A Yeast Polyamine Acetyltransferase*

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An uncharacterized yeast gene has been shown to encode a polyamine acetyltransferase and named PAA1. The recombinant Paa1 protein readily acetylates various polyamines such as putrescine, spermidine, and spermine. paa1 mutants are viable and grow normally under standard conditions. The mutants are sensitive to hydroxyurea, and they are synthetically temperature-sensitive with a rad53-21 mutation. The mutants also show genetic interactions with components of the transcriptional co-activator complex, SAGA, and partially suppress Spt- phenotypes of two spt mutants. These phenotypes suggest that acetylation of polyamines removes them from chromatin and makes the chromatin more accessible. It is known that spermine, but not acetyl spermine, is a precursor in the pathway for synthesis of coenzyme A in yeast. When Paa1 is overexpressed, leading to a lower level of spermine, cells show a growth dependence on either of two downstream compounds in the coenzyme A pathway, pantothenate or β-alanine. This demonstrates that spermine and perhaps other polyamines are the in vivo targets of Paa1.

Polymains such as spermidine, spermine, and putrescine are positively charged small molecules that bind tightly to both RNA and DNA and also to some proteins (1–3). Although polyamines are abundant and ubiquitous components of all cells, relatively little is known about their function. In mammalian cells, there is a positive correlation between rapid growth and high levels of polyamines (4, 5). Furthermore, addition of growth factors to quiescent cells leads to a rapid induction of polyamine synthesis.

Acetylation of polyamines appears to be necessary for their breakdown and export from cells (6). Mammalian spermidine/spermine N\(^+\)-acetyltransferase is a well-characterized cytoplasmic enzyme that catalyzes the rate-limiting step in polyamine catabolism by transferring the acetyl group from acetyl-CoA to the N\(^+\) position of spermidine or spermine (reviewed in Ref. 6). Acetylated polyamines are exported from cells or metabolized by the constitutive flavin adenine dinucleotide-requiring polyamine oxidase (6). In addition to the cytoplasmic polyamine acetyltransferase, mammalian cells also have a nuclear enzyme of unknown function that acetylates the N\(^+\) amino group of spermidine (7). Escherichia coli also has a polyamine acetyltransferase; it can acetylate both the N\(^+\) and the N\(^2\) positions on spermidine (8–10). Mutations in the gene for this enzyme lead to an accumulation of spermidine in the cell and an inhibition of growth. Thus, in both bacteria and mammalian cells, acetylation of polyamines is necessary for their catabolism and/or excretion from cells. The genes for polyamine acetyltransferases have been cloned from both E. coli and mammalian sources, but not from yeast (8, 11). We were unable to find an obvious yeast homolog of either the bacterial or mammalian enzyme by investigation of the yeast genome. Therefore, we examined uncharacterized yeast genes that were members of the GNAT superfamily (12). This family includes histone acetyltransferases and other enzymes that acetylate amino groups on various proteins and even sugars. Here we describe a yeast gene that encodes a polyamine acetyltransferase.

MATERIALS AND METHODS

Plasmids—The PAA1 open reading frame (ORF)‡ was amplified from yeast genomic DNA by PCR and cloned into E. coli expression vector pET28a as an Nhel-Xhol fragment to create pLBS12. This allowed expression of full-length Paa1 with a His tag at the N terminus. The protein was purified from E. coli extracts by Ni\(^2+\) affinity chromatography. It migrated as a 21-kDa protein on SDS-PAGE gels, consistent with its predicted molecular mass. It was dialyzed into 50 mM sodium phosphate, pH 7.2, and stored at −80 °C after addition of glycerol to a final concentration of 10%.

For expression of Paa1 from plasmids in yeast, a BamHI-HindIII PCR fragment was amplified from yeast genomic DNA by PCR and cloned into E. coli expression vector pRS424, which contains a BamHI-HindIII fragment of the yeast paa1 gene. The fragment was cloned into the CEN vector pRS315 to create pLBS23 and into the 2-μm vector pRS425 to create pLBS25. For overexpression in yeast, the PAA1 ORF was cloned as an EcoRI-Xhol fragment into a vector related to pRS423, behind the TEP promoter, to create pLBS21.

Acetyltransferase Assays—The filter assay for polyamine acetyltransferase activity was essentially as described previously (13). Reactions were carried out in 50 μl containing 3 mM polyamine, 100 mM Tris-HCl, pH 7.9, 0.5 μCi of \(^3\)H-acetyl-CoA (5.6 Ci/mmol; Amersham Biosciences) with 0.5–1.0 μg of recombinant Paa1 enzyme. Reactions were incubated at 30 °C for 30 min and terminated by the addition of 10 μl of 1 M hydroxylamine. Samples were boiled for 3 min and centrifuged to remove precipitated protein. Whatman P81 paper filters (1 cm\(^2\)) were spotted with 40 μl of the supernatant, and the filters were air dried and then washed four times with H\(_2\)O and finally with 100% ethanol to remove any remaining \(^3\)H-acetyl-CoA. The filters were dried in an oven, and radioactivity was determined in a liquid scintillation counter.

A paper chromatographic assay for polyamine acetyltransferase activity was also employed (15). The conditions were the same as those described above, except that the total volume was 25 μl and 0.125 μCi of [1,\(^14\)C]acetyl-CoA (57 Ci/mmol; Amersham Biosciences) was used. After centrifugation, 15 μl of the supernatant was spotted on Whatman No. 1 paper. Chromatography used a solvent consisting of 1-butanol/water/pyridine/glacial acetic acid (20:10:5:10) for 3–4 h. To visualize the compounds, the paper was sprayed with a ninhydrin solution (100 mg of ninhydrin + 70 μl of ethanol + 2.9 μl of 2,4,6-collidine + 21 μl of acetic acid). The paper was placed at 90 °C for 10 min so that the positions of the polyamines could be visualized. The paper was then exposed to film or a phosphorimager so that the amount of \(^14\)C-labeled acetylated polyamine could be determined. A phosphorimager was used to quantify the results shown in Fig. 6B.

* This work was supported by National Institutes of Health Grant GM28220. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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The thin layer chromatography assay for tryptamine acetyltransferase activity followed the previously published procedure (16). A phosphorimager was used to quantitate the results.

**Yeast Strains and Growth Media—**The yeast strains used in this study are listed in Table 1. Deletion mutants were constructed by transformation using methods described by Longtine et al. (14). The gene mutants were obtained from Fred Winston, and the gcn5 mutants were obtained from Steve Elledge. Standard yeast peptone dextrose and synthetic complete media were used for most experiments. A pantetheine-free medium was used for the experiment shown in Fig. 7 (17).

### RESULTS

Several previously uncharacterized members of the yeast GNAT superfamily (12) were expressed in *E. coli*, and the recombinant proteins were purified and assayed for their ability to acetylate polyamines. One of them, the product of yeast ORF YDR071c, had robust activity and was able to acetylate putrescine, spermine, and spermine (Fig. 1A). We called the gene for this protein *PAA1* (polyamine acetyltransferase). Paa1 protein also acetylated N²- or N⁶-acetyl spermidine and N¹-acetyl spermine, indicating that the enzyme could diacetylate polyamines (Fig. 1A). Diacetylation occurred with low efficiency, however. For example, putrescine was only monoacetylated under our standard assay conditions, but the diacetylated product could be detected if N²-acetyl putrescine was used as the substrate (Fig. 1B). The acetylation activity of Paa1 appeared to be specific for polyamines because the enzyme could not acetylate histones, nucleosomes, or the high mobility group-like proteins, Nhp6a, Nhp6b, Hmo1, Hmo2, or Abf2 (data not shown).

In order to learn more about the function of Paa1, a mutant with a complete deletion of the ORF was constructed. The mutant was viable and grew at a normal rate at 25 °C, 30 °C, and 37 °C in both rich and synthetic medium and also in medium containing galactose or glycerol as a carbon source. Many mutants in genes for HATs or other transcriptional co-activators are defective in transcription of the INO1 gene, leading to an inositol auxotrophy (18). However, the *paa1* mutant grew on a medium lacking inositol. The mutant also had no silencing defect at the *HM loci* or the telomeres.

The *paa1* mutant was somewhat sensitive to hydroxyurea (Fig. 2A). This sensitivity was complemented by introducing a plasmid expressing Paa1 under its own promoter on either a CEN or a 2-μm plasmid (Fig. 2B). The sensitivity to hydroxyurea suggested that the *paa1* mutant might be slightly defective in DNA replication. We therefore checked whether the mutant might be slightly defective in transcriptional induction (19).

### Table I

| Strain     | Genotype                                      |
|------------|-----------------------------------------------|
| W303-1a    | MATα ade2-1 his3-11,15 leu2-3,112 can1-100 ura3-1 trp1-1 |
| W303-1b    | MATα ade2-1 his3-11,15 leu2-3,112 can1-100 ura3-1 trp1-1 |
| YLS81      | W303-1a fms1::kanMX4                          |
| YLS83      | W303-1a paa1::kanMX4                         |
| YLS84      | W303-1b paa1::kanMX4                         |
| Y301       | MATα rad53-21 ade2-1 his3-11,15 can1-100 ura3-1 trp1-1 |
| YLS818     | Y301 paa1::his5⁻                             |
| YLS20      | W303-1b gen5::his5⁻                           |
| YLS23      | W303-1a gen5::his5⁻ paa1::kanMX4             |
| FW942      | MATα his 4-9178 lys2-173R2 ura3-52            |
| YLS29      | FW942 paa1::kanMX4                           |
| FW1259     | MATα spit15-21 his 4-9178 lys2-173R2 trp1Δ1 ura3-52 |
| YLS30      | FW1259 paa1::kanMX4                          |
| FW1342     | MATα spit8-308 his 4-9178 leu2-3,112 ura3-52  |
| YLS34      | FW1342 paa1::kanMX4                          |
| FW1087     | MATα spit7-217 lys2-173R2 trp1Δ1 cpyR1        |
| YLS33      | FW1087 paa1::kanMX4                          |

**FIG. 1.** Recombinant Paa1 has polyamine acetyltransferase activity. A, bar graph of activity with various polyamines, using the filter assay described under “Materials and Methods.” B, autoradiogram of a thin layer chromatogram showing activity of Paa1 with putrescine and less activity with acetyl putrescine. PAA1 would exhibit a genetic interaction with RAD53, a gene crucial for the S-phase checkpoint. Indeed, a *paa1 rad53-21* double mutant was unable to grow at 37 °C, whereas both single mutants could (Fig. 3). Interestingly, a recent report describing a tandem affinity tag purification of Rad53-containing protein complexes identified Paa1 (at that time an uncharacterized product of ORF YDR071c) as a member of a Rad53 complex (19).

As described under “Introduction,” polyamines bind tightly to nucleic acids. Furthermore, it was previously demonstrated that polyamine depletion in yeast partially suppressed a *gcn5* mutation, suggesting that polyamines play a role in repressing transcription (20). Because acetylation of polyamines leads to their breakdown via oxidation by polyamine oxidase, at least in mammalian cells, we reasoned that a *paa1* mutant would have more polyamines bound to chromatin and hence might be somewhat defective in transcriptional induction. As mentioned above, the mutant grew normally on rich or poor carbon sources, and it could grow in inositol-free medium, so it clearly
did not have a major transcriptional defect. Several genetic interactions suggested that PAA1 does indeed play a role in transcription, however. Such interactions were observed with GCN5 and SPT8, genes for two proteins present in histone acetyltransferase complexes important for transcriptional activation. A paa1 gcn5 double mutant could not grow on rich medium at 37 °C, whereas each single mutant could (Fig. 4A). A similar interaction was observed with a paa1 spt8 double mutant; again, the single mutants could grow at 38 °C, whereas the double mutant could not (Fig. 4B). Gcn5 is a histone acetyltransferase present in several protein complexes important for transcriptional activation (reviewed in Ref. 21). Spt8 is a component of one of these complexes, the SAGA complex. A genetic interaction was also observed with the SPT15 gene that encodes the essential general transcription factor TATA-binding protein. A paa1 spt15-21 double mutant was temperature-sensitive, whereas the single mutants grew normally at the high temperature (Fig. 4C).

We tested whether a paa1 mutation would lead to an Spt− phenotype by moving the mutation into a strain, FW942, carrying Ty1 δ insertions in the HIS4 and LYS2 genes (22). The paa1 derivative of FW942 had the same His− Lys+ phenotype as FW942, showing that the mutation does not cause a Spt− phenotype (Fig. 5). The paa1 mutation was able to partially suppress the Spt− phenotype of the spt15-21 and spt7-217 strains, however. These strains also have Ty1 δ insertions in the promoters of the HIS4 and LYS2 genes, causing a His+ Lys− phenotype (Fig. 5). Addition of the paa1 mutation causes each of the two strains to become Lys− but remain His+ (Fig. 5). That is, the paa1 mutation suppressed the Spt− phenotype at the LYS2 locus but not at the HIS4 locus. This suppression of the lys2-173R2 δ insertion allele but not the his4 δ insertion...

FIG. 2. Hydroxyurea (HU) sensitivity of paa1 mutants. A, 10-fold serial dilutions of wild-type (WT; W303-1a and W303-1b) and paa1 (YLBS3 and YLBS4) cells on synthetic complete (SC) medium without and with 150 mM hydroxyurea. B, centromere or 2-μm plasmids expressing Paa1 complement the hydroxyurea sensitivity of a paa1 mutant (YLBS3).

FIG. 3. Synthetic temperature sensitivity of paa1 rad53-21 double mutants. Strains used were W303-1b (WT), Y301 (rad53-21), and their respective paa1 derivatives, YLBS4 and YLBS18.

FIG. 4. Synthetic temperature sensitivity of paa1 with various genes involved in transcription. A, paa1 gcn5 double mutant. The strains used were the four haploid segregants of a tetratype tetrad from a cross of YLBS20 (gcn5) with YLBS3 (paa1). B, paa1 spt8–308 double mutant. The strains used were FW942 (WT), FW1342 (spt8), and their respective paa1 derivatives, YLBS29 and YLBS34. C, paa1 spt15-21 double mutant. The strains used were FW942 (WT), FW1259 (spt15), and their respective paa1 derivatives, YLBS29 and YLBS30.

FIG. 5. Partial suppression of the Spt− phenotype of two spt mutations, spt7-217 and spt15-21. These two spt mutations cause a His+ Lys− phenotype in a strain with the appropriate Ty δ insertions (22). The presence of the paa1 mutation leaves the His+ phenotype unchanged but causes a Lys− phenotype. The plates were incubated at 30 °C for this experiment. Strains used were FW942 (SPT7), FW1087 (spt7), FW1259 (spt15), and their respective paa1 derivatives, YLBS29, YLBS3, and YLBS30.
is characteristic of weak suppressors of spt mutations. In summary, the paa1 mutation partially suppresses the Spt phenotype of two different mutants carrying the spt15-21 and spt7-217 alleles, respectively.

While our work on Paa1 was in progress, a study was published reporting that this protein was a homolog of the mammalian arylalkylamine N-acetyltransferase that converts serotonin to acetylserotonin, a precursor of melatonin (16). The authors reported that the recombinant yeast protein was able to acetylate arylalkylamines such as tryptamine and serotonin, and they suggested that the enzyme was an authentic arylalkylamine N-acetyltransferase. The specific activity was significantly lower and the substrate specificity was somewhat broader than that of the authentic mammalian enzyme, however. In view of this report, we compared the specific activity of the Paa1 enzyme on various polyamines versus tryptamine. As can be seen in Fig. 6, Paa1 indeed had significant ability to acetylate tryptamine, although the specific activity was somewhat higher on polyamines, particularly putrescine.

In view of these results we did an experiment to test whether polyamines, rather than arylalkylamines, were the true in vivo substrate for Paa1. It is known that spermine is oxidized in yeast by a polyamine oxidase that is the product of the FMS1 gene (17, 23, 24). One of the products of the oxidation is 3-aminopropanal, which is then converted to β-alanine. The β-alanine is a precursor of pantothenate, which is in turn a precursor of coenzyme A. In yeast, this is the major pathway for synthesizing coenzyme A, and thus fms1 mutants require β-alanine or pantothenate for growth (17) (Fig. 7A). When we introduced a multicopy plasmid overexpressing Paa1 from a strong promoter into yeast, there was a significant inhibition of growth on a medium lacking pantothenate (Fig. 7B). This inhibition was only seen on medium lacking pantothenate and not on rich or synthetic complete medium. In contrast, when β-alanine was added to the medium lacking pantothenate, not only was an fms1 mutant able to grow (as expected), but the growth of the wild-type strain with the Paa1-overproducing plasmid was greatly improved. That is, overproduction of Paa1 in the cell greatly inhibited growth, but only in medium lacking pantothenate or β-alanine.

Our interpretation of these results is that when there is excess Paa1 in the cell, more putrescine, spermidine, and spermine are acetylated, leading to a lower amount of spermine in the cell (Fig. 7A). Most of the polyamines and their acetylated derivatives can be oxidized by Fms1, but only spermine oxidation yields 3-aminopropanal, the precursor of β-alanine and hence coenzyme A (17, 23). The depletion of spermine by excess Paa1 leads to the growth defect seen in the absence of β-alanine or pantothenate. This experiment provides strong evidence that spermine is an in vivo substrate of Paa1.

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2 F. Winston, personal communication.
We also tried a more direct experiment to test whether Paa1 acetylated spermine in vivo. We exposed yeast cells to \[^{14}C\]spermine for 16 h, extracted polyamines, and looked for \[^{14}C\]acetyl spermine by thin layer chromatography. We compared \(PAA1\) and \(paa1\) strains, both with \(spe4\) and \(fms1\) mutations. Spe4 catalyzes the final step in spermine synthesis, and Fms1 breaks down spermine (Fig. 7A), and thus a strain lacking those enzymes should increase the intracellular specific activity of the \[^{14}C\]spermine. Nevertheless, we were unable to detect any \[^{14}C\]acetyl spermine in the wild-type \(PAA1\) strain, even when it was carrying the multicopy plasmid overexpressing \(PAA1\) described above (Fig. 7B). Another laboratory also has been unable to detect intracellular acetyl spermine in yeast.\(^3\) The concentration of acetyl spermine is also very low in mammalian cells (25). It must either be rapidly broken down or secreted from the cell.

**DISCUSSION**

Although the genes for polyamine acetyltransferases had been identified in both mammalian cells and bacteria, no obvious homolog was apparent in the yeast genome. We therefore examined uncharacterized members of the GNAT superfamily, consisting of various \(N\)-acyetyltransferases, to find one that acetylated polyamines. The product of yeast ORF \(YDR071c\) clearly had such an activity (Fig. 1) and hence was named \(PAA1\). The Paa1 protein acetylates putrescine, spermidine, and spermine in vitro. It also can acetylate the monoacetylated derivatives of these compounds, leading to the diacetylated products (Fig. 1). It is not clear whether all or only some of these polyamines are in vivo substrates, but, as discussed below, spermine definitely is a substrate.

Are spermine and other polyamines the only in vivo substrates of Paa1? The recombinant enzyme could not acetylate histones or various small basic proteins associated with chromatin, suggesting that proteins are not substrates. However, as described above, while our work on \(PAA1\) was under way, a report was published that the recombinant protein acetylated arylalkylamines such as tryptamine and serotonin (16). We were able to confirm this observation (Fig. 6). However, the experiment shown in Fig. 7 strongly suggests that Paa1 acts on polyamines in vivo. In yeast, the pathway for the biosynthesis of coenzyme A involves the oxidation of spermine to 3-aminopropanal and spermidine by Fms1, followed by the conversion of 3-aminopropanal to \(\beta\)-alanine (17). The \(\beta\)-alanine is a precursor of pantothenate, which in turn is a precursor of coenzyme A (Fig. 7A). Overexpression of Paa1 would be expected to cause a decrease in the concentration of unacetylated spermine and hence lead to less coenzyme A. Indeed, that is what was observed. The overexpression plasmid led to growth inhibition in a medium lacking pantothenate, but not in rich media that have large amounts of polyamines and pantothenate. This growth inhibition could be reversed by adding pantothenate or \(\beta\)-alanine to the medium (Fig. 7B). These results indicate that spermine is a substrate of Paa1 in yeast, and perhaps other polyamines are also substrates.

We think it is unlikely that arylalkylamines are in vivo substrates of Paa1. First, there is no evidence that serotonin exists in yeast. Furthermore, there are no homologs in yeast of the two mammalian enzymes that convert tryptophan to tryptamine and then to serotonin. Thus, there is no reason to think that yeast needs an arylalkylamine acetyltransferase. The in vitro activity of Paa1 on arylalkylamines is probably due to the similarity of the ethyl amine moiety present on arylalkylamines to the propyl and butyl amines present on polyamines.

Polyamines bind strongly to nucleic acids through electrostatic interactions and are thought to condense chromatin through these interactions (26). The effects of polyamines on chromatin are likely to be complicated, however. For example, in vitro studies show that histone acetylation of nucleosomes, generally associated with open chromatin and transcriptional activation, is stimulated by low concentrations of polyamines but inhibited at high concentrations of polyamines (27). The interpretation of this result is that at low concentrations, the polyamines compete with the binding of the histone N-terminal tails to DNA in chromatin, thus freeing the tails for acetylation. Thus, binding of polyamines to chromatin may, under some circumstances, actually open the chromatin instead of condensing it.

Evidence for a role in transcriptional repression by polyamines in yeast comes from a search for suppressors of the transcription defect of a yeast \(gcn5\) mutant. Gen5 is a transcriptional co-activator that is the catalytic subunit of several histone acetyltransferase complexes (21). Pollard et al. (20) showed that yeast \(spe1\) mutations or overproduction of ornithine transcarbamoylase, both of which reduced the level of

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\(^3\) H. Tabor, personal communication.
polyamines, suppressed the transcription defects of *gcn5* mutants. This suggested that polyamines act as repressors of transcription, presumably by binding tightly to the chromatin and thus counteracting the effects of transcriptional activation by histone acetylation.

Acetylation of polyamines reduces or removes their positive charge and thus would be expected weaken their affinity for DNA. It is known that acetylation of polyamines in mammals leads to their breakdown and export from the cell (6). Therefore, it is predicted that *paa1* mutants should lead to a higher level of unacetylated polyamines on chromatin, and this should affect transcription and replication. The various genetic interactions we observed are consistent with this view. We found that combining *paa1* mutations with *gcn5*, *spt8*, and *spt15* mutations greatly increased the growth defect caused by those mutations (Fig. 4). *Gcn5* and *Spt8* are subunits of histone acetyltransferase complexes that act as transcriptional co-activators, and *SPT15* codes for the general transcription factor TATA-binding protein. Therefore, the results suggest that transcriptional activation is even more defective in the absence of polyamine acetylation than when these transcription factors alone are absent from the cell. The partial suppression of the Spt  phenotype of *spt7-217* and *spt15-21* mutants by deletion of *PAA1* (Fig. 5) also suggests a role for *Paa1* in regulating transcription.

The increased sensitivity of *paa1* mutants to hydroxyurea (Fig. 2) could also be due to a greater concentration of unacetylated polyamines bound to chromosomal DNA. Hydroxyurea inhibits ribonucleotide reductase and thus slows down replication fork movement and induces a RAD53-dependent checkpoint. The additional burden of a high level of polyamines bound to the DNA could slow replication even more, leading to the increased sensitivity to hydroxyurea. Consistent with this is the observation that the *paa1* mutation greatly exacerbated the checkpoint defect of a *rad53-21* mutation (Fig. 3).

In conclusion, we have identified a gene, *PAA1*, that encodes a polyamine acetyltransferase. The phenotypes associated with the *paa1* mutant suggest that the role of Paa1 in yeast is to regulate the levels of polyamines on chromosomal DNA. Acetylation removes them from DNA, thus changing chromatin structure and hence affecting both transcription and replication.

**Acknowledgments**—We thank Craig Peterson and Aaron Neiman for advice and Fred Winston and Steve Elledge for strains.

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