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N^{α}-acetyltransferase deficiency alters protein synthesis in *Saccharomyces cerevisiae*

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Acetylation is the most frequently occurring chemical modification of the α-NH_2 group of eukaryotic proteins and is catalyzed by a N^{α}-acetyltransferase. Two-dimensional gel electrophoresis was used to compare the soluble proteins synthesized in wild type and a mutant (*aaa1*) yeast cells lacking N^{α}-acetyltransferase. Among 855 soluble proteins identified in wild type and mutant, ~20% of the proteins in the mutant either disappeared or were shifted to higher p*I* without a change of molecular mass, and 27 proteins were observed only in the mutant. In addition, the synthesis of another 12% of the proteins in the mutant was either diminished or enhanced, suggesting that the acetylation of certain regulatory proteins may affect their expression. This is the first demonstration of the broad-based functional role of N^{α}-acetylation in eukaryotic protein synthesis.

Acetyltransferase; Acetylation; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

α-Amino acetylation is an important co- and post-translational modification of proteins in prokaryotic and eukaryotic cells, and acetylation is the most common chemical modification of the α-NH_2 group of eukaryotic proteins (reviewed in [1,2]). It is mediated by an N^{α}-acetyltransferase, which catalyzes the transfer of an acetyl group from acetyl coenzyme A to the α-NH_2 group of proteins and peptides. Since the first identification that an acetyl moiety was the NH_2-terminal blocking group of tobacco mosaic virus coat protein [3] and α-melanocyte-stimulating peptide [4], a large number of proteins from various organisms have been shown to possess acetylated NH_2-terminal residues (e.g., mouse L-cells and Ehrlich ascites cells have about 80% of their intracellular, soluble proteins N^{α}-acetylated [5,6]). In lower eukaryotic organisms (*Saccharomyces* and *Neurospora*) only about 50% of the soluble proteins are N^{α}-acetylated [7]. It is not clear how N^{α}-acetylation affects eukaryotic translation and processing [8] and protects against proteolytic degradation [9,10]. Further, the rate of protein turnover mediated by the ubiquitin-dependent degradation system apparently depends on a free α-NH_2 group at the NH_2-terminus of model proteins [11,12], and this dependence indicates that N^{α}-acetylation plays a crucial role in impeding protein turnover.

Serine and alanine are the most frequently observed N-terminal residues in acetylated proteins, and these residues, together with methionine, glycine, threonine, valine, and aspartic acid account for almost all N^{α}-acetylated residues [1,2,13–15]. However, since not all proteins with these residues at their N-termini are acetylated, the basis by which certain proteins become acetylated remains unclear.

Although many cell types contain N^{α}-acetyltransferases [2], only the N^{α}-acetyltransferase from *Saccharomyces cerevisiae* was purified to homogeneity [16]. The enzyme is encoded by a single gene (*AAA1*; also called *NAT1* [17]) [18]. A null mutation deficiency created by gene replace-

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ment (*aaa1*), while not lethal, makes cells grow slowly and heterogeneously, sporulate defectively, become sensitive to heat shock, fail to enter the stationary phase, and display reduced a-type functions [17,19]. In order to study the effect of N^{α}-acetyltransferase deficiency on protein synthesis in yeast, a comparison between the soluble proteins, isolated and then separated by two-dimensional gel electrophoresis, from wild type and *aaa1* mutant was carried out by computer-based analysis of two-dimensional protein gels.

2. MATERIALS AND METHODS

2.1. *Strains and media*

Yeast strains T3A (*MATα his3, leu2, ura3, AAA1*) and T3A-a (*MATα his3, leu2, ura3, aaa1-1*) were used [19]. Yeast culture media were prepared as described by Sherman et al. [20]: YPD contained 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% glucose. YNB medium: succinate, 10.0 g/l; NaOH, 6.0 g/l; $(NH_4)_2SO_4$, 5.0 g/l; yeast nitrogen base (without amino acids and $(NH_4)_2SO_4$), 1.7 g/l; 18 amino acids (without methionine and cysteine), 12.5 mg/l each; adenine and uracil, 10 mg/l each; and glucose, 20 g/l.

2.2. *Metabolic labeling of yeast proteins with [^{35}S]methionine*

Yeast were grown overnight on a rotary shaker at 25°C, 200 rpm in YNB medium to A_{660} = 0.75. Yeast proteins were labeled by adding [^{35}S]methionine (~1200 Ci/mmol) to a 10 ml yeast culture at a concentration of 10 μCi/ml and shaken for another 20 min at 25°C. After adding ice to chill the culture, the cells were isolated by centrifugation (7000 × *g*) at 4°C for 5 min in 15 ml Corex tubes, washed once with cold distilled water, and centrifuged. 300 μl of cold distilled water were added to the cell pellet, followed by the addition of 0.45 mm glass beads up to the meniscus. The cells were disrupted by vortexing vigorously for 30 s (4 times) with chilling on ice for 1 min between each two 30 s bursts. The homogenate was removed from the glass beads with an Eppendorf pipet and placed in a 1.5 ml microfuge tube. The glass beads were washed twice with 100 μl of distilled water, and the washes were added to the homogenate. 40 μl (1/10 vol.) of a solution containing 0.3% SDS, 1.0% β-mercaptoethanol, 50 mM Tris-HCl, pH 8.0, was added. The solution was heated in the boiling water bath for 2 min and then cooled on ice. 50 μl of a solution containing 1 mg/ml DNase I, 500 μg/ml RNase A, 50 mM $MgCl_2$ in 50 mM Tris-HCl, pH 7.0, was added to the lysate, and the solution was incubated on ice for 10 min. The lysate was centrifuged in the microfuge for 8 min, and the supernatant was transferred to a fresh tube and frozen in liquid nitrogen.

2.3. *Computer-based two-dimensional gel analysis*

Pairs of two-dimensional gels were run for two different sample preparations and computer-analyzed by Protein Databases Inc. (Huntington Station, NY). The gels were prepared according to the method of Garrels [21]. Lysates containing ~400000 cpm were loaded onto each gel. The ampholine range of the isoelectric focusing (first dimension) was pH 4–7. The polyacrylamide concentration of the sodium dodecyl sulfate (second dimension) was 12.5%. The gels were processed for fluorography. Three sets of exposures were prepared for each sample in two experiments (3-, 6-, 12-day). The films were scanned with an Optronics P-1000 scanner interfaced to a PDP-11/60 computer. The data were transferred to a PDQuest workstation. The protein spots were identified, quantitated, and compared with the PDQuest system, which is based on the system of Garrels and Franza [22].

3. RESULTS AND DISCUSSION

The effects of N^{α}-acetyltransferase deficiency on protein synthesis were examined by a comparison of the two-dimensional gel electrophoretic pattern of the soluble proteins from wild type and *aaa1* mutant yeast cells (fig.1). 855 discrete protein spots were detected by computer analysis of the gels. Without a change in their molecular mass, 48 proteins, identified in wild type cells, were observed to have higher p*I* values in the *aaa1* mutant cells. Such shifts to higher p*I*s likely result from protonation of the α-NH_2 group in proteins, lacking an acetyl group, and 22 representative proteins of this class are indicated in fig.1. In addition, the *aaa1* mutant cells contained 144 fewer proteins than the wild type cells.

Hershko et al. [11] showed that N^{α}-acetylated proteins are degraded by the ubiquitin/ATP-dependent system less rapidly than proteins with a free N-terminus and suggested that N^{α}-acetylation may prevent degradation by this system. It is possible that the 144 proteins no longer detected in the *aaa1* mutant may have been degraded by this pathway. However, most of the 'shifted' proteins were not more labile. Hence, N^{α}-acetylation cannot be the only factor involved in preventing protein degradation.

Interestingly, 27 new proteins appeared in *aaa1* mutant. We propose that the synthesis of these proteins may result from derepression or activation of genes regulated by regulatory proteins, which are no longer acetylated, as has been suggested to be the case for a protein regulating a-specific mating type genes in the *aaa1* mutant [17,19].

Furthermore, a comparison between the proteins of wild type and *aaa1* mutant revealed that 71 proteins of *aaa1* mutant were decreased by >50%

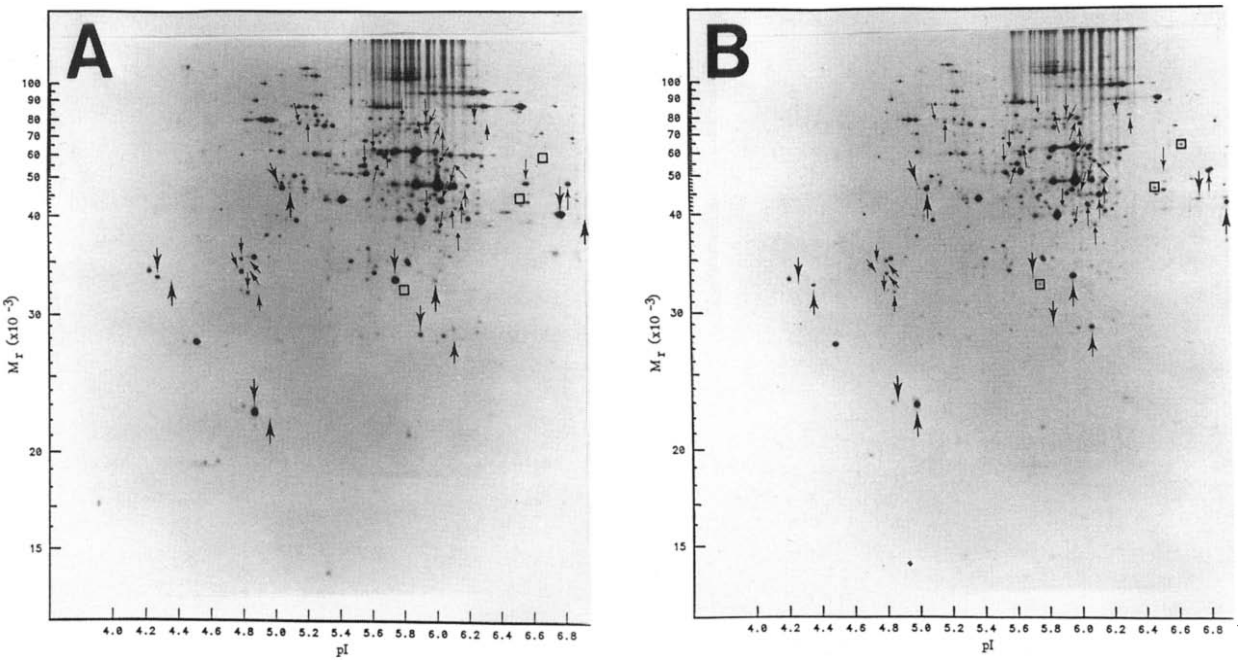

Fig.1. Two-dimensional gels of [^{35}S]methionine-labeled proteins from wild type and *aaa1* yeast strains. (A) Wild type (strain T3A). (B) *aaa1* mutant (T3A-a). Cell lysates containing ~400000 cpm were loaded onto each gel and separated by two-dimensional gel electrophoresis. The ampholine range of the isoelectric focusing (first dimension) was pH 4 to 7. The polyacrylamide concentration of the sodium dodecyl sulfate (second dimension) was 12.5%. The gels were treated for fluorography and exposed for 6 days. Twenty-two representative proteins that shifted toward higher p*I* in the *aaa1* mutant are indicated by their position in the wild type (down arrow) and in the mutant (up arrow). Three representative proteins which appear only in the *aaa1* mutant are marked (square boxes).

and 34 proteins were increased by >200%. Such diminished or enhanced synthesis might also be controlled by regulatory proteins lacking N^{α}-acetylation.

We have demonstrated that only 20% of the soluble proteins were either shifted or disappeared in the *aaa1* mutant (i.e., indicating that they probably lacked a N^{α}-acetyl group), although it has been suggested that 50% of the soluble proteins in yeast are N^{α}-acetylated [7]. The presence of additional N^{α}-acetyltransferases may account for this apparent difference.

N^{α}-acetylation is a common and important chemical modification of eukaryotic proteins, as indicated by the large number of proteins whose synthesis is altered by deleting the N^{α}-acetyltransferase gene.

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