Published in final edited form as:
Biochemistry. 2010 June 29; 49(25): 5225–5235. doi:10.1021/bi100428x.

Identification of Protein N-Terminal Methyltransferases in Yeast and Humans†

Kristofor J. Webb[§], **Rebecca S. Lipson**[§], **Qais Al-Hadid**[§], **Julian P. Whitelegge**[§,‡], and **Steven G. Clarke**[§,*]

[§] Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, 607 Charles E. Young Drive East, Los Angeles, California 90095

[‡] Pasarow Mass Spectrometry Laboratory, NPI-Semel Institute for Neuroscience and Human Behavior, UCLA, Los Angeles, California 90024

Abstract

Protein modification by methylation is important in cellular function. We show here that the *Saccharomyces cerevisiae* YBR261C/*TAE1* gene encodes an N-terminal protein methyltransferase catalyzing the modification of two ribosomal protein substrates, Rpl12ab and Rps25a/Rps25b. The YBR261C/Tae1 protein is conserved across eukaryotes; all of these proteins share sequence similarity with known seven beta strand class I methyltransferases. Wild type yeast cytosol and mouse heart cytosol catalyze the methylation of a synthetic peptide (PPKQQLSKY) that contains the first eight amino acids of the processed N-terminus of Rps25a/Rps25b. However, no methylation of this peptide is seen in yeast cytosol from a ΔYBR261C/*tae1* deletion strain. Yeast YBR261C/*TAE1* and the human ortholog METTL11A genes were expressed as fusion proteins in *Escherichia coli* and were shown to be capable of stoichiometrically dimethylating the N-terminus of the synthetic peptide. Furthermore, the YBR261C/Tae1 and METTL11A recombinant proteins methylate variants of the synthetic peptide containing N-terminal alanine and serine residues. However, methyltransferase activity is largely abolished when the proline residue in position 2 or the lysine residue in position 3 is substituted. Thus, the methyltransferases described here specifically recognize the N-terminal X-Pro-Lys sequence motif and we suggest designating the yeast enzyme Ntm1 and the human enzyme NTMT1. These enzymes may account for nearly all previously described eukaryotic protein N-terminal methylation reactions. A number of other yeast and humans proteins also share the recognition motif and may be similarly modified. We conclude that protein X-Pro-Lys N-terminal methylation reactions catalyzed by the enzymes described here may be widespread in nature.

Posttranslational and cotranslational modification of the N-terminus of proteins is well established, but the biological function is known only in a few cases (1–6). Acetylation, the most extensive modification, occurs on approximately 77% of cytosolic proteins in mammals and about 50% of cytosolic proteins in *Saccharomyces cerevisiae* (3). Methylation of the N-terminus has also been observed in a variety of proteins in both prokaryotes and eukaryotes (2). A major chemical consequence of such modification is controlling the

†This work was supported by National Institutes of Health grant GM026020. R.S.L. was supported by the UCLA Cellular and Molecular Biology Training Program funded by NIH Grant GM007185. Mass spectrometry was performed in the UCLA Molecular Instrumentation Center supported by Grant S10RR024605 from the National Center for Research Resources. The purchase of the LTQ FT Ultra mass spectrometer was made possible by NIH/NCRR grant S10 RR023045.

*To whom correspondence should be addressed. Tel: 310 825-8754. Fax: 310 825-1968. clarke@mbi.ucla.edu.

SUPPORTING INFORMATION AVAILABLE

A list of yeast strains used and lists of yeast and human open reading frames containing the X-Pro-Lys N-terminal methyltransferase recognition motif. Additional material is available free of charge via the Internet at http://pubs.acs.org.

charge at the N-terminus. For acetylated proteins, the partial positive charge on the amino group is neutralized; for fully methylated N-terminal residues, the positive charge is fixed. In both cases, these modifications abolish the nucleophilicity of the α-amino nitrogen (2).

Eukaryotic N-terminal methylation has been detected to date on one large subunit ribosomal protein in fungal and plant species, one small subunit ribosomal protein in *Saccharomyces cerevisiae*, one large subunit ribosomal protein in higher plant chloroplasts, the small subunit of Rubisco in higher plant chloroplasts, histone H2B proteins in several invertebrates, cytochrome c-557 species from one protozoan genus, myosin light chain proteins from mammals, and the RCC1 protein of humans (Table 1). Interestingly, with the exception of the two chloroplast species, the N-terminal tetrapeptide sequences of all of these proteins are similar. All contain an N-terminal methionine residue that is predicted to be cleaved (24), followed by a proline, alanine, or serine residue in the second position, and apparently invariant proline and lysine residues in the third and fourth positions respectively. Most of these species are fully methylated (dimethylated proline, trimethylated alanine) to give a quaternary alpha amino group. It has been proposed that a single enzyme that recognizes an X-Pro-Lys N-terminal sequence could account for all of these methylation reactions (2). However, no gene product has been associated to date with this activity.

We have been interested in the modification of ribosomal proteins in the yeast *S. cerevisiae* and in identifying the genes that encode the enzymes that catalyze these reactions. We have taken the approach of analyzing intact ribosomal proteins by mass spectrometry from extracts of mutant yeast lacking putative methyltransferases to detect unmodified or undermodified species. Using this method, we have identified four SET domain-containing protein side-chain lysine methyltransferases, Rkm1, Rkm2, Rkm3, and Rkm4 and their large ribosomal protein substrates Rpl23ab, Rpl12ab, and Rpl42ab (9,25,26) as well as Rmt1-dependent protein arginine methylation of Rps2 (27). The distinct methylation of N-terminal proline residues has recently been shown in the fission yeast (7) and plant (8) homologs of budding yeast Rpl12ab; we later confirmed the presence of this modification in *S. cerevisiae* (9).

In this work, we have used the approach of intact mass spectrometric profiling of ribosomal proteins from yeast cells deficient in putative methyltransferases (28) to now identify the gene necessary for protein N-terminal methylation. We demonstrate that the loss of the *S. cerevisiae* ORF YBR261c/*TAE1*, encoding a candidate seven-beta strand methyltransferase, results in the loss of the N-terminal methylation of both Rpl12ab and Rps25a/Rps25b. The YBR261C/*TAE*1 product had been previously linked to a translational function by a chemical genetic profile analysis (29), but its specific function was not identified. We demonstrate that recombinant fusion proteins YBR261C/Tae1 and METTL11A (the human homolog) are active methyltransferases with specificity for stoichiometric modification of X-Pro-Lys N-terminal sequences.

EXPERIMENTAL PROCEDURES

Purification of S. cerevisiae Ribosomal Proteins from Wild Type and Mutant Strains

The strains used in this study were obtained from the Saccharomyces Genome Deletion Project and included the parent strains BY4742, BY4741 and the YBR261C/*tae1* deletion strain in each background, as well as the deletion strains listed in Supplemental Table 1. Large and small ribosomal proteins were isolated from wild type and ΔYBR261C/*tae1* strains as described previously (9).

Liquid Chromatography with Electrospray Ionization Mass Spectrometry of Intact Ribosomal Proteins

Ribosomal proteins were fractionated using reverse-phase liquid chromatography as described previously (9). The resulting effluent was directed into a QSTAR Elite (Applied Biosystems/MDX SCIEX) electrospray mass spectrometer running in MS only mode. The instrument was calibrated using external peptide standards to yield a mass accuracy of 40 ppm or better.

Localization of Methylation Sites by Top Down Mass Spectrometry Using Collisionally Activated Dissociation or Electron Capture Dissociation

Ribosomal proteins Rpl12ab and Rps25a/Rps25b from the ΔYBR261C/*tae1* deletion and wild type strains were separated by HPLC as described above and fractions containing the isolated intact proteins were directly infused on a hybrid linear ion-trap/FTICR mass spectrometer (LTQ FT Ultra, Thermo Fisher, Bremen, Germany) and fragmented using collisionally activated dissociation or electron capture dissociation as described previously (9). All FTICR MSMS spectra were processed using ProSightPC software (Thermo Fisher, San Jose, CA) in single protein mode with a 15 ppm mass accuracy threshold. The root mean square deviations for assigned fragments were less than 5 ppm. The reference database for the ProSightPC software included the *S. cerevisiae* proteome from Swiss-Prot. Intact mass measurements were processed using the manual Xtract program, version 1.516 (Thermo Fisher, San Jose, CA). All top down experimental data obtained for this study is available online at http://www.proteomecommons.org.

Preparation of Cytosolic Extracts

S. cerevisiae cytosol was isolated from wild-type (BY4742) and ΔYBR261C/*tae1* strains. These strains were grown at 30°C in 500 ml YPD to an OD_{600} of 0.7. The cells were then centrifuged at 5,000 × g for 5 min at 4°C. The resulting pellet was resuspended in 4 ml of phosphate-buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4) and 100 μM phenylmethylsulfonyl fluoride. The cells were lysed by vortexing for 1 min in the presence of 0.2 g of baked zirconium beads (Biospec Products; Bartlesville, OK), followed by cooling on ice for 1 min, for a total of 7 cycles. A crude cytosolic fraction was then obtained by centrifuging for 15 min at 12,000 × *g* at 4 °C. Mouse heart cytosol was obtained as previously described (30).

Plasmid Construction and Preparation of the Recombinant Yeast YBR261C/Tae1 and Human METTL11A Fusion Proteins

The BG1805-amp vector containing the yeast YBR261C/*TAE1* gene and the pSPORT1 vector containing the full length human cDNA for METTL11A (Accession number NM_014064) were purchased from Open Biosystems (Huntsville, AL). The two open reading frames were cloned into the pET-100/D-TOPO *E. coli* expression vector according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). Proper insertion into the pET100D vector was verified by full DNA sequencing of both strands (GENEWIZ, NJ) using the standard T7 forward and T7 reverse primers. The vector pET-100/D-TOPO carries a His_6 tag in the linker region MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKDHPFT which is incorporated before the initiator methionine residue of the cloned protein. The constructed plasmids were transformed into *E. coli* BL21(DE3). The recombinant proteins were overexpressed by growing two liters of the transformed *E. coli* cells at 37 °C in LB media with 100 μg/ml ampicillin to an OD_{600} of 0.7. Recombinant protein production was induced for four hours by the addition of isopropyl β-D-L-thiogalactopyranoside to a final concentration of 0.4 mM. Washed cells were resuspended in 20 ml of PBS and 100 μM phenylmethylsulfonyl

fluoride and subsequently broken by 10 30-s sonicator pulses (50% duty; setting 4) on ice with a Sonifier cell disruptor W-350 (SmithKline Corp.). The lysate was centrifuged for 50 min at 13,000 × *g* at 4 °C. The resulting supernatant was loaded onto a 5 ml HisTrap HP nickel affinity column (GE Healthcare part number 17-5248-1) and the fusion proteins were purified using a gradient of 30 to 500 mM imidazole per the manufacturer's specifications.

Protein Concentration Determination

A modified Lowry procedure was used to determine protein concentrations of recombinant proteins and cell cytosol following precipitation with 1.0 ml of 10% (w/v) trichloroacetic acid (31). A stock solution of bovine serum albumin was used as a protein standard.

In Vitro Methylation of Peptides

Synthetic nonapeptides were prepared by Biosynthesis, Inc. (Lewisville, TX). These peptides were based on the sequence of the first eight amino acids of the N-terminus of the *S. cerevisiae* ribosomal protein Rps25a/Rps25b with an additional tyrosine residue added at the C-terminus for UV detection. Peptides were prepared with N-terminal proline (PPKQQLSKY), alanine (APKQQLSKY), and serine (SPKQQLSKY) residues. A peptide mixture was synthesized where equal amounts of each of the twenty protected amino acid derivatives were used for the first position (X_{20}PKQQLSKY). A similarly prepared mixture was made except that proline and alanine were not included (X_{18}PKQQLSKY). Two additional peptide mixtures were prepared: one designed to contain equal amounts of each of the twenty amino acids at the second position except proline (PX_{19}KQQLSKY), and one designed to contain equal amounts of the twenty amino acids at the third position except lysine (PPX_{19}QQLSKY). Methylation radiolabelling experiments were carried out in the presence of 0.5 μM *S*-adenosyl- L-[*methyl*-^{3}H]methionine ([^{3}H]AdoMet; 70.8 Ci/mmol from a 7.8 μM stock solution in 10 mM H_2SO_4/ethanol 9:1; PerkinElmer, Inc.). Non-radiolabelled methylation reactions were carried out in 200 μM *S*-adenosyl-L-methionine p-toluenesulfonate salt (Sigma; from a 10 mM stock solution in 10 mM HCl). All methylation reactions were performed in 100 mM NaCl and 100 mM sodium phosphate buffer at pH 7.0 in a total reaction volume of 200 μl. Reactions were terminated by the addition of 20 μl of 10% trifluoroacetic acid. After centrifugation at 20,000 × *g* for 10 min at room temperature, the supernatant was fractionated by HPLC. A PLRP-S polymeric column was used with a pore size of 300 A, bead size of 5 μm, and dimensions of 150 × 2 mm (Polymer Laboratories, Amherst, MA). The column was maintained at 50 °C and initially equilibrated in solvent A (0.1% trifluoroacetic acid in water) at 95% and solvent B (0.1% trifluoroacetic acid in acetonitrile) at 5%. Peptides were eluted at a flow rate of 200 μl/min using a program of 5 min at 5% B, followed by a 20-min gradient from 5 to 60% B; half minute fractions were collected.

The incorporation of ^{3}H-methyl radioactivity into peptides was determined either by direct counting or using an inline scintillation counter as described in the figure legends. For the non-isotopically labelled reaction mixtures, methylated peptides were analyzed by MALDI (matrix-assisted laser desorption ionization) mass spectrometry. An aliquot of the HPLC peptide peak fraction or fractions (1 μl) was mixed with 1 μl of 70% acetonitrile/0.1% trifluoroacetic acid containing 1 mg/ml α-cyano-4-hydroxycinnamic acid as the MALDI matrix and spotted onto a MALDI target plate. The samples were analyzed using a Voyager-DE STR MALDI-TOF mass spectrometer (Applied Biosystems).

RESULTS

Yeast YBR261C/Tae1 is Required for the Methylation of Two Ribosomal Proteins

After the loss of the initiator methionine residue (24), *S. cerevisiae* ribosomal proteins Rpl12ab and Rps25a/Rps25b share a common N-terminal sequence Pro-Pro-Lys. Rpl12ab represents the identical polypeptides encoded by the yeast *RPL12A* and *RPL12B* genes; Rps25a and Rps25b are encoded by the yeast *RPS25A* and *RPS25B* genes and differ only at a single amino acid residue. The N-terminal proline residue in each of these proteins has been shown to be modified at the alpha amino group by dimethylation (9,10), although the enzyme or enzymes that catalyze the methylation reaction have not been identified. The Rpl12ab protein is modified by methylation at two additional sites; trimethylation at lysine 3 catalyzed by the Rkm2 enzyme (9,26), and delta-monomethylation at arginine 66 catalyzed by the Rmt2 enzyme (32). All of these modifications appear to be stoichiometric and no evidence for additional types of modifications has been observed (9).

To identify the enzymes responsible for the N-terminal proline modification, we measured the intact mass of Rpl12ab and Rps25a/Rps25b from large and small ribosomal proteins prepared from wild type yeast cells and from 37 yeast deletion strains lacking known or putative methyltransferase genes (28,33–35) (Supplemental Table 1). In this screen, purified ribosomal proteins from small or large subunits were fractionated by HPLC and analyzed by electrospray mass spectrometry as described in “Experimental Procedures”. We found that Rpl12ab from the wild type strain (Fig. 1A) and from 34 of the 37 deletion strains contained the full complement of methyl groups (data not shown; Supplemental Table 1). As expected, the intact mass of the Rpl12ab protein from the *rkm2* deletion strain was reduced by 42 Da consistent with the loss of trimethylation at Lys-3. Furthermore, the intact mass of Rpl12ab from the *rmt2* deletion strain was reduced by 14 Da consistent with the loss of monomethylation at Arg-66 (data not shown). However, in the Rpl12ab protein prepared from the YBR261C/*tae1* deletion strain, we did not detect the mass of the fully modified protein (average mass 17775) but instead found a series of products differing by the replacement of three to five methyl groups with hydrogen atoms at 17733 Da ($-3\ CH_2$), 17719 Da ($-4\ CH_2$), and 17705 Da ($-5\ CH_2$) (Fig. 1A). This result suggests that YBR261C/*TAE1* encodes a gene needed for the methylation of Rpl12ab at the N-terminal proline residue. These losses were verified in an independent YBR261C/*tae1* deletion strain in the BY4741 background (data not shown). The appearance of a mixture of species lacking 3, 4, and 5 methyl groups suggests that the affinity of Rkm2 for Lysine 3 is decreased when the N-terminal modification of the proline residue is not present.

We additionally observed that the intact masses of the small ribosomal proteins Rps25a (average mass 11936 Da) and Rps25b (average mass 11906 Da) were consistent with the presence of an N-terminal dimethylproline residue (10) in the wild type strain (Fig. 1B). Rps25a and Rps25b differ only at position 104; a threonine residue is present in the former and an alanine residue in the latter. These forms were also detected in 36 of the 37 deletion strains examined (data not shown; see Supplemental Table 1). However, Rps25a/Rps25b was found to have an intact mass reduced by 28 Da (loss of 2 methyl groups) in the YBR261C/*tae1* deletion strain (Fig. 1B). This loss was also verified in the independent YBR261C/*tae1* deletion strain in the BY4741 background (data not shown). The results obtained with Rpl12ab and Rps25a/Rps25b suggests that the YBR261C/*TAE1* product is necessary for the formation of the dimethylproline residue in each of these ribosomal proteins.

YBR261C/Tae1-Dependent N-terminal Methylation of Rpl12ab and Rps25a/Rps25b

To identify the residue(s) where loss of methylation occurs in the YBR261C/*tae1* deletion strain we performed intact mass top down fragmentation analysis. This approach allows an unbiased assignment of posttranslational modifications across the entire polypeptide chain (36). Ribosomal proteins were fractionated using reverse phase HPLC and purified fractions containing Rpl12ab and Rps25a/Rps25b were collected for offline top down analysis. In Rpl12ab isolated from the YBR261C/*tae1* deletion strain we found that the major species present was monomethylated (Fig. 1A). When this species was fragmented using collisionally activated dissociation, we localized the site of methylation to the region between residues 35 and 73 containing the known monomethylation site at arginine 66 (Fig. 2A). Additionally, we observed that the N-terminal region was unmodified (Fig. 2A). The intact dimethylated Rpl12ab species (average mass 17719) isolated from the YBR261C/*tae1* deletion strain was fragmented and a b_{20} ion was detected with the addition of one methyl group, suggesting partial methylation of lysine 3 (data not shown). Neither of these species were dimethylated at the N-terminal residue.

Wild type Rps25a/Rps25b was isolated and subjected to the same top down analysis to verify the location and extent of methylation. Our data is in agreement with the observations of Meng and colleagues (10), isolating the dimethylation to the N-terminal region of Rps25a/Rps25b (Fig. 2B). We also found no evidence for unmethylated or monomethylated species of Rps25a/Rps25b. Rps25a/Rps25b isolated from the YBR261C/*tae1* deletion strain is 28 Da less than the wild type and the fragmentation data indicate no methylation is present (Fig. 2C). The intact mass data from Rpl12ab and Rps25a/Rps25b in Fig. 1 and the fragmentation data in Fig. 2 from wild type and YBR261C/*tae1* deletion strains confirm that the YBR261C/Tae1 protein is necessary for the stoichiometric methylation of the N-terminus of both Rpl12ab and Rps25a/Rps25b.

YBR261C/Tae1 and the N-terminus of Ribosomal Proteins Rpl12ab and Rps25a/Rps25b are Conserved Across Eukaryotes

Previously the YBR261C/*TAE1* locus was identified as encoding a hypothetical methyltransferase (28,33–35) containing the seven beta strand class I conserved AdoMet-dependent methyltransferase motif I, post-I, motif II, and motif III (Fig. 3). A BLAST search revealed homologs to the YBR261C/*TAE1* gene in multiple eukaryotic, but not prokaryotic, organisms. Two human homologs were found, METTL11A and METTL11B (Fig. 3). Additionally, we noted that human ribosomal proteins L12 and S25 (the homologs of Rpl12ab and Rps25a/Rps25b) share the same N-terminal tripeptide sequence as the yeast proteins. Human S25 has been reported to be modified by the addition of two methyl groups, although their location in the polypeptide chain has not been established (37).

The YBR261C/TAE1 and Human METTL11A Genes Encode N-terminal Protein Methyltransferases

The results above suggest that YBR261C/Tae1 is the methyltransferase that directly modify the ribosomal proteins. However, the possibility remains that YBR261C/Tae1 may indirectly control methylation, for example as a necessary non-catalytic subunit of a methyltransferase complex. To address this issue, we developed an *in vitro* assay for N-terminal methyltransferase activity (Fig. 4). We observed the presence of an activity in yeast and mammalian cytosolic extracts capable of methylating a synthetic peptide constructed using the sequence of the first eight amino acids of the Rps25a/Rps25b protein and a C-terminal tyrosine residue, PPKQQLSKY. Here, the cytosolic extracts were incubated with this peptide and [^{3}H]AdoMet. The reaction mixture was fractionated by HPLC and the ^{3}H-methyl groups in the peptide were determined by scintillation counting (Fig. 4A, B). In the wild type cytosolic extract radioactivity migrated with the synthetic peptide indicating the

presence of an AdoMet dependent methyltransferase. In the cytosol from the ΔYBR261C/*tae1* strain no radioactivity migrated with the peptide. This result shows that the synthetic peptide is a good substrate for the endogenous methyltransferase. We also detected methylation of the peptide with the cytosolic extract of mouse heart, a tissue containing high transcript levels of the METTL11A gene (Fig. 4C).

Using this validated assay, we next directly tested whether the purified recombinant forms of YBR261C/Tae1 and the human homolog METTL11A prepared as fusion proteins in *E. coli* could independently methylate the synthetic peptide. In a control, we showed no activity with an unrelated fusion protein prepared in parallel from *E. coli*. As shown in Fig. 4D for the yeast protein and in Fig. 4E for the human protein, both were capable of rapidly transferring methyl groups from [^{3}H]AdoMet to the peptide. This result now clearly demonstrates that YBR261C/Tae1 and METTL11A are methyltransferases that directly catalyze the methylation reaction in the absence of other protein species. We have not been able to prepare a stable recombinant form of human METTL11B; whether this protein is also a similar methyltransferase remains to be seen.

To determine the extent of methylation catalyzed by these enzymes, the PPKQQLSKY peptide was incubated with recombinant YBR261C/Tae1 or recombinant METTL11A for an extended time in the presence of a high concentration of AdoMet to drive methylation to completion (Fig. 5). Both recombinant YBR261C/Tae1 and recombinant METTL11A were found to completely dimethylate the PPKQQLSKY peptide (Fig. 5A, B, and C). The N-terminal location of the methylation was verified by MSMS analysis of the peptide product, localizing the dimethylation site within the first two proline residues (data not shown).

Taken together, these results indicate that YBR261C/Tae1 and METTL11A are N-terminal methyltransferases that catalyze the formation of N-terminal dimethylproline residues.

Substrate Specificity of N-terminal Protein Methyltransferases

Non-chloroplast eukaryotic proteins, including Rpl12ab and Rps25a/Rps25b, are modified by N-terminal methylation on a common sequence motif (Table 1). In each of these proteins, an N-terminal proline, alanine or serene residue is followed by a Pro-Lys sequence. The conservation of the X-Pro-Lys sequence has led to the postulation that one enzyme may be responsible for the modification of all of these proteins (2). In this regard, recent results of the modification of the human RCC1 protein containing a Ser-Pro-Lys N-terminal sequence are of interest (5). Here, RCC1 was found to be mono, di, and trimethylated at the N-terminal serine residue. The authors expressed variants of the N-terminal sequence in HeLa cells where proline and alanine residues replaced the serine residue. They found the proline form was completely dimethylated; the alanine and serine forms were found in states of methylation from unmodified to trimethylated (5). The authors also found that changing the residues in the second and third position had a large effect on methylation.

These results encouraged us to directly test the specificity of the YBR261C/Tae1 and METTL11A forms of the N-terminal methyltransferase in both cytosolic extracts and as purified recombinant proteins. When the N-terminal proline of the Rps25a/Rps25b-derived peptide PPKQQLSKY was replaced with an alanine or a serine residue and incubated with [^{3}H]AdoMet and recombinant YBR261C/Tae1 (Fig. 4D) or recombinant METTL11A (Fig. 4E), peptide methylation was observed but to a lower extent than with the N-terminal proline peptide. Methylation of the N-terminal variants APKQQLSKY and SPKQQLSKY was greatly reduced compared to PPKQQLSKY in cytosolic extracts of yeast (Fig. 4A, B). These results suggest that the yeast enzyme appears to prefer the Pro-Pro-Lys N-terminal sequence over the Ala-Pro-Lys or Ser-Pro-Lys sequence. The relative extent of methylation of the Ala-Pro-Lys and Ser-Pro-Lys peptides in Fig. 4D may be overestimated with

recombinant enzyme because methylation may have been limited by the amount of AdoMet present. The mammalian enzyme may be less specific because relatively more methylation of the Ala-Pro-Lys and Ser-Pro-Lys species is seen with the recombinant METTL11A enzyme compared to the yeast recombinant enzyme (Fig. 4D, E). This trend is also observed when mouse heart cytosol is used as an enzyme source (Fig. 4C), although it is possible that other methyltransferase species (such as METT11B, see Fig. 3) may contribute to the modifications. The absence of methylation of any of the peptides catalyzed by the yeast cytosol from the ΔYBR261c/*tae1* deletion strain suggests that only one N-terminal methyltransferase of this type is present in yeast.

The extent of methylation of the APKQQLSKY and SPKQQLSKY species was determined by incubating peptide with recombinant YBR261C/Tae1 or recombinant METTL11A for an extended time in order to drive methylation to completion, as described in the legend of Fig. 5. Recombinant YBR261C/Tae1 and recombinant METTL11A nearly completely trimethylated the APKQQLSKY peptide, with only a small fraction remaining in the dimethylated form (Fig. 5D, E, F). The methylation of SPKQQLSKY was less extensive, with YBR261C/Tae1 producing mono, di, and trimethylated forms (Fig. 5H). METTL11A predominately trimethylated the SPKQQLSKY peptide (Fig. 5I). Taken together these data suggest that the human METTL11A methyltransferase, and to a slightly lesser extent the YBR261c/Tae1 methyltransferase, can recognize species with N-terminal alanine and serine residues in addition to those with proline residues.

Enzyme preference for the amino acid in the first position of the N-terminal X-Pro-Lys sequence was further tested by comparing the methylation catalyzed by recombinant YBR261C/Tae1 or recombinant METTL11A with mixtures of synthetic peptides designed to contain equal amounts of each of the 20 amino acids at the first position (X_{20}PKQQLSKY) or each of the twenty amino acids at the first position except proline and alanine (X_{18}PKQQLSKY) (Fig. 6A). The substantial incorporation of radioactivity into the mixed peptides, where each species is present at only 1/20 or 1/18 the concentration of the unmixed peptides, suggests that these enzymes can recognize substrates with a variety of amino acids in the first position. However, in yeast cytosol where the activity is present at lower levels, the highest methylation levels are seen in the PPKQQLSKY peptide suggesting that the proline residue in position one is a preferred substrate (Fig. 6B). In mouse heart cytosol, each of these peptide mixtures is recognized to a similar level (Fig. 6C). As described above, this may reflect the activity of additional methyltransferases such as METT11B. The recombinant enzyme reactions were repeated using the X_{20}PKQQLSKY and X_{18}PKQQLSKY mixtures in the reaction conditions described in Fig. 5 and subjected to MALDI analysis. Although the analysis of the data was limited by overlapping signals in the MALDI spectrum, signals were detected for the methylation of peptides with alanine, proline, and glycine at the N-terminal position (data not shown).

Finally, we investigated the effect of substituting other amino acids for either the proline residue in the second position or the lysine residue in the third position of the X-Pro-Lys N-terminal sequence. With the recombinant yeast and human methyltransferases, we found little or no methylation over background for a peptide mixture containing each of the twenty amino acids except lysine at the third position (Fig. 6A) (PPX_{19}QQLSKY). A very small amount of methylation was observed for the peptide mixture containing each of the twenty amino acid except proline at position two (Fig. 6A) (PX_{19}KQQLSKY). Similarly, we observed little or no methylation over background for either of these peptide mixtures for yeast or mouse heart cytosolic preparations (Fig 6B, C). These results suggest that the major specificity factor for recognition of N-terminal polypeptides by the yeast YBR261C/Tae1 or the human METTL11A enzymes is the presence of a proline residue in position 2 and a lysine residue in position 3. All of the known N-terminally methylated species in eukaryotes

contain this motif. We thus designate this enzyme Ntm1 as the X-Pro-Lys N-terminal protein methyltransferase.

DISCUSSION

Phenotypes of Yeast Lacking the X-Pro-Lys N-terminal Protein Methyltransferase

A phenotype of increased sensitivity to paromomycin, an antibiotic from *Streptomyces* that targets the ribosome, was previously found for the YBR261C/*tae1* deletion strain in a functional genomics profile of yeast non-essential genes (29). This result suggests that the modification may be important in helping to protect yeast against microbial attack. Further analysis of this mutant showed an increased sensitivity to the histidine biosynthesis inhibitor 3-amino-1,2,4-triazole, a reduction in polysomes, and a corresponding increase in the 80 S and 60 S subunits as compared to wild type strain. In addition, the mutant demonstrated decreased translational efficiency and fidelity. For these reasons, the YBR261C gene was designated *TAE1* for "**T**ranslational **A**ssociated **E**lement 1" (29). However, we note that these phenotypes may not be a direct affect of loss of N-terminal methylation on ribosomal proteins Rpl12ab and Rps25a/Rps25b. In addition to these three ribosomal proteins, there are 16 other yeast proteins with the (Pro/Ala/Ser)-Pro-Lys N-terminal methyltransferase recognition sequence including the ribosomal associated protein Tma46, the Lsg1 protein involved in 60S ribosomal subunit biosynthesis, and the Loc1 60 S ribosomal subunit assembly protein (Supplementary Table 2). All of these proteins may be modified by N-terminal methylation and their function(s) may be affected by the loss of the YBR261C/Tae1 methyltransferase. It will be important to determine whether some or all of these 16 yeast proteins with N-terminal (Pro/Ala/Ser)-Pro-Lys sequences are in fact methylated at the N-terminus in reactions catalyzed by YBR261C/Tae1.

Biological Significance of N-terminal Methylation in Other Cell Types

In Drosophila cells, evidence has been presented that the degree of monomethylation of the N-terminal proline residue of histone H2B can be increased by heat shock treatment (15). This result opens the possibility that this methylation reaction can be regulated as a response to stress. In human cell lines N-terminal methylation of a serine residue in the Regulator of Chromatin Condensation 1 (RCC1) protein has been observed to yield mono, di, and trimethylated derivatives (5). RCC1 is a guanine nucleotide-exchange factor that plays roles in protein transport through the nuclear envelope, nuclear envelope assembly, and mitosis. In higher primates the N-terminal sequence of RCC1 after methionine removal is Ser-Pro-Lys and in other mammals it is Pro-Pro-Lys. N-terminal serine residues are often targets of acetylation (24), raising the possibility that there may be competition between the acetylation and methylation reactions. However, at least in yeast, it is clear that none of the three N-terminal acetyltransferases catalyze modification at N-terminal Ser-Pro (or Ala-Pro, Cys-Pro, Thr-Pro, or Val-Pro) sequences (24), thus making the alpha-amino group potentially available to the methyltransferase. RCC1 mutants with the lysine at position 3 substituted with a glutamine residue blocked N-terminal methylation and resulted in less efficient chromatin binding during mitosis and increased spindle-pole defects as compared to wild type protein (5). *In vitro* DNA binding experiments using methylated and unmodified RCC1 peptides containing the first 34 amino acids showed an increased affinity for DNA when the N-terminal methylation was present (5).

N-terminal methylation may also protect proteins from attack by aminopeptidases(2). Interestingly the human X-prolyl aminopeptidase, XPNPEP1, has a Pro-Pro-Lys N-terminal sequence that may lead to its methylation and thus protection against self digestion. In Supplemental Table 3, we list human proteins that have the N-terminal (Pro/Ala/Ser)-Pro-

Lys recognition sequence for the N-terminal methyltransferase all of these proteins are candidates for modification.

Prokaryotic Protein N-Methylation Reactions

The *E. coli* large subunit ribosomal protein L11 is a distant homolog of the yeast Rpl12ab protein. Interestingly, L11 is α-*N*-trimethylated at its N-terminal alanine residue and is ε-*N*-trimethylated at Lys-3 and Lys-39 in reactions reportedly catalyzed by the PrmA methyltransferase as reviewed in Ref. 4. This situation is remarkably similar to the dimethylation of the N-terminal proline residue and the trimethylation of Lys-3 in yeast Rpl12ab. However the N-terminal sequence of L11 is Ala-Lys-Lys, different from the Pro-Pro-Lys sequence seen in yeast and higher eukaryotes. Additionally, there is very little sequence similarity between PrmA and YBR261C/Tae1 or METTL11A. BLAST searches reveal that non-related methyltransferases in yeast and human are more similar to PrmA than the N-terminal methyltransferases. The three methylated L11 residues in *E. coli* are apparently modified by the single PrmA methyltransferase, while N-terminal methylation in yeast Rpl12ab is dependent on the YBR261C/Tae1 protein, the trimethylation at Lys 3 is dependent upon Rkm2 and the monomethylation at Arg 66 is dependent upon Rmt2.

N-terminal methylation also occurs in four other *E. coli* ribosomal proteins. Monomethylmethionine is found in L16 and L33, while monomethylalanine is found in L33 and S11 (4). Monomethylmethionine is also found in the *E. coli* initiator factor IF3 and in the CheZ chemotaxis protein (2). Interestingly, monomethylalanine (22) and monomethylmethionine (23) have been found in eukaryotic chloroplast proteins, possibly reflecting the prokaryotic origin of this organelle (Table 1). The enzymes catalyzing these reactions have not been described to date. However, the modification of pilin proteins from a number of prokaryotic species by monomethylation at the N-terminal phenylalanine residues has been shown to be catalyzed by a bifunctional enzyme that results in endopeptidic cleavage of a leader peptide followed by the methylation reaction (38). A similar enzyme may be present in chloroplasts (6). Importantly, none of these proteins share the X-Pro-Lys N-terminal sequence (2). These results suggest that a distinct enzymatic mechanisms are used for prokaryotic and chloroplast N-terminal methylation reactions. Finally, we note that major targets of N-terminal methylation reactions in both prokaryotes and eukaryotes include proteins in translation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Drs. Joseph Loo, Jane Strouse, and Kym Faull for their continuing support and helpful advice on mass spectrometry.

Abbreviations

The abbreviations used are:

[^{3}H]AdoMet	*S*-adenosyl-L-[*methyl*-^{3}H]methionine
AdoMet	*S*-adenosyl-L-methionine
FTICR	Fourier-transform ion cyclotron resonance
MSMS	tandem mass spectrometry

References

1. Wold F. In vivo chemical modification of proteins (post-translational modification). Annu Rev Biochem. 1981; 50:783–814. [PubMed: 6791580]
2. Stock A, Clarke S, Clarke C, Stock J. N-terminal methylation of proteins: structure, function and specificity. FEBS Lett. 1987; 220:8–14. [PubMed: 3301412]
3. Arnesen T, Van Damme P, Polevoda B, Helsens K, Evjenth R, Colaert N, Varhaug JE, Vanderkerckhove J, Lillehaug JR, Sherman F, Gevaert K. Proteomics analyses reveal the evolutionary conservation and divergence of N-terminal acetyltransferases from yeast and humans. Proc Natl Acad Sci U S A. 2009; 106:8157–8162. [PubMed: 19420222]
4. Polevoda B, Sherman F. Methylation of proteins involved in translation. Mol Microbiol. 2007; 65:590–606. [PubMed: 17610498]
5. Chen T, Muratore TL, Schaner-Tooley CE, Zhabanowitz J, Hunt DF, Macara IG. N-terminal alpha-methylation of RCC1 is necessary for stable chromatin association and normal mitosis. Nat Cell Biol. 2007; 5:596–603. [PubMed: 17435751]
6. Houtz RL, Magnani R, Nayak NR, Dirk LMA. Co- and post-translational modifications in Rubisco: unanswered questions. J Exp Bot. 2008; 59:1635–1645. [PubMed: 18353761]
7. Sadaie M, Shinmyozu K, Nakayama J. A conserved SET domain methyltransferase, Set11, modifies ribosomal protein Rpl12 in fission yeast. J Biol Chem. 2008; 283:7185–7195. [PubMed: 18195021]
8. Carroll AJ, Heazlewood JL, Ito J, Millar AH. Analysis of the Arabidopsis cytosolic ribosome proteome provides detailed insights into its components and their post-translational modification. Mol Cell Proteomics. 2008; 2:347–369. [PubMed: 17934214]
9. Webb KJ, Laganowsky A, Whitelegge JP, Clarke SG. Identification of two SET domain proteins required for methylation of lysine residues in yeast ribosomal protein Rpl42ab. J Biol Chem. 2008; 283:35561–35568. [PubMed: 18957409]
10. Meng F, Du Y, Miller LM, Patrie SM, Robinson DE, Kelleher NL. Molecular-level description of proteins from *Saccharomyces cerevisiae* using quadrupole FT hybrid mass spectrometry for top down proteomics. Anal Chem. 2004; 76:2852–2858. [PubMed: 15144197]
11. Martinage A, Briand G, van Dorsselaer A, Turner CH, Sautiere P. Primary structure of histone H2B from gonads of the starfish Asterias rubens. Identification of an N-dimethylproline residue at the amino-terminal. Eur J Biochem. 1985; 147:351–359. [PubMed: 3882426]
12. Kmiecik D, Belaiche D, Sautiere P, Loucheux-Lefebvre MH, Kerckaert JP. Complete sequence of *Sipunculus nudus* erythrocyte histone H2B and its gene. Identification of an N,N-dimethylproline residue at the amino-terminus. Eur J Biochem. 1991; 198:275–283. [PubMed: 2040294]
13. Shimizu T, Hozumi K, Horiike S, Nunomura K, Ikegami S, Takao T, Shimonishi Y. A covalently crosslinked histone. Nature. 1996; 380:32–32. [PubMed: 8598899]
14. Strickland M, Strickland WN, von Holt C. The histone H2B from the sperm cell of the starfish *Marthasterias glacialis*. Eur J Biochem. 1980; 106:541–548. [PubMed: 7398625]
15. Desrosiers R, Tanguay RM. Methylation of Drosophila histones at proline, lysine, and arginine residues during heat shock. J Biol Chem. 1988; 263:4686–4692. [PubMed: 3127388]
16. Smith GM, Pettigrew GW. Identification of N,N-dimethylproline as the N-terminal blocking group of *Crithidia oncopelti* cytochrome c557. Eur J Biochem. 1980; 110:123–130. [PubMed: 6254758]
17. Hill GC, Pettigrew GW. Evidence for the amino-acid sequence of *Crithidia fasciculata* cytochrome c555. Eur J Biochem. 1975; 57:265–271. [PubMed: 170111]
18. Bergmüller E, Gehrig PM, Gruissem W. Characterization of post-translational modifications of histone H2B-variants isolated from *Arabidopsis thaliana*. J Proteome Res. 2007; 6:3655–3668. [PubMed: 17691833]
19. Medzihradszky KF, Zhang X, Chalkley RJ, Guan S, McFarland MA, Chalmers MJ, Marshall AG, Diaz RL, Allis CD, Burlingame AL. Characterization of *Tetrahymena* histone H2B variants and posttranslational populations by electron capture dissociation (ECD) Fourier transform ion cyclotron mass spectrometry (FT-ICR MS). Mol Cell Proteomics. 2004; 3:872–886. [PubMed: 15199121]
20. Nomoto M, Kyogoku Y, Iwai K. N-Trimethylalanine, a novel blocked N-terminal residue of *Tetrahymena* histone H2B. J Biochem. 1982; 92:1675–1678. [PubMed: 6818230]

21. Henry GD, Dalgarno DC, Marcus G, Scott M, Levine BA, Trayer IP. The occurrence of alpha-N-trimethylalanine as the N-terminal amino acid of some myosin light chains. FEBS Lett. 1982; 144:11–15. [PubMed: 7106295]
22. Kamp RM, Srinivasa BR, von Knoblauch K, Subramanian AR. Occurrence of a methylated protein in chloroplast ribosomes. Biochemistry. 1987; 26:5866–5870.
23. Grimm R, Grimm M, Eckerskorn C, Pohlmeyer K, Rohl T, Soll J. Postimport methylation of the small subunit of ribulose-1,5-bisphosphate carboxylase in chloroplasts. FEBS Lett. 1997; 408:350–354. [PubMed: 9188792]
24. Polevoda B, Sherman F. N-terminal acetyltransferases and sequence requirements for N-terminal acetylation of eukaryotic proteins. J Mol Biol. 2003; 325:595–622. [PubMed: 12507466]
25. Porras-Yakushi TR, Whitelegge JP, Clarke SG. A novel SET domain methyltransferase modifies ribosomal protein Rpl23ab in yeast. J Biol Chem. 2005; 280:34590–34598. [PubMed: 16096273]
26. Porras-Yakushi TR, Whitelegge JP, Clarke SG. A novel SET domain methyltransferase in yeast: Rkm2-dependent trimethylation of ribosomal protein L12ab at lysine 10. J Biol Chem. 2006; 281:35835–35845. [PubMed: 17005568]
27. Lipson RS, Webb KJ, Clarke SG. Rmt1 catalyzes zinc-finger independent arginine methylation of ribosomal protein Rps2 in Saccharomyces cerevisiae. Biochem Biophys Res Commun. 2010; 391:1658–1662. [PubMed: 20035717]
28. Petrossian TC, Clarke S. Bioinformatic identification of novel methyltransferases. Epigenomics. 2009; 1:163–175. [PubMed: 20582239]
29. Alamgir M, Eroukova V, Jessulat M, Xu J, Golshani A. Chemical-genetic profile analysis in yeast suggests that a previously uncharacterized open reading frame, YBR261C, affects protein synthesis. BMC Genomics. 2008; 9:583–596. [PubMed: 19055778]
30. Lowenson JD, Kim E, Young SG, Clarke S. Limited accumulation of damaged proteins in L-isoaspartyl (D-aspartyl) *O*-methyltransferase-deficient mice. J Biol Chem. 2001; 276:20695–20702. [PubMed: 11279164]
31. Bailey, J. Techniques in Protein Chemistry. 2. Elsevier Science Publishers B. V.; Amsterdam: 1967.
32. Chern MK, Chang KN, Liu LF, Tam TC, Liu YC, Liang YL, Tam MF. Yeast ribosomal protein L12 is a substrate of protein-arginine methyltransferase 2. J Biol Chem. 2002; 277:15345–15353. [PubMed: 11856739]
33. Niewmierzycka A, Clarke S. *S*-Adenosylmethionine-dependent methylation in *Saccharomyces cerevisiae. Identification of a novel protein arginine methyltransferase*. J Biol Chem. 1999; 274:814–824. [PubMed: 9873020]
34. Katz JE, Dlakic M, Clarke S. Automated identification of putative methyltransferases from genomic open reading frames. Mol Cell Proteomics. 2003; 2:525–540. [PubMed: 12872006]
35. Petrossian TC, Clarke SG. Multiple Motif Scanning to identify methyltransferases from the yeast proteome. Mol Cell Proteomics. 2009; 8:1516–1526. [PubMed: 19351663]
36. Pesavento JJ, Bullock CR, LeDuc RD, Mizzen CA, Kelleher NL. Combinatorial modification of human histone H4 quantitated by two-dimensional liquid chromatography coupled with top down mass spectrometry. J Biol Chem. 2008; 283:14927–14937. [PubMed: 18381279]
37. Yu Y, Ji H, Doudna JA, Leary JA. Mass spectrometric analysis of the human 40S ribosomal subunit: Native and HCV IRES-bound complexes. Protein Sci. 2005; 14:1438–1446. [PubMed: 15883184]
38. Lory S, Storm MS. Structure-function relationship of type-IV prepilin peptidase of *Pseudomonas aeruginosa*--a review. Gene. 1997; 192:117–121. [PubMed: 9224881]

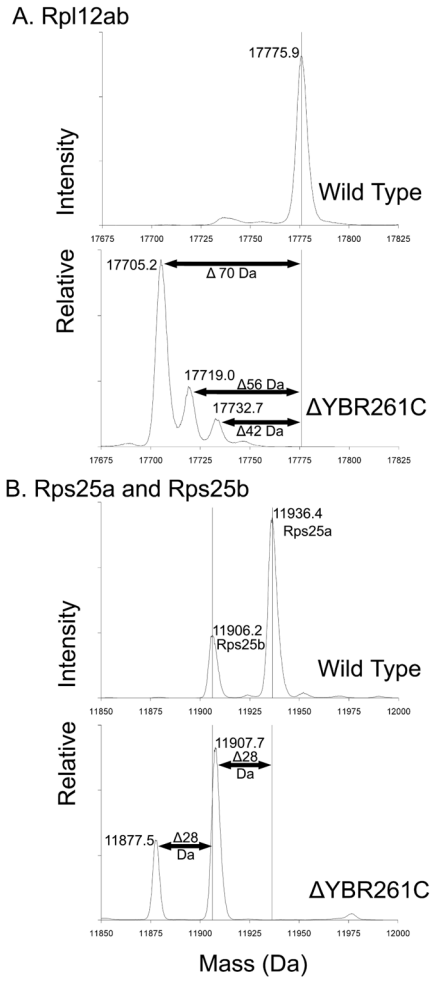

FIGURE 1.

Loss of methylation of Rpl12ab, Rps25a/Rps25b in yeast ΔYBR261C/*tae1* mutants. Ribosomal proteins were isolated and separated by reverse phase HPLC from wild type (BY4742) and a YBR261C/*tae1* deletion mutant described in the “Experimental Procedures” section. The HPLC effluent was directed to the source of an electrospray mass spectrometer (QSTAR Elite). The resulting deconvoluted MS spectra are displayed with the average masses of the significant peaks shown. Changes in mass of the proteins from mutant and wild type cells are indicated by double arrows.

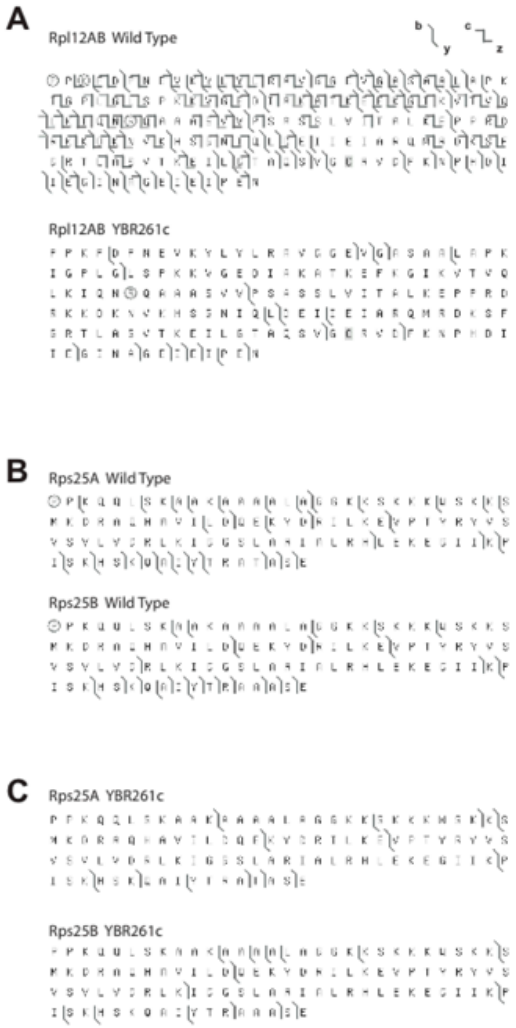

FIGURE 2.

Localization of methylation sites in intact ribosomal proteins. Fragmentation patterns for ribosomal protein Rpl12ab from the wild type (BY4742) strain and for the major form of Rpl12ab from the ΔYBR261C/*tae1* strain are shown in panel A. Similar fragmentation patterns for ribosomal proteins Rps25a/Rps25b from wild type (BY4742) and ΔYBR261C/*tae1* strains are shown in panels B and C, respectively. “b” type cleavages are shown by the upper hash mark, “y” type cleavages are shown by the lower hash mark, “c” type cleavages are indicated by the upper horizontal line, and “z” type cleavages are indicated by the lower horizontal line. Ribosomal proteins were isolated by reverse phase HPLC as described under “Experimental Procedures.” Fractions containing Rpl12ab and Rps25a/Rps25b isolated from wild type and ΔYBR261C/*tae1* strains were subjected to top down analysis where the intact protein was collisionally activated to produce fragment ions. Dimethylation of the N-terminal proline residue in Rpl12ab and Rps25a/Rps25b is represented by the circled P, trimethylation of lysine 3 of Rpl12ab is indicated by the circled K, and monomethylation of arginine 66 of Rpl12ab is indicated by the circled R.

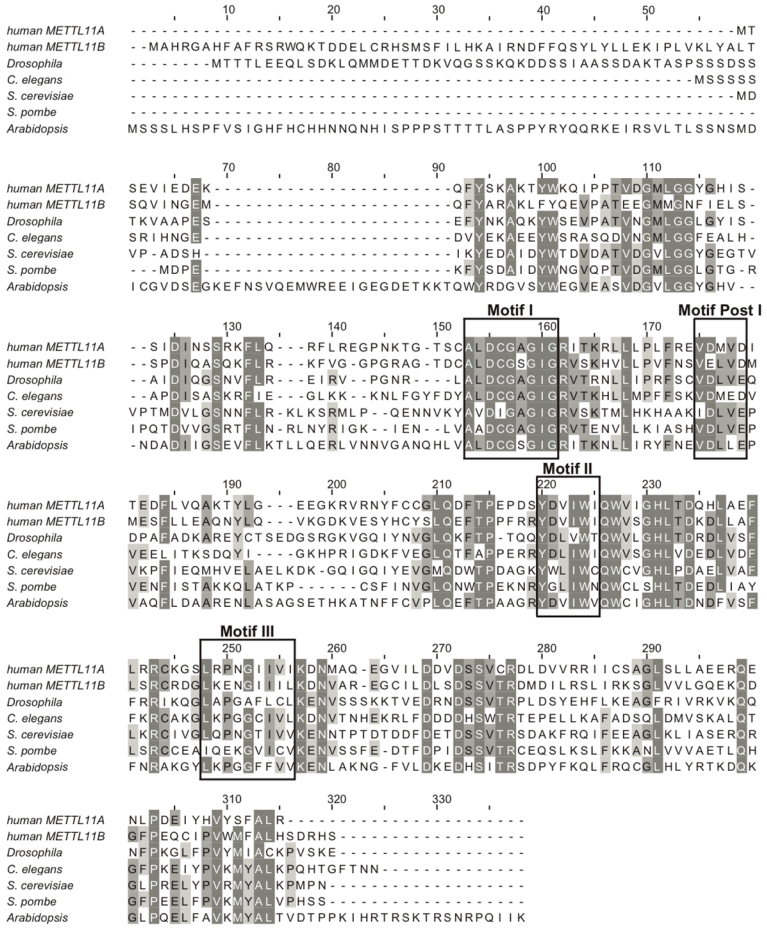

FIGURE 3.

Yeast YBR261C/Tae1 homologs in eukaryotes. The signature methyltransferase motifs common to all class I protein methyltransferases are boxed. The accession numbers used in this alignment and the corresponding BLAST expect values related to the yeast YBR261C/Tae1 protein are as follows: human METTL11A (*Homo sapiens*, NP_054783, expect value 6e-36), human METTL11B (*Homo sapiens*, NP_001129579, expect value 6e-27), Drosophila (*Drosophila melanogaster*, NP_610528, expect value 9e-37), *C. elegans* (*Caenorhabditis elegans*, NP_490660, expect value 3e-35), *S. cerevisiae* (*Saccharomyces cerevisiae*, NP_009820), *S. pombe* (*Schizosaccharomyces pombe*, NP_594227, expect value 1e-49), Arabidopsis (*Arabidopsis thaliana*, NP_199258, expect value 2e-42).

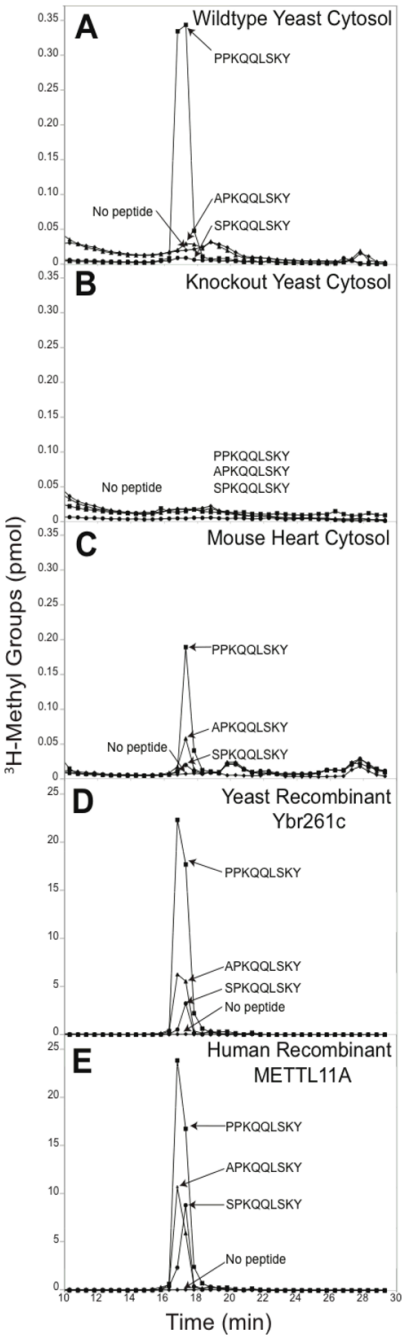

FIGURE 4.

Specificity of methylation for synthetic peptides containing the X-Pro-Lys N-terminal sequence. Nonapeptides (22 μg, 100 μM) containing the first eight amino acids of the N-terminus of Rps25a/Rps25b or with alanine or serine substituted at the N-terminal position were incubated with cytosolic extracts containing 90 μg of protein in the presence of 0.5 μM [^{3}H] AdoMet in a final volume of 200 μl for 1 h and then subjected to reverse phase HPLC as described under “Experimental Procedures.” (A) Cytosol from yeast wild type BY4742 cells was incubated at 30 °C; (B) cytosol from yeast ΔYBR261C/*tae1* cells was incubated at 30 °C; (C) cytosol from mouse heart was incubated at 37 °C. Radioactivity was detected by adding 75 μl from each HPLC fraction to 10 ml of scintillation fluor and counted on a

Beckman LS6500 instrument; the number of methyl groups was determined from a calculated specific radioactivity of 78,000 cpm/pmol (50% counting efficiency). (D) Identical to A except the yeast extract was replaced with 1 μg of recombinant YBR261C/Tae1 fusion protein. (E) Identical to A except the yeast extract was replaced with 1 μg of recombinant human METTL11A fusion protein and the incubation temperature was 37 °C.

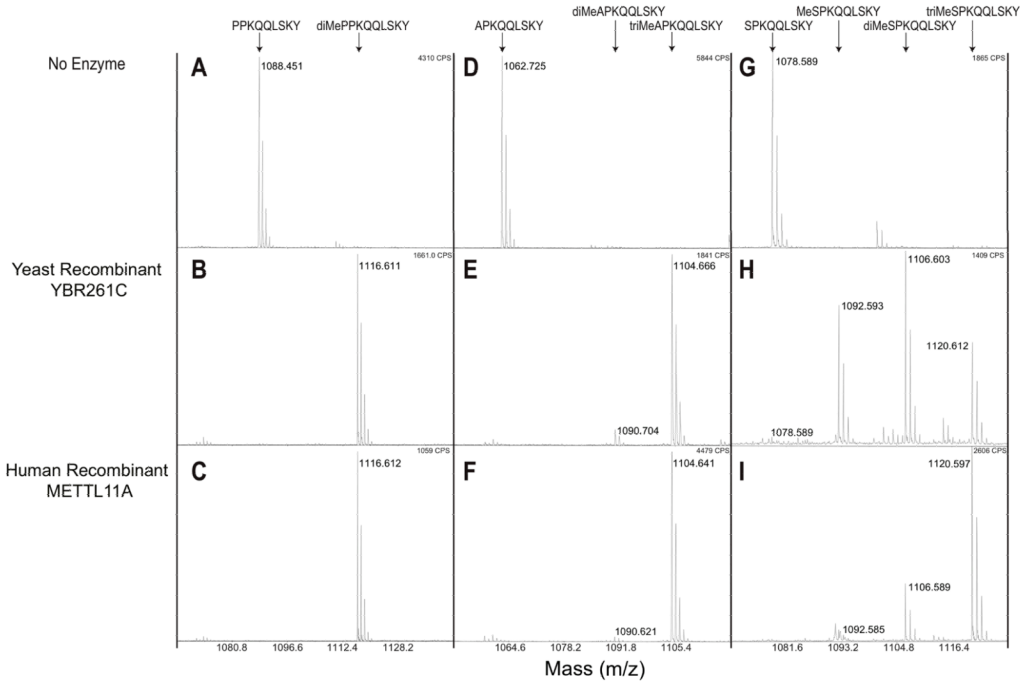

FIGURE 5.

Extent of modification of peptides with Pro-Pro-Lys, Ala-Pro-Lys, Ser-Pro-Lys N-terminal sequences by recombinant YBR261C/Tae1 and METTL11A. 10 μg of recombinant YBR261C/Tae1 or recombinant METTL11A were incubated with 10 μM of peptide in the presence of 200 μM AdoMet for 4.5 h at 30 °C and 37 °C, respectively. Shown are the MALDI mass spectra results of PPKQQLSKY(A, B, and C), APKQQLSKY (D, E, and F), SPKQQLSKY (G, H, and I) after incubation and HPLC purification with buffer only (No Enzyme, A, D and G), yeast recombinant YBR261C/Tae1 (B, E and H), or human recombinant METTL11A (C, F and I).

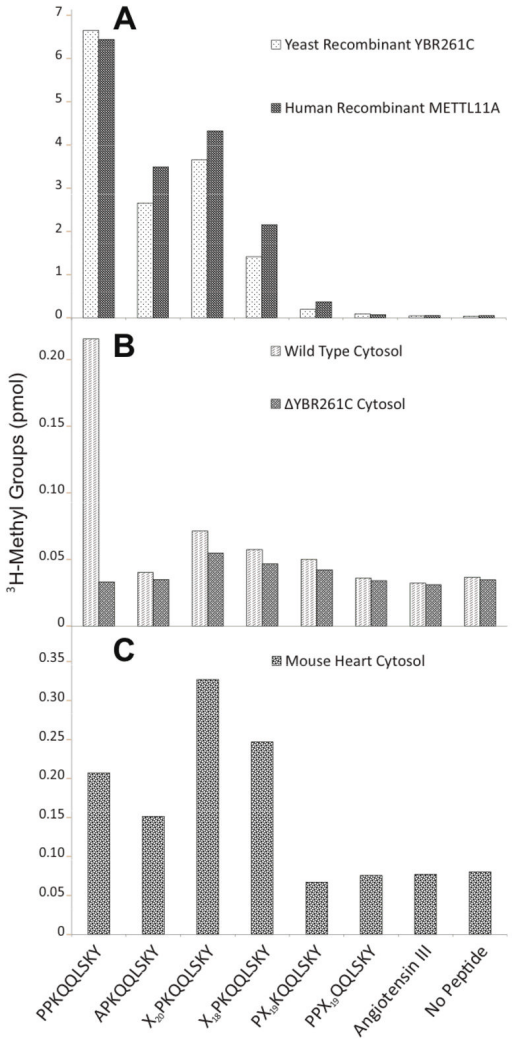

FIGURE 6.

Importance of the N-terminal X-Pro-Lys sequence in methyltransferase substrates. Recombinant YBR261c/Tae1, recombinant METTL11A (A), wild-type yeast cytosol, ΔYBR261C/*tae1* yeast cytosol (B), and mouse heart cytosol (C) were incubated at the same concentrations as in Fig. 4 with 22 μg (~100 μM) of a synthetic peptide (PPKQQLSKY or APPKQQLSKY) or synthetic peptide mixtures based on these sequences. The mixtures included one containing all twenty amino acids at position one (denoted X_{20}PKQQLSKY), one containing all twenty amino acids at position one except proline and alanine (denoted X_{18}PKQQLSKY), one all amino acids at position two except proline (denoted PX_{19}KQQLSKY), and one containing all amino acids at position three except lysine (denoted PXK_{19}QQLSKY). Reactions were carried out in a final volume of 200 μl for 1 h in the presence of 0.5 μM [^{3}H] AdoMet at 37° C for the human and mouse enzymes and at 30° C for the yeast enzymes as described in the “Experimental Procedures” section. A portion (50 μl) of the reaction mixture was applied to the HPLC column as described in the “Experimental Procedures” section and radioactivity was detected using a Beta-RAM model 5 inline scintillation counter under low flow mode with a window of 2 s for all samples except the mouse cytosol where the window was 5 s (LabLogic Inc.). Radioactivity was summed over the elution times of all peptides used (from minute 12.5 to minute 20.5); the

number of methyl groups was determined from a calculated specific radioactivity of 62,400 cpm/pmol (40% counting efficiency).

Table 1

Known Eukaryotic N-Terminally Methylated Proteins

Protein Name	N-Terminal Encoded Sequence	Organism	Known N-terminal Modification and Reference
Rpl12	MPPKFDPNEV	*Schizosaccharomyces pombe*	N,N-dimethylproline (7)
L12	MPPKLDPSQI	*Arabidopsisthaliana*	N,N-dimethylproline(8)
Rpl12ab	MPPKFDPNEV	*Saccharomyces cerevisiae*	N,N-dimethylproline (9)
Rps25a/Rps25b	MPPKQQLSKA	*Saccharomyces cerevisiae*	N,N-dimethylproline (10)
Histone H2B	MPPKPSGKGQ	*Asterias rubens*	N,N-dimethylproline (11)
Histone H2B	MPPKPSAKGA	*Sipunculus nudus*	N,N-dimethylproline (12)
Histone H2B	MPPKPSGKGQ	*Asterinapectinifera*	N,N-dimethylproline (13)
Histone H2B	MPPKSGKGQK	*Marthasterias glacialis*	N,N-dimethylproline (14)
Histone H2B	MPPKTSGKAA	*Drosophila melanogaster*	N-methylproline(15)
Cytochrome c-557	MPPKAREPLP	*Crithidia oncopelti*	N,N-dimethylproline (16)
Cytochrome c-557	MPPKARAPLP	*Crithidia fasciculata*	N,N-dimethylproline (17)
Histone H2B	MAPKAEKKPA	*Arabidopsis thaliana*	N-methyl; N,N-dimethylalanine; N,N,N-trimethylalanine (18)
Histone H2B	MAPKKAPAAA	*Tetrahymena thermophila*	N,N,N-trimethylalanine (19)
Histone H2B	MAPKKAPAAA	*Tetrahymena pyriformis*	N,N,N-trimethylalanine (20)
Fast skeletal myosin light chain 2	MAPKKAKRRA	*Oryctolagus cuniculus*	N,N,N-trimethylalanine (21)
Myosin light chain 1, skeletal muscle isoform	MAPKKDVKKP	*Oryctolagus cuniculus*	N,N,N-trimethylalanine (21)
Myosin light chain 3	MAPKKPDPKK	*Bos taurus*	N,N,N-trimethylalanine (21)
Regulator of chromosome condensation 1	MSPKRIAKRR	*Homo sapiens*	N-methylserine; N,N-dimethylserine; N,N,N-trimethylserine (5)
Chloroplast ribosomal protein L2	MAIHLYKTST	*Spinacia oleracea*	N-methylalanine (22)
Chloroplast rubisco small subunit	MQVWPPIGKK	*Pisum sativum*	N-methylmethionine (23)