Identification of a Gene Encoding a Yeast Histone H4 Acetyltransferase*

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A collection of yeast temperature-sensitive mutants was screened by an enzymatic assay to find a mutant defective in the acetylation of histone H4. The assay used a fractionated cell extract and measured acetylation of a peptide corresponding to amino acids 1–28 of H4. There are at least two activities in this fraction that acetylate the peptide. A mutation, hat1-1, that eliminates one of the activities was identified and mapped to a locus near the centromere of chromosome XVI. The HAT1 gene was cloned and found to encode a protein of 374 amino acids. Analysis of the peptide used in the assay demonstrated that the HAT1 enzyme acetylates lysine 12 of histone H4. hat1-1 mutants have no obvious growth defects or phenotypes other than the enzyme defect itself. The HAT1 protein expressed in Escherichia coli gave histone acetyltransferase activity in vitro, demonstrating that HAT1 is the structural gene for the enzyme.

The N-terminal tails of all four core histones are subject to several post-translational modifications including the reversible acetylation-deacetylation of ε-amino groups of specific lysine residues. Three distinct roles have been proposed for histone acetylation. One is in gene activation and the regulation of transcription (1, 2). A clear correlation exists between a high degree of acetylation and a high level of transcription (3) but whether the acetylation is a cause or an effect of gene activation is unclear. Another role proposed for histone acetylation is during histone synthesis and deposition. It is thought that a specific transient acetylation of histones may be necessary for their deposition during DNA replication (4, 5). Finally, histone acetylation appears to play a role in histone replacement during germ cell maturation (6).

Histone acetyltransferases have been purified and characterized from a number of mammalian sources (7, 8) as well as yeast (9, 10). Fractionation studies suggest that there are at least two such enzymes, one cytoplasmic that acetylates free histones, and one nuclear that perhaps acetylates histones in chromatin (11). It is still not clear, however, whether different enzymes exist for each histone or even for specific lysine residues on a given histone. To date, no histone acetyltransferase genes or mutants have been reported. In order to learn more about the in vivo roles of histone acetylation and about the specificities of the enzymes, we looked for a yeast mutant defective in this process. A collection of yeast temperature-sensitive mutants was screened by an in vitro enzymatic assay that measured acetylation of an H4 peptide corresponding to amino acids 1–28. In this report, we describe the identification of a mutant specifically defective in the acetylation of lysine 12 in this peptide. The cloning of the wild type gene is also described.

Materials and Methods

Yeast Strains, Media, and Strain Manipulations—The following yeast strains were used: W303-1a: MATα ade2 ura3 leu2 trp1 his3 can1; W303-1b: MATα, otherwise isogenic to W303-1a; W303-3: diploid from W303-1a and W303-1b; SK56: isogenic to W303-1a plus hat1-1; TRP1; SK57: isogenic to W303-1b plus hat1-2; TRP1; A364a: MATα ade2 ade2 ura1 tyr1 lys2 his3 gal1. (This is the parent strain for the collection of temperature-sensitive mutants.) The four centromere mapping strains CSH87L, CSH89L, X3144-11A, and X3382-3A have been described (12). Yeast cells were grown at 30°C in YPD or SC medium (13) plus appropriate supplements unless otherwise stated. Strain constructions, genomic DNA isolation, sporulation, tetrad dissection, and analysis were performed as described (13). The hat1-1 mutation was followed through genetic crosses by the HAT1 enzyme assay. Yeast transformations were carried out by the lithium acetate method (14).

Preparation of Yeast Cell Extracts—Yeast cells were grown at 23°C in 20–40 ml of liquid medium to a cell density of 2–8 × 10⁷ cells/ml, shifted to 37°C for 1 h, spun down, washed with 5 ml of H2O, and resuspended in 200 μl of 20 mM Tris-HCl, pH 7.5, 1 mM EDTA, pH 8.0, 500 mM KCl, 10% glycerol, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. Cell suspensions were transferred to microcentrifuge tubes containing 100 μl glass beads (300-μm diameter), chilled, and sonicated for 12 s three times using a Heat Systems Ultrasonics cell disrupter with a microtip at 2.0 output. After centrifugation for 15 min at 30,000 × g, the supernatants were dialyzed into buffer A (20 mM Tris-HCl, pH 8.3, 100 mM NaCl, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 5 mM 2-mercaptoethanol) using a microdialysis chamber (Bethesda Research Laboratories).

Fractionation of Extracts—The dialyzed extracts were fractionated in small batches in microcentrifuge tubes by loading 175 μl of extract on 200 μl of Q-Sepharose FF resin (Pharmacia), equilibrated with buffer A, and mixed gently for 15 min at 4°C. After the resin had settled, the supernatant was discarded, washed with 400 μl of buffer A, and eluted with 200 μl of buffer B (same as buffer A but 625 mM NaCl). The supernatant, referred to as the Q380 fraction, was used in the histone acetyltransferase (HAT) assays. Protein concentrations were determined by a microassay (Bio-Rad) using bovine serum albumin as a standard; ~5 μg of protein were used per assay.

Histone Acetyltransferase Assay—The assays were carried out in 50 μl of 75 mM Tris-HCl, pH 8.7, 0.1 mM EDTA, pH 8.0, and a final concentration of 150 mM NaCl. The peptide concentration in the assay was 50 μM, and [3H]acetyl coenzyme A was added to a final concentration of 3.0 μM at 2.3 Ci/mmol. The reaction mixture was preincubated for 30 s at 37°C, the Q380 fraction was added, and the incubation continued at 37°C for 15 min. The reaction was stopped by the addition of 1.5 ml of cold 20% trichloroacetic acid. Precipitated peptide and proteins were collected on glass fiber filters (Schleicher & Schuell), washed twice with 5% trichloroacetic acid, once with methanol, dried, and counted in a scintillation counter.

Amino Acid Sequence Analysis—In order to obtain enough peptide

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This paper is dedicated to the memory of Hal Weintraub.

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The abbreviations used are: HAT, histone acetyltransferase; kb, kilobase(s); bp, base pair(s).
for sequence analysis, several HAT assays of an extract were pooled. Reactions were terminated by incubating them at 65 °C for 5 min instead of by trichloroacetic acid precipitation. The samples were bound to Immobilon PVDF membranes (Millipore) and sequenced on an automatic protein Sequencer. The eluates from each cycle were collected, dried, and counted for 10 min in a scintillation counter.

Cloning, Sequencing, and Null Mutation—The preparation of λ-DNA and cloning of the genomic inserts from the λ-DNA into the shuttle vector pBM2384 were done as described (15). Subclones from the initial plasmid, p5358, were cloned into YEp352 or M13mp18 using standard protocols (16). Nucleotide sequence was determined from M13 single-stranded DNA by the dideoxy method. The hat1-2:TRP1 null mutant plasmid was generated by cloning the EcoRI-BglII fragment from the upstream region of the HAT1 gene into the EcoRI and BamHI of the disruption vector pRS304 (17), and the Stu-NruI fragment from the 3′ end of the gene into a blunt-ended XhoI site of the same vector. The resulting plasmid, p52K, was linearized with Sall, transformed into a wild type diploid (W303), which after sporulation and dissection gave rise to strains SK56 and SK57. The same plasmid was also used to transform strain MX1-4c, which resulted in strain SK55. The correct disruption vector pRS304 (17), and the Stu-NruI9-BglI10-EcoI11 of the correct gene replacements were verified by Southern blot. The hat1-2:TRP1 null mutation deletes 50 bp upstream of the HAT1 open reading frame (ORF) as well as amino acids 1–266.

HAT1 Expression in Escherichia coli—The HAT1 gene was cloned into the T7 expression vector pET3b (18) to yield pSTT21. This plasmid encoded a fusion protein consisting of the first 11 amino acids of T7 gene 10 protein, 4 linker amino acids, and the entire 374 amino acids of HAT1, all downstream of a T7 RNA polymerase promoter. E. coli strain BL21-DE3 containing pSTT21 was grown in LB plus ampicillin medium, induced with isopropyl-1-thio-D-galactopyranoside for 2 h, and harvested. Cells were resuspended in 200 μl of cold buffer B, sonicated, and centrifuged for 1 min in a desk top centrifuge. The supernatant was used directly for HAT assays.

RESULTS AND DISCUSSION

Identification of the hat1-1 Mutant—The hat1-1 mutant was found by screening a collection of temperature-sensitive (ts) yeast mutants (19, 20) for histone H4 acetyltransferase activity in vitro. Previous studies in our laboratory (21) as well as published partial purification protocols for acetyltransferases from yeast suggested the presence of more than one such activity in yeast (10). Since multiple activities contributing to an in vitro assay might obscure the identification of a mutation in only one activity, we attempted to gain specificity by making two improvements: first, instead of detecting the acetyltransferase activity in crude extracts, we fractioned the extracts over a Q-Sepharose anion exchange resin and assayed a high salt eluate (this fraction is referred to as Q380); second, we used as a specific substrate a peptide corresponding to amino acids 1–20 of yeast histone H4 (aa 1–20) with an N-terminal tritylbutoxycarbonyl group at its N terminus (H4 aa 81–20) in Table I. Both strains exhibit a slight reduction in activity compared to the longer, unblocked H4 aa 1–28 peptide, but the hat1-1 mutant still exhibits a 40% reduction in activity compared to wild type with this substrate. We attribute the overall reduction in HAT activity in both strains with the blocked peptide substrate to a lower affinity of the HAT enzyme(s) for this substrate. As another test for the specificity of the assay, we used as a substrate a 21-amino acid peptide, corresponding to the N terminus of histone H3 (kindly provided by D. Allis, Syracuse University). The wild type and hat1-1 strains showed the same amount of activity with this substrate (Table I), but this amount corresponds to only 15% of the activity found with the 28-amino acid H4 substrate. Apparently, the Q380 fraction of the yeast extract contains only a minor activity that recognizes the H3 substrate, but this activity is not affected by the hat1-1 mutation. A different fraction of the yeast extract, containing much more acetylation activity specific for H3, also is unaffected by the hat1-1 mutation. The Q380 subfraction used here seems enriched for an H4 specific activity.

HAT1 Is a Heat-sensitive Acetyltransferase Activity—The hat1-1 mutant still showed 60% HAT activity when compared to a wild type strain (Table I). This remaining activity could be attributed either to a leaky phenotype of the hat1-1 allele or to a second activity that is not affected by the mutation. To distinguish between these two possibilities, we examined the heat inactivation profile of the HAT activity by subjecting the Q380 fraction to 45 °C for various periods of time. Fig. 1 shows that the wild type activity has a biphasic heat inactivation profile, with about 25% of the activity being rapidly inactivated and the remainder inactivated more slowly. HAT assays were performed as described under “Materials and Methods” with Q380 fractions from wild type and hat1–1 strains. The peptide substrates used correspond to: yeast histone H4 amino acids 1–28 (H4 aa 1–28); yeast histone H4 amino acids 1–20 with an N-terminal tritylbutoxycarbonyl group (H4 aa 81–20); Tetrahymena histone H3 amino acids 1–21 (H3 aa 1–21). The activity for wild type on each of the H4 peptides is set at 100%.

| Strain | Substrate  | Specific activity | % activity |
|--------|------------|-------------------|-----------|
| Wild type | H4 aa 1–28 | 2342 | 100 |
| hat1–1 | H4 aa 1–28 | 1332 | 64 |
| Wild type | H4 aa 81–20 | 1499 | 100 |
| hat1–1 | H4 aa 81–20 | 879 | 66 |
| Wild type | H3 aa 1–21 | 308 | 100 |
| hat1–1 | H3 aa 1–21 | 357 | 100 |

Fig. 1. HAT activity after heat inactivation of Q380 fractions from wild type and a hat1 mutant. Q380 fractions prepared from a wild type strain (●) and a hat1-1 mutant (○) were incubated at 45 °C for the indicated times and then assayed for HAT activity in the standard assay.

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remaining 75% being inactivated much more slowly. On the other hand, all the activity remaining in the mutant (60% of the initial wild type activity) is very heat-resistant. Reproducibly, the activity remaining in the mutant after heating is always lower than that remaining in the wild type fraction (Fig. 1). We interpret these data to show that the Q380 fraction has at least two HAT activities, one heat-sensitive and one or more heat-resistant. The hat1 mutant lacks the heat-sensitive activity and also appears to have partially lost the heat-resistant activity. Perhaps the HAT1 protein is a component of two different HAT enzymes, one heat-sensitive and one heat-resistant. The Q380 fraction clearly contains a significant heat-resistant HAT activity unaffected by the hat1 mutation.

The HAT1 Activity Acetylates Lysine 12 of Histone H4—In order to determine which lysine residues of the H4 peptide were being acetylated, a standard assay with [3H]acetyl coenzyme A in the standard HAT assay, using either the wild type (○) or the hat1-1 mutant Q380 fraction (□) was performed. The peptide was subjected to microsequence analysis, and the amount of radioactivity in each cyde (amino acid) was determined. Since the amount of radioactivity in each of the two samples differed (wild type, 4107 cpm; mutant, 1022 cpm), the counts/min were arbitrarily normalized to be equal at amino acid 8. No matter how the results are graphed, they clearly demonstrate that the hat1 mutant is deficient at acetylation of lysine 12.

Cloning the HAT1 Gene—Analysis of the initial crosses between the hat1-1 mutant and a HAT1 strain indicated that the hat1-1 mutation was centromere-linked. This conclusion was based on the observation that there was a paucity of tetratype tetrads between hat1-1 and trpl, a centromere-linked marker on chromosome IV. In order to localize the hat1-1 mutation to a particular chromosome, we crossed the hat1-1 mutant with several mapping strains carrying many centromere-linked markers. In these crosses, the hat1-1 mutation was followed by the enzymatic assay. The results showed linkage of hat1-1 to aro7, a marker near the centromere and on the right arm of chromosome XVI. A three-factor cross involving hat1, aro7, and rad1, a centromere-linked marker on the left arm of chromosome XVI, mapped the hat1 mutation to the interval between rad1 and aro7, close to the centromere (data not shown). Next, we obtained three overlapping λ phages containing yeast genomic DNA from the region spanning centromere XVI (24). The genomic inserts from these phages were introduced into a specialized yeast shuttle vector developed for this purpose (15). We used the 2μ plasmid pBM2384 for these constructions to avoid generating a plasmid with two centromeres, since one of the phages contained centromere XVI. Q380 fractions from a hat1-1 mutant carrying a plasmid containing the genomic insert from λ phage p5538 (p5538) restored activity to the mutant to about 90% of the wild type level (data not shown). A 6-kb Sall subclone from p5538 also restored activity to the mutant. We determined the DNA sequence of short stretches from this subclone and aligned them to a stretch of 10 kb of preliminary chromosome XVI DNA sequence, kindly provided to us by H. Bussey, McGill University. ORFs within the 6-kb Sall fragment were identified and used to direct further subcloning. A subclone with a 2.3-kb Stul-EcoRI fragment (p160RF4), containing only one ORF, was found to restore HAT activity to the mutant. We named the gene corresponding to this ORF HAT1. The DNA sequence of HAT1 and the surrounding region has recently been deposited in GenBank™ as part of the yeast genome sequencing project (accession number Z48493). Analysis of the DNA sequence near HAT1 revealed that the gene is immediately adjacent to the centromere on the left arm of chromosome XVI, with its direction of transcription toward the centromere. The 374-amino acid sequence predicted for the HAT1 protein is shown in Fig. 3. The protein has an unusually large number (23) of phenylalanine residues and a molecular mass of 44 kDa. A search of the GenBank™ data base using the Blast algorithm revealed no significant homology between HAT1 and other proteins. Further analysis revealed that this HAT1 does show homology to a bipartite consensus sequence found previously in a group of N-terminal acetyltransferases (23). The two regions of homology to the consensus are underlined in the HAT1 sequence shown in Fig. 3. We speculate that these regions of homology may constitute an acetyl coenzyme A binding site.

A hat1-2 null mutant was constructed by deleting the ORF from amino acids 1-266 plus an additional upstream 50 bp of 5'-untranslated region and replacing it with the selectable marker TRP1. Strains carrying the hat1-2:TRP1 allele are viable and show the same amount of HAT activity (60% of wild type) as the originally identified hat1-1 allele (data not shown). This result confirmed that the residual activity seen in hat1-1 mutants is caused by a second activity and is not due to a leaky allele. The hat1-2 null mutant has no obvious phenotype; thus far, we have not found any differences between the two hat1 alleles in in vitro assays or in other phenotype studies.

HAT1 Expression in E. coli—The HAT1 gene was cloned into a T7 expression vector (18) and transformed into a suitable E. coli strain (see "Materials and Methods"). After induction, a large amount of soluble HAT1 protein was produced, as judged by the appearance of a band of the expected mobility on an
SDS-polyacrylamide gel (data not shown). Extracts prepared from these cells had very high levels of HAT activity (Fig. 4). Even a 1:1000 dilution of the extract had detectable activity. A control extract from an E. coli strain without the HAT1 plasmid had no activity. These results show that HAT1 is the structural gene for the enzyme and that no other subunits are required for activity.

In summary, we have identified a mutant and cloned the gene (HAT1) for a histone acetyltransferase that acetylates lysine 12 of H4. It remains to be seen whether other enzymes exist in yeast with the same or overlapping specificity. The fact that the hat1 mutant has no obvious growth defect makes the latter possibility quite likely.

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Fig. 4. Expression of HAT1 activity in E. coli. HAT1 was expressed in E. coli and assayed for enzymatic activity in vitro by the standard assay. Activity is shown for various dilutions of the original extract.
