaryotic translation and processing (50) and protects against proteolytic degradation (16, 33). Further, the rate of protein turnover mediated by the ubiquitin-dependent degradation system apparently depends on the presence of a free α-$NH_2$ group (2, 12), and this dependence indicates that $N^{\alpha}$ acetylation may play a crucial role in impeding protein turnover.

Recently, we purified an $N^{\alpha}$-acetyltransferase from *Saccharomyces cerevisiae* and characterized it as a dimeric protein, whose subunit $M_r$ was 95,000 and which would effectively transfer an acetyl group to various synthetic peptide substrates (including adrenocorticotropin [amino acid residues 1 to 24], human superoxide dismutase [residues 1 to 24], and yeast alcohol dehydrogenase [residues 1 to 24] (20). Further, we demonstrated that this enzyme would not transfer an acetyl group to the ε-amino group of lysyl residues in various peptide substrates and histones. By using amino acid sequences of tryptic peptides derived from the purified enzyme, we have cloned its cDNA, demonstrated that the enzyme is encoded by a single gene (*AAA1*, amino-terminal α-amino acetyltransferase), and localized this gene to chromosome IV (21). This yeast DNA forms the basis for elucidating the biological function and regulation of $N^{\alpha}$ acetylation in eucaryotic protein synthesis and degradation. In this paper, we describe the creation of a null mutation (*aaa1-1*) by one-step gene replacement and the testing of various null mutants for entrance into stationary phase, sporulation efficiency, temperature sensitivity, and mating functions. The results indicate that $N^{\alpha}$ acetylation affects one or more proteins involved in each of these vital cell functions.

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## 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,

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.

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.

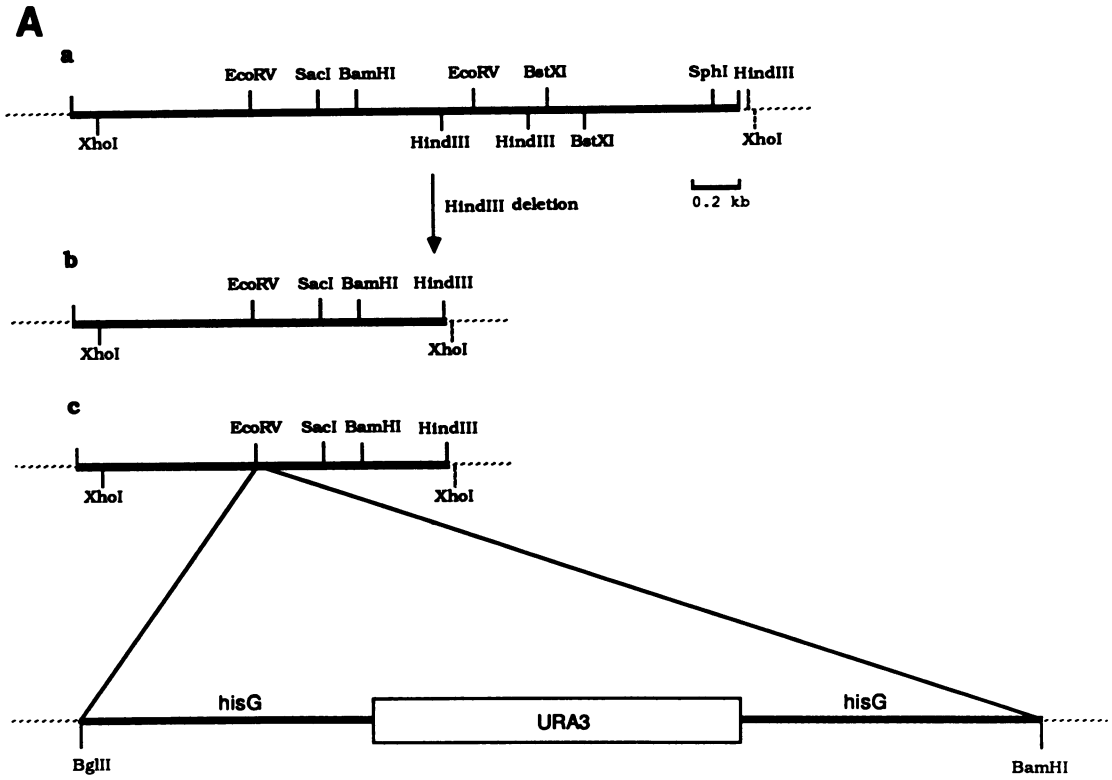
**DNA blot analysis.** All restriction enzymes were purchased from New England BioLabs, Inc. DNA markers were obtained from Bethesda Research Laboratories, Inc. GeneScreen Plus membrane was from New England Nuclear Corp. Yeast genomic DNA was isolated (34), digested with restriction enzymes, electrophoresed 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$^\alpha$-acetyltransferase.** Crude yeast lysates were prepared, and $N^\alpha$-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 (~5 × $10^6$) 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 *MATa*) were tested for α-factor response. Cells were grown over-



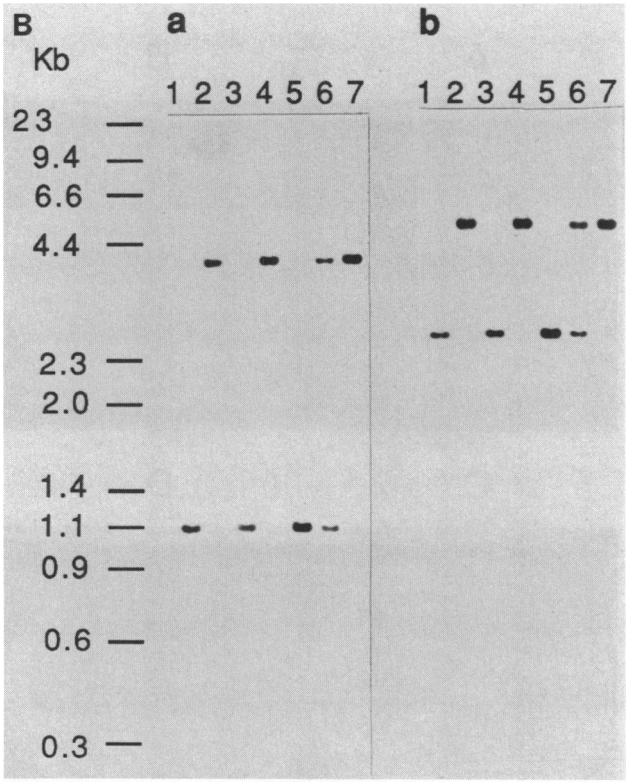




FIG. 1. Disruption of *AAA1* gene. (A) Construction of disrupted *AAA1* gene. (a) Full-length *AAA1* cDNA clone (pBN9). ▬, cDNA; ——, Bluescript. (b) Deletion of 3′ *Hin*dIII fragment from pBN9 (pBNH9). (c) Insertion of the 3.8-kb *hisG-URA3-hisG* fragment at the *Eco*RV site of pBNH9 (pBNHU9). (B) Analysis 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.

night 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 × *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

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] $N^{\alpha}$-acetyltransferase activity was determined as described in reference 20.

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 GeneScreen 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.

## RESULTS

***AAA1* gene disruptions.** In order to study the biological role of the $N^{\alpha}$ acetylation of proteins in yeast cells, a disruption mutation of the *AAA1* gene encoding the $N^{\alpha}$-acetyltransferase was made by single-step gene replacement (32). A *Hin*dIII fragment was removed from the 3′ end of the *AAA1* gene, thereby deleting ~45% of the gene, and the 3.8-kb *hisG-URA3-hisG* gene fragment was inserted into an *Eco*RV site (Fig. 1A). A DNA fragment containing the *aaa1*::*hisG-URA3-hisG* sequence was then transformed into the *ura3/ura3* diploid yeast (MGD502) (Table 1) (14). Ura$^+$ transformants were isolated and sporulated, and the resulting asci were dissected for tetrad analysis. Most diploid cells gave rise to four viable spores. However, each complete tetrad (20 tetrads) consisted of two wild-type-sized colonies and two smaller colonies (data not shown). Characterization of complete tetrads indicated that larger colonies consisted of Ura$^-$ cells and that smaller colonies consisted of Ura$^+$ cells. DNA blot analysis of tetrads confirmed that Ura$^-$ spores contained either 1.1-kb *Xho*I-*Bam*HI or 2.5-kb *Xho*I-*Sph*I fragments found in *AAA1*, while the Ura$^+$ spores contained an additional 3.8 kb, corresponding to the *hisG-URA3-hisG* gene (Fig. 1B). Since each of the strains (diploid and haploid) containing the *aaa1-1* disruption (*aaa1*::*hisG-URA3-hisG*) was viable, *AAA1* is not an essential gene. Enzyme assays of protein extracts from several of these strains confirmed that the Ura$^+$ strains contained no detectable $N^{\alpha}$-acetyltransferase activity, while untransformed diploid (+/+), heterozygous diploid (+/*aaa1-1*), and Ura$^-$ strains had normal enzyme activity (Table 2). We also transformed the DNA fragment containing the *aaa1*::*hisG-URA3-hisG* sequence into the *ura3* haploid yeast strains (MGD502.4b, MGD502.4c, AB18, and T3A) (Table 1). Ura$^+$ transformants of haploid strains were isolated, and DNA blot analyses and enzyme assays confirmed that the *AAA1* gene was also disrupted (data not shown).

**Phenotypes of *aaa1* strains.** The specific growth rates of haploid cells (wild type and *aaa1* mutant) were determined.

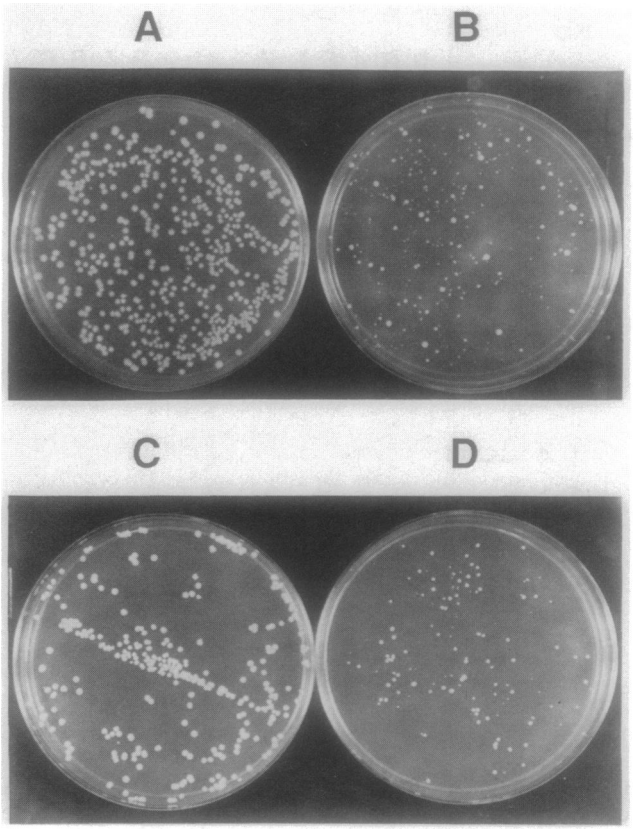



FIG. 2. Morphology of wild-type and *aaa1* strains. Wild-type strains MGD502.4b (A) and MGD502.4c (C) and *aaa1* strains MGD502.4a (B) and MGD502.4d (D) were grown on YPD plates at 30°C for 5 days. The genotypes of the strains are shown in Table 1.

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.

The *aaa1* mutants had a 40 to 60% decrease in specific growth rate in comparison with wild-type cells (Table 2). In order to assess whether the *aaa1* mutation affects the entrance of a cell into stationary phase, the ratio of budded to unbudded cells, the survival percentage in stationary phase, and the glycogen accumulation were determined. Cultures of *aaa1* cells of either mating type exhibited ratios of budded to unbudded cells characteristic of exponentially growing cultures, whereas the wild-type strains had bud ratios characteristic of stationary-phase cultures (Table 2). In addition, multiple-site budding and aberrant budding were frequently observed in the mutant cells (data not shown). The survival percentage in stationary phase showed that the nonproliferating cultures of the *aaa1* strains lost viability more rapidly than did cultures of wild-type strains (Table 2). In addition, only the wild-type cells showed glycogen accumulation characteristic of stationary-phase cells.

When 3-day-old cultures were plated onto YPD plates, the *aaa1* colonies were varied in size and misshapen, and ~80% were smaller than the wild-type colonies (Fig. 2). Multiple mutant colonies of different sizes were picked up, grown in YPD for 3 days, and then plated onto a YPD plate. These *aaa1* mutant colonies were also varied in size and misshapen (data not shown). Since large colonies failed to breed true, they are not likely to be pseudorevertants of the *aaa1-1* disruption.

***AAA1* gene is required for sporulation.** Sporulation in yeast cells, initiated upon nitrogen starvation of *MAT***a**/*MAT*α diploid cells, represents a regulated program of differentiation (8). In order to assess whether $N^{\alpha}$ acetylation plays a role in sporulation, two sets of genotype diploid yeast strains (MGD502 and MS) (Table 1), representing wild type (+/+), heterozygous disrupted (+/*aaa1-1*), and homozygous disrupted (*aaa1-1/aaa1-1*) diploid, were used. Neither homozygous (*aaa1-1/aaa1-1*) diploid strain (MS-2a and MGD502-2a) sporulated efficiently (Table 3).

***aaa1* mutants are sensitive to heat shock.** $N^{\alpha}$ acetylation has been suggested to play a role in protecting various proteins against intracellular proteolytic degradation (16, 33). The rate of protein turnover mediated by the ubiquitin-dependent degradation system also has been documented to depend on the presence of a free $\alpha$-$NH_2$ group at the $NH_2$ terminus of model proteins (2, 12), and in yeast cells, polyubiquitin has been demonstrated to be a heat shock protein (9, 41). However, it has not been shown whether $N^{\alpha}$ acetylation of proteins plays a role in resistance to heat shock. Therefore, exponentially growing cells of eight haploid strains (four *aaa1* mutants and four wild type) were heat shocked at 54°C, and the survival percentages were determined at various

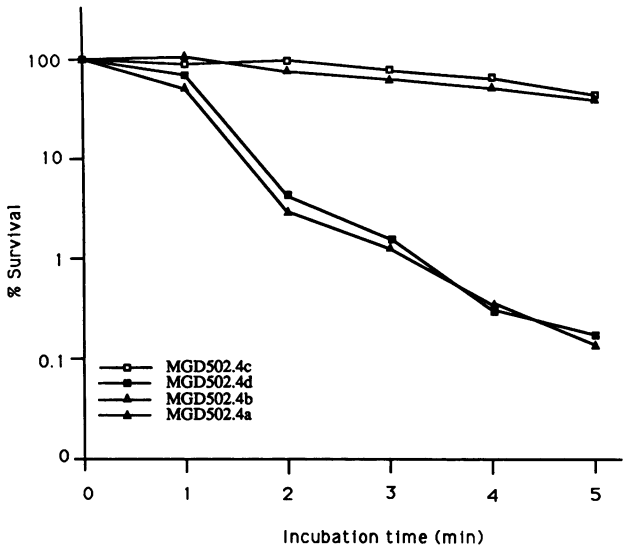



FIG. 3. Heat shock sensitivity of *aaa1* strains. Cells were grown to late log phase in YPD medium, diluted in SD medium, and incubated at 54°C. The survival percentage was determined at each indicated time. The genotypes of the strains are shown in Table 1.

times. *aaa1* strains (MGD502.4a and MGD502.4d) are more sensitive to heat shock than are wild-type strains (MGD502.4c and MGD502.4b) (Fig. 3). Other *aaa1* strains (AB18-a and T3A-a) were also more sensitive than wild-type strains (AB18 and T3A) (data not shown).

***AAA1* gene is required for a-specific mating-type functions.** Haploid *S. cerevisiae* cells occur in two mating types, **a** and α, determined by the *MAT* locus (for a review, see reference 29). Cells of opposite mating types can participate in a mating reaction that results in cell fusion and creation of a diploid cell (3, 37, 43). Several proteins are responsible for the mating process, although it is unclear whether $N^{\alpha}$ acetylation of any of these occurs.

Matings were carried out by gently mixing an *aaa1* strain (MGD502.4d [α], MGD502.4a [**a**], T3A-a [α], or AB18-a [**a**]) with a strain of the opposite mating type. Surprisingly, the *MAT***a** *aaa1* mutant strains (AB18-a and MGD502.4a) did not agglutinate as well as wild-type *MAT***a** cells when mixed with wild-type *MAT*α cells (data not shown). Quantitative mating tests indicated that the mating efficiency of *MAT***a** *aaa1* cells was significantly reduced, although not ablated (Table 4). Two *MAT*α *aaa1* mutants (T3A-a and MGD502.4d) produced α factor at levels similar to those of the wild-type strains (T3A and MGD502.4c) (Fig. 4A, spots a through d). However, two *MAT***a** *aaa1* mutants (AB18-a and MGD 502.4a) (Fig. 4A, spots f and i) produced less **a** factor than the wild-type strains (AB18 and MGD502.4b) (Fig. 4A, spots h and e). *MAT***a** *aaa1* mutants (MGD502.4a) produced at least 30-fold less **a**-factor than the wild-type strains (MGD502.4b) (4B). Similar results were found for AB18-a in comparison with AB-18 (data not shown).

*MAT***a** cells are known to secrete the *BAR1* gene product, so-called barrier activity, which degrades α factor (13, 19, 26, 38), and *MAT***a** *aaa1* mutant strains have only a slight reduction of barrier activity in comparison with wild-type cells and supersensitive cells (Fig. 5). In addition, *MAT***a** *aaa1* mutants (AB18-a and MGD502.4a) failed to arrest in G1 phase when the cells were suspended in YPD containing 1 μM α factor (data not shown).

***AAA1* gene is not regulated by glucose, growth phase, and**

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

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

[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.

**heat shock.** Total RNA was prepared from cells grown in YPG medium at mid-log phase and in YPD medium at early log phase, at mid-log phase, at stationary phase, and after heat shock at 37°C for 2 h. RNA blot analysis was carried out with a random-primed, $^{32}$P-radiolabeled *AAA1* and yeast β-tubulin probes (Fig. 6). There was no effect of glucose repression, growth phase, or heat shock on the levels of transcription of the *AAA1* gene in comparison to levels of the β-tubulin gene (30).

**Expression of *AAA1* gene can complement *aaa1* mutation.** Various *aaa1* mutants were transformed with plasmid pLA1 or pUA1 containing the *AAA1* gene. The MGD502-2a/T transformant (containing pLA1) restored the sporulation deficiency found in MGD502-2a (Table 3). In addition, the introduction of pLA1 into MGD502.4a (*MAT***a** *aaa1-1*) and pUA1 into AB18-ap (*MAT***a** *aaa1-2*) restored the mating efficiency (Table 4). These transformants also expressed **a** mating factor as abundantly as wild type (Fig. 4A, spots g and j). Moreover, the specific growth rates of these transformants were restored, and the cells were able to enter the stationary phase (data not shown).

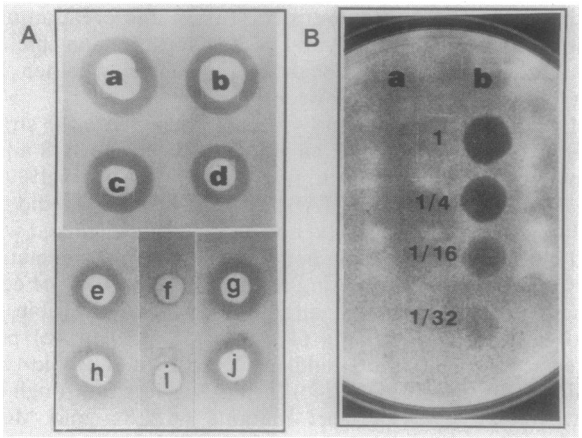



FIG. 4. Mating-type factor expression. (A) Production of mating factor in wild-type and *aaa1* strains. For the α-factor assay, late-log-phase MGD502.4c (*MATα AAA1*) (a), MGD502.4d (*MATα aaa1-1*) (b), T3A (*MATα AAA1*) (c), and T3A-a (*MATα aaa1-1*) (d) were spotted on a lawn of strain F676 (*MAT***a** *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 (*MATα 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 (*MATα sst2-1*) and incubating it for 36 to 48 h at 23°C.

## DISCUSSION

We have found that yeast strains lacking the $N^{\alpha}$-acetyltransferase gene, *AAA1*, grew as smaller, variably sized, and misshapen colonies in comparison with wild-type strains and that cells from these strains budded multiply and abnormally. In addition, *AAA1* was demonstrated to be required for entrance into stationary phase, sporulation, resistance to heat shock, and **a**-specific mating-type functions (i.e., decreased **a**-factor production, decreased barrier activity, and failed G1 arrest with α factor).

Several other genes involved in the cyclic AMP pathway have been found to regulate the G1 arrest in response to nutritional limitation (44). In particular, *RAS2*$^{Val19}$ and *bcy1* cells also show abnormal multibudded cells, failure of starv-

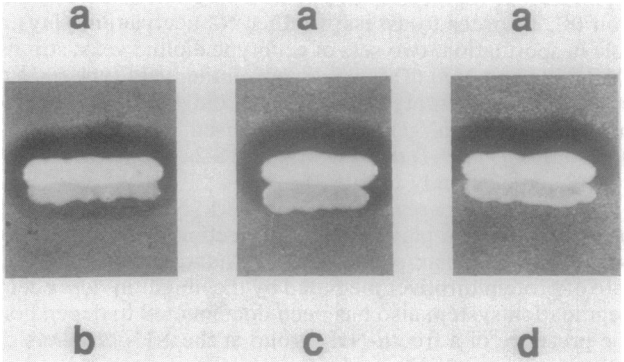



FIG. 5. Barrier activity of wild-type and *aaa1* strains. Cells of strain F676 (*MAT***a** *sst1*) were spread onto a YPD (pH 4.5) plate before the *MAT***a** strains to be tested were streaked. Streaks of barrier-producing cells disturbed the halo zone which was formed by inhibition of the growth of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

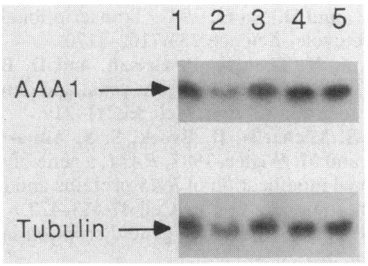



FIG. 6. RNA blot analysis of yeast cells at different growth conditions. Total RNA was prepared from cells grown in YPG medium at mid-log phase (lane 1) or in YPD medium at early log phase (lane 2), mid-log phase (lane 3), or stationary phase (lane 4) and after heat shock at 37°C for 2 h (lane 5). RNA (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.

ing cells to arrest in G1 phase, lack of glycogen accumulation, inability to sporulate, and sensitivity to heat, although neither displays a-specific sterility (27, 44). It is likely that one or more proteins involved in the regulation of cyclic AMP requires $N^{\alpha}$ acetylation for its function.

Perhaps the most remarkable finding concerning the identified phenotypes of the *aaa1* mutants is that *MAT***a** but not *MAT*α *aaa1* mutants mated less efficiently. It has been observed previously that mutations in *STE2*(α-factor receptor) (11, 15), *STE6* (49), *STE14* (L. C. Blair, Ph.D. thesis, University of Oregon, Eugene, 1979; J. D. Rine, Ph.D. thesis, University of Oregon, Eugene, 1979), *STE16* (31) (genes required for a-factor maturation), MFa1, and MFa2 (**a** factor) (28) resulted in a million-fold reduction of mating-type efficiency. In contrast, the *aaa1* mutation resulted in a 1,000- to 2,000-fold reduction of mating-type efficiency. Thus, it is likely that the *aaa1* mutation reduced, but did not abolish, the expression of certain a-specific gene products, as has been observed for the *ard1* mutation (48). Moreover, *ard1* cells also bear all the other phenotypes associated with *aaa1* cells. The *ARD1* gene product has been suggested to act, directly or indirectly, at the *HML* locus and to repress its expression (47). We have also demonstrated that neither *MAT***a** *HML***a** *HMR***a** *aaa1-1* nor *MAT***a** *HML***a** *HMR*α *aaa1-1* strains were mating defective (unpublished data). Therefore, we propose that a protein necessary for repression of the *HML* locus may require $N^{\alpha}$ acetylation in order to be functional. Further, the sequences of the *AAA1* and *ARD1* genes bear no relationship to one another (21, 48), and the relationship between them will require further investigation.

Hershko et al. (12) showed that $N^{\alpha}$-acetylated proteins are degraded by the ATP-dependent ubiquitin system less rapidly than proteins with free N termini, and they suggested that $N^{\alpha}$ acetylation is involved in protection from protein degradation. In addition, induction of the *UBI4* gene (encoding ubiquitin) by heat shock suggested that ubiquitin plays a role in the heat shock response and that its physiological role may be to degrade altered or toxic proteins generated by environmental stress (9, 41). We have demonstrated indirectly that $N^{\alpha}$ acetylation plays a role in resistance to heat shock. However, how many $N^{\alpha}$-acetylated proteins are involved in protection from heat shock and whether an exposed α-$NH_2$ group in one or more of these proteins forms a recognition signal for ubiquitin conjugation and ubiquitin-mediated degradation are at present unknown.

Different levels of acetylation have been observed for several eucaryotic proteins (10, 17, 23, 24, 35, 39, 40). Furthermore, isoenzymes (ADH I and ADH II) have also been shown to differ in their levels of acetylation (18). This differential acetylation may be due to differences in primary structure between the isozymes, a lack of available acetyl coenzyme A, or differences in level of enzyme activity. However, synthetic peptides mimicking residues 1 through 24 of both alcohol dehydrogenase isoenzymes were equally acetylated by the yeast $N^{\alpha}$-acetyltransferase, which suggests that under conditions in which acetyl coenzyme A is in excess, effective acetylation of both isoenzymes should proceed (20). Furthermore, RNA blot analysis reveals that there was no major effect of glucose repression, different growth phase, or heat shock on the transcriptional regulation of the *AAA1* gene.

The expanded usage of these *aaa1* mutants and the *AAA1* gene forms the basis for elucidating the biological function and regulation of $N^{\alpha}$ acetylation in yeast cells.

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## ADDENDUM

After the review of the manuscript, a study describing the identification and characterization of a gene (*NAT1*) affecting $N^{\alpha}$ 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*.

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