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Identification of Methionine N^{α}-Acetyltransferase from *Saccharomyces cerevisiae**

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N^{α}-Acetylation is the most frequently occurring chemical modification of the α-NH_2 group of eukaryotic proteins and was believed until now to be catalyzed by a single N^{α}-acetyltransferase. The transfer of an acetyl group from acetyl coenzyme A to the α-amino group of five NH_2-terminal residues (serine, alanine, methionine, glycine, and threonine) in proteins accounts for ~95% of acetylated residues. We have found that a crude lysate from *Saccharomyces cerevisiae* mutant (*aaa1*) deficient in N^{α}-acetyltransferase activity can effectively transfer an acetyl group to peptides containing NH_2-terminal methionine but not to serine or alanine. This methionine N^{α}-acetyltransferase has been extensively purified, and this purified enzyme can selectively transfer an acetyl group to various model peptides containing an NH_2-terminal methionine residue and a penultimate aspartyl, asparaginyl, or glutamyl residue. Such specificity of N^{α}-acetylation of methionine has been previously observed based on the analysis of eukaryotic protein sequences (Persson, B., Flinta, C., Heijne, G., and Jornvall, H. (1985) *Eur. J. Biochem.* 152, 523–527; Arfin, S. M., and Bradshaw, R. A. (1988) *Biochemistry* 27, 7979–7984). The identification of this methionine N^{α}-acetyltransferase provides an explanation as to why two distinct classes of N^{α}-acetylated proteins exist in nature: (i) those whose initiator methionine is acetylated and (ii) those whose penultimate residue is acetylated after cleavage of the initiator methionine.

EXPERIMENTAL PROCEDURES

Materials—[^{3}H]Acetyl coenzyme A was from Amersham Corp., and unlabeled acetyl coenzyme A was from Pharmacia LKB Biotechnology Inc. Reagents and solvents for amino acid analysis and Ready-Solv EP scintillation mixtures were obtained from Beckman. SP membrane discs were from Cuno Inc. Reagents and solvents for protein sequence analysis were from Applied Biosystems. Reagents for peptide synthesis were obtained from Applied Biosystems, Inc., and solvents for peptide synthesis were from Anachem. Boc-amino acids were from Peninsula. All other chemicals were reagent grade or better.

Strains and Media—Yeast strains T3A (MATα *his3*, *leu2*, *ura3*, *AAA1*) and T3A-a (MATα *his3*, *leu2*, *ura3*, *aaa1-1*) (21) were used in this study. Yeast culture media were prepared, as described by Sherman *et al.* (23); YPD contained 1% Bacto-yeast extract, 2% Bacto-peptone, and 2% glucose.

Preparation of Yeast Lysates and N^α-Acetyltransferase Activity Assay—Yeast were grown overnight on a rotary shaker at 30 °C, 200 rpm in YPD medium to A_{600} = 6.0. Yeast crude lysates were prepared, and N^α-acetyltransferase activity was determined as previously described (17). Aliquots of the lysate were added to 1.5-ml Eppendorf tubes containing a reaction mixture of 50 mM HEPES,[1] pH 7.4, 150 mM KCl, 1 mM DTT, 25 μM [^{3}H]acetyl coenzyme A (0.5 μCi), and 50 μM synthetic peptide 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 chilled in an ice bath. The reaction samples were filtered through SP membrane discs and then

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[1] The abbreviations used are: HEPES, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; DTT, dithiothreitol; MES, 2-(*N*-morpholino)ethanesulfonic acid.

washed with 0.5 M acetic acid on a Millipore 1225 sampling manifold. The partially dried membranes were placed in scintillation mixture and counted with a Beckman LS 3801 scintillation counter. One unit of activity was defined as the amount of enzyme able to transfer 1 pmol of [^{3}H]acetyl group from [^{3}H]acetyl coenzyme A to synthetic peptide per min under standard enzyme assay conditions defined above.

Peptide Synthesis and Analysis—Peptides were synthesized on an Applied Biosystems model 430A peptide synthesizer and characterized by methods previously described (24). Peptide sequence analysis was carried out by using an Applied Biosystems 470A protein Sequencer and an Applied Biosystems 120A phenylthiohydantoin analyzer (25).

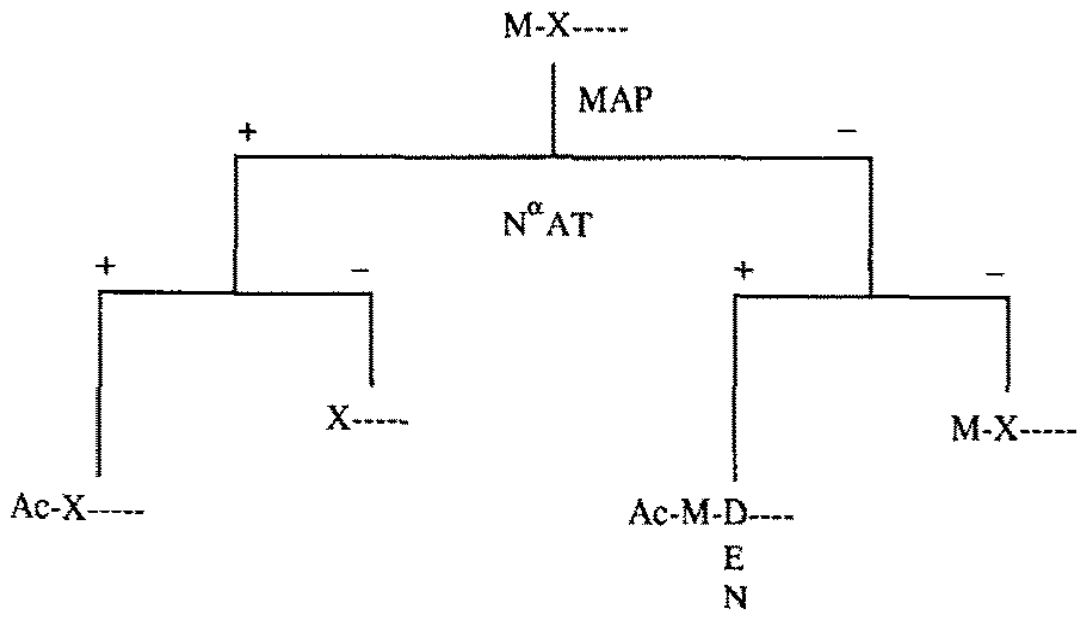

FIG. 1. **Proposed pathway for the co-translational modification of eukaryotic proteins involving methionine aminopeptidase (*MAP*) and a single N^{α}-acetyltransferase ($N^{\alpha}AT$), as previously proposed by Arfin and Bradshaw (26).** MAP removes the initiator methionine from most but not all proteins, and a single N^{α}-acetyltransferase acetylates certain proteins from each class. The initiator methionine, penultimate residue, and acetyl group at the α-amino group are indicated by *M*, *X*, and *Ac*, respectively. The nascent polypeptide is indicated (---). The positive (+) and negative (–) actions of the enzymes for the protein substrates are indicated.

TABLE I

N^{α}-Acetylation of various peptide substrates by crude yeast lysates from wild type and aaa1 mutant strains

The wild type (containing endogenous N^{α}-acetyltransferase activity) and the *aaa1* (lacking endogenous N^{α}-acetyltransferase activity) strains were T3A and T3A-a, respectively (21). Human adrenocorticotropic hormone-(1–24), yeast alcohol dehydrogenase-(1–24), and human superoxide dismutase-(1–24) have either serine or alanine as amino-terminal residues, while proteinase A inhibitor 3 has a methionine residue. The synthesis of the peptide substrates, the preparation of the crude yeast lysates, and the assay of enzyme activity were as described under "Experimental Procedures." The numbers in parentheses refer to the residue numbers, and the letters in parentheses specify the amino acid sequence. Data are reported as mean activity ± S.D. (n = 3–5) and are normalized to the activity of the lysate (~2.0 enzyme units) toward human adrenocorticotropic hormone-(1–24) (ACTH-(1–24)).

Substrate (amino acid sequence)	Activity	
	AAA1^{+}	*aaa1*$^{-}$
	%	
ACTH-(1–24) (human) (S-Y-S-M-E-H-F-R-W-G-K-P-V-G-K-K-R-R-P-V-K-V-Y-P)	100 ± 5	0
Alcohol dehydrogenase I-(1–24) (yeast) (S-I-P-E-T-Q-K-G-V-I-F-Y-E-S-H-G-K-L-E-Y-K-D-I-P)	102 ± 5	0
Superoxide dismutase-(1–24) (human) (A-T-K-A-V-C-V-L-K-G-D-G-P-V-Q-G-S-I-N-F-E-Q-K-E)	80 ± 6	0
Proteinase A inhibitor 3-(1–24) (yeast) (M-N-T-D-Q-Q-K-V-S-E-I-F-Q-S-S-K-E-K-L-Q-G-D-A-K)	75 ± 5	80 ± 5

TABLE II

Purification of methionine-specific N^{α}-acetyltransferase from S. cerevisiae (strain T3A-a)

Yeast culture (10 × 1 liter) was grown at 30 °C, 200 rpm in YPD medium to A_{600} = 6.0. Yeast crude lysates were prepared, and N^{α}-acetyltransferase activity using proteinase A inhibitor 3-(1–24) as substrate was determined as previously described (17). The supernatant solution of crude lysates was concentrated to a volume of 10 ml, using a PM-30 ultrafiltration membrane, and dialyzed overnight against 2 × 2 liters of HDG (20 mM HEPES, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol, and 0.02% NaN_3) buffer containing 0.2 M KCl. The dialyzed supernatant fluid was applied to DEAE-Sepharose CL-6B (2.5 × 55 cm) equilibrated with HDG buffer containing 0.2 M KCl and eluted with same buffer. The fractions (4 ml) containing enzyme activity were pooled, concentrated to a volume of 5 ml, dialyzed overnight against 2 × 2 liters of HDG buffer containing 0.05 M KCl, and then applied to a DE52 cellulose column (2.5 × 55 cm) equilibrated in HDG buffer containing 0.05 M KCl. The column was eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in HDG buffer. Fractions (3.5 ml) containing enzyme activity were pooled, concentrated to a volume of 2.5 ml, dialyzed overnight against 2 × 2 liters of MDG (20 mM MES, pH 6.7, 0.5 mM DTT, 10% (v/v) glycerol, and 0.02% NaN_3) buffer containing 0.05 M KCl, and then applied to a CM-52 cellulose column (2.5 × 50 cm) equilibrated in MDG buffer containing 0.05 M KCl. The column was eluted with a linear gradient of 0.05 M (250 ml) to 0.5 M (250 ml) KCl in MDG buffer. Fractions (3.0 ml) were collected, and the fractions containing enzyme activity (at ~0.15 M KCl) were pooled, concentrated to a volume of 1.0 ml, and applied to a Sepharose CL-6B column (2.0 × 90 cm) equilibrated with 0.1 M potassium phosphate buffer, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3. The column was eluted with the same buffer. Fractions (3.5 ml) containing enzyme activity were pooled, concentrated to a volume of 0.5 ml, and applied to a hydroxylapatite (Bio-Rad) column (2.0 × 15 cm) equilibrated with 0.1 M potassium phosphate buffer, pH 7.4, 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3. The column was eluted with a linear gradient of 0.1 M (100 ml) to 0.6 M (100 ml) potassium phosphate buffer, pH 7.4, containing 0.5 mM DTT, 10% (v/v) glycerol, 0.02% NaN_3. Fractions (2.0 ml) were collected, and the fractions containing enzyme activity (at ~0.45 M KH_2PO_4) were pooled and concentrated to a volume of 0.5 ml. Protein was determined by the Bradford assay (Bio-Rad) (33) with bovine serum albumin as the standard.

Step	Activity	Protein	Specific activity	Purification	Yield
	units	*mg*	*units/mg*	*-fold*	*%*
1. Crude extract	6400	2200	2.9	1.0	100
2. DEAE-Sepharose	3760	410	9.2	3.2	59
3. DE52-cellulose	2450	110	22.3	7.7	38
4. CM52-cellulose	1400	2.2	636	219	22
5. Sepharose CL-6B	940	0.8	1180	407	15
6. Hydroxylapatite	380	0.04	9500	3280	6

TABLE III

N^{α}-Acetylation of various NH_2-terminal methionine-containing peptide substrates

The synthesis of the peptide substrates and the assay of enzyme activity were as described under "Experimental Procedures." The methionine N^{α}-acetyltransferase was purified as described in Table II. The numbers in parentheses refer to the residue numbers, and the letters in parentheses refer to the amino acid sequence. Data are reported as mean activity ± S.D. (n = 3–5) and are normalized to the activity of the enzyme (~4.0 enzyme units) toward yeast proteinase A inhibitor 3.

Substrate (amino acid sequence)	Activity
	%
Proteinase A inhibitor 3-(1–24) (yeast) (M-N-T-D-Q-Q-K-V-S-E-I-F-Q-S-S-K-E-K-L-Q-G-D-A-K)	100 ± 5
[Met^{-1}]Actin-(1–24) (yeast) (M-D-S-E-V-A-A-L-V-I-D-N-G-S-G-M-C-K-A-G-F-A-G-D)	65 ± 5
Protein-tyrosine-phosphatase-(1–24) (human) (M-E-M-E-K-E-F-E-Q-I-D-K-S-G-S-W-A-A-I-Y-Q-D-I-R)	56 ± 5
[$Met^{-1}Asp^{1}$]Iso-1-cytochrome *c*-(1–24) (yeast) (M-D-E-F-K-A-G-S-A-K-K-G-A-T-L-F-K-T-R-C-L-Q-C-H)	75 ± 5
[Met^{-1}]Iso-1-cytochrome *c*-(1–24) (yeast) (M-T-E-F-K-A-G-S-A-K-K-G-A-T-L-F-K-T-R-C-L-Q-C-H)	0
[$Met^{-1}Ala^{1}$]Iso-1-cytochrome *c*-(1–24) (yeast) (M-A-E-F-K-A-G-S-A-K-K-G-A-T-L-F-K-T-R-C-L-Q-C-H)	0
[$Met^{-1}Leu^{1}$]Iso-1-cytochrome *c*-(1–24) (yeast) (M-L-E-F-K-A-G-S-A-K-K-G-A-T-L-F-K-T-R-C-L-Q-C-H)	0
[Met^{1}]Alcohol dehydrogenase I-(1–24) (yeast) (M-I-P-E-T-Q-K-G-V-I-F-Y-E-S-H-G-K-L-E-Y-K-D-I-P)	0

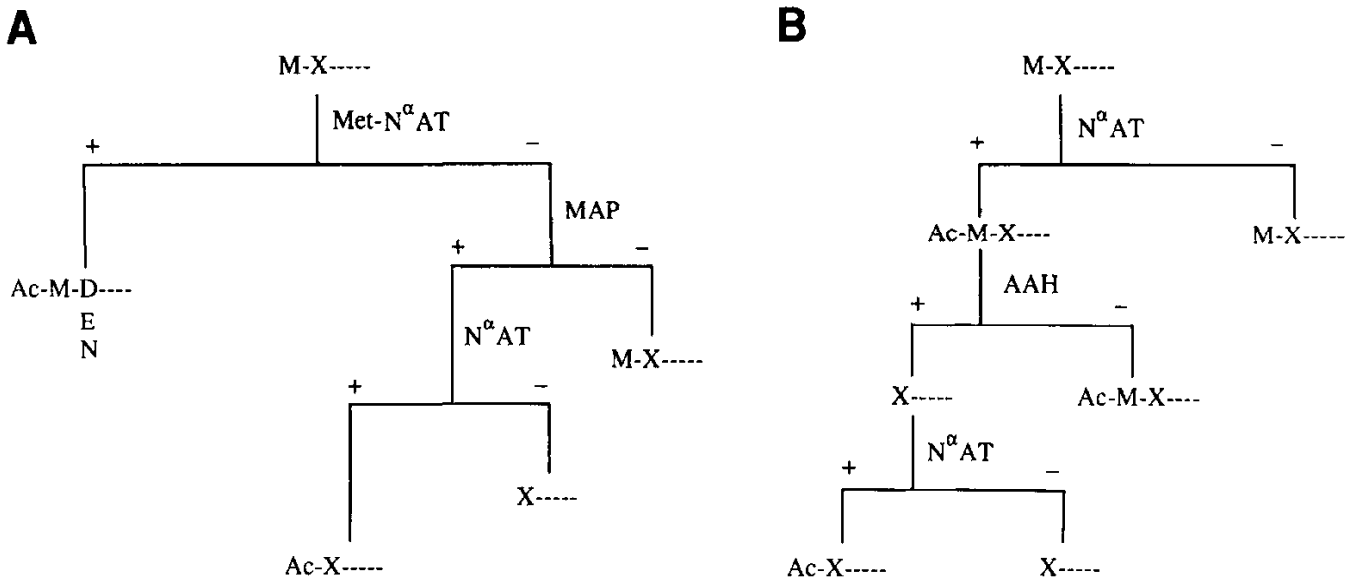

FIG. 2. *A*, proposed pathway for the co-translational modification of eukaryotic proteins mediated by methionine N^{α}-acetyltransferase (*Met-N$^{\alpha}$AT*), methionine aminopeptidase (*MAP*), and N^{α}-acetyltransferase (*N$^{\alpha}$-AT*). *B*, alternative pathway involving a single N^{α}-acetyltransferase acting at two different stages of acetylation and acylamino acid hydrolyase (*AAH*), as previously proposed by Wold (3). This pathway does not require the action of methionine aminopeptidase. The *symbols* are as indicated in Fig. 1.