The Ribosomal L1 Protuberance in Yeast Is Methylated on a Lysine Residue Catalyzed by a Seven-β-strand Methyltransferase

Kristofer J. Webb1, Qais Al-Hadid1, Cecilia I. Zurita-Lopez, Brian D. Young, Rebecca S. Lipson, and Steven G. Clarke*

From the Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90095-1569

Modification of proteins of the translational apparatus is common in many organisms. In the yeast Saccharomyces cerevisiae, we provide evidence for the methylation of Rpl1ab, a well conserved protein forming the ribosomal L1 protuberance of the large subunit that functions in the release of tRNA from the exit site. We showed that the intact mass of Rpl1ab is 14 Da larger than its calculated mass with the previously described loss of the initiator methionine residue and N-terminal acetylation. We determined that the increase in mass of yeast Rpl1ab is consistent with the addition of a methyl group to lysine 46 using top-down mass spectrometry. Lysine modification was confirmed by detecting 3H-N-ε-monomethyllysine in hydrolysates of Rpl1ab purified from yeast cells radiolabeled in vivo with S-adenosyl-L-[methyl-3H]methionine. Mass spectrometric analysis of intact Rpl1ab purified from 37 deletion strains of known and putative yeast methyltransferases revealed that only the deletion of the YLR137W gene, encoding a seven-β-strand methyltransferase, resulted in the loss of the +14-Da modification. We expressed the YLR137W gene as a His-tagged protein in Escherichia coli and showed that it catalyzes N-ε-monomethyllysine formation within Rpl1ab on ribosomes from the ΔYLR137W mutant strain lacking the methyltransferase activity but not from wild-type ribosomes. We also showed that the His-tagged protein could catalyze monomethyllysine formation on a 16-residue peptide corresponding to residues 38–53 of Rpl1ab. We propose that the YLR137W gene be given the standard name RKM5 (ribosomal lysine (K) methyltransferase 5). Orthologs of RKM5 are found only in fungal species, suggesting a role unique to their survival.

Proteins of the eukaryotic translational apparatus are often targets for post-translational covalent modifications (1). One of the major types of these reactions is the transfer of methyl groups from S-adenosylmethionine to a variety of residues including arginine (2–5), lysine (6–11), glutamine (12), histidine (13), and N-terminal (14, 15) residues. Ribosomal proteins (1–5, 7–11, 13, 14, 16), elongation factor 1A (1, 6, 17), and translational release factors (12) are common substrates of protein methyltransferases. These modifications are known to enhance resistance to ribosome-targeting antibiotics, facilitate ribosomal protein transport into and out of the nucleus, allow efficient ribosomal assembly, and increase translational accuracy (1, 5).

We have been interested in understanding the role of protein methylation in ribosomal function and assembly in Saccharomyces cerevisiae using a proteomics-guided approach. Previous efforts have identified modifications within proteins of the large ribosomal subunit. Interestingly, mass spectrometric analysis suggested that six proteins, Rpl1ab, Rpl3, Rpl12ab, Rpl23ab, Rpl42ab, and Rpl43ab, were subject to methylation (18). Further analysis has localized the methylation sites on four of these proteins. Rpl3 is modified to form a 3-methylhistidine residue at position 243 (13), whereas Rpl12ab is modified at three positions: a dimethylproline residue is present at the N terminus (14), an ε-trimethyllysine residue is found at position 3 (9, 19), and an unusual δ-monomethylarginine residue is found at position 66 (2). Rpl23ab is modified by ε-dimethyllysine formation at positions 105 and 109 (7, 17), and Rpl42ab is modified by ε-monomethyllysine formation at positions 39 and 55 (9). The proposed methylation at Rpl43ab has not been observed to date in our studies. The only protein remaining to be analyzed is Rpl1ab.

In this work, we demonstrate that Rpl1ab from S. cerevisiae is modified at lysine 46 by the addition of a single methyl group. This protein is a member of the ribosomal L1 family that is conserved in most organisms and forms the “L1 protuberance” that is involved in tRNA exit from the E site of the large subunit (20–22). This protein binds not only the large subunit rRNA, but its own mRNA, the latter presumably in a regulatory role (21, 23). Interestingly, the protein structure appears to be flexible; no clear electron density is seen for L1 in the 2.4 Å map of the large subunit from the archaeon Haloarcula marismortui (24).

We show here that methylation of the yeast L1 protein is absent in a deletion mutant of the putative seven-β-strand methyltransferase encoded by the YLR137W gene. We show that the expressed YLR137W protein can catalyze N-ε-monomethyllysine formation in yeast ribosomes containing

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S The on-line version of this article (available at http://www.jbc.org) contains supplemental Table S1.

1 Both authors contributed equally to this work.

2 To whom correspondence should be addressed: Dept. of Chemistry and Biochemistry and the Molecular Biology Institute, UCLA, 607 Charles E. Young Dr. East, Los Angeles, CA 90095-1569. Tel.: 310-825-8754; Fax: 310-825-1968; E-mail: clarke@mbi.ucla.edu.

3 Yeast ribosomal proteins are identified based on the gene designation. In cases where two genes encode identical protein products, the protein is designated “ab.” For example, the Rpl1ab protein is the identical translation product of the RPL1A and RPL1B genes.
Methyllysine Formation in Yeast Ribosomal Protein L1

unmethylated Rpl1ab and in a synthetic peptide corresponding to the methylated region on Rpl1ab. To date, most protein lysine methyltransferases have been identified as members of the SET domain methyltransferase family. However, the YLR137W gene product, now designated Rkm5, now joins four other protein lysine methyltransferases of the seven-β-strand superfamily: the Dot1 methyltransferase acting on histone H3 (25, 26), the CaM KMT acting on calmodulin (27), the See1 methyltransferase acting on elongation factor 1A (17), and the PrmA methyltransferase acting on prokaryotic ribosomal protein L11 (28).

EXPERIMENTAL PROCEDURES

Isolation of Ribosomal Proteins from S. cerevisiae Wild-type and Mutant Strains—The wild-type BY4742 and the ΔYLR137W/rkm5 deletion strain in the BY4742 background, as well as the deletion strains listed in supplemental Table S1, were obtained from the Saccharomyces Genome Deletion Project via Open Biosystems (Huntsville, AL). Intact S ribosomes and large ribosomal subunits were isolated from the wild-type and ΔYLR137W/rkm5 deletion strains as described previously (9). Proteins were extracted from the large ribosomal subunits with ethanol and acetic acid as described (29).

Localization of Methylation Sites by Top-down Mass Spectrometry of Intact Ribosomal Proteins—Ribosomal proteins extracted from the large subunit 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 30 ppm or better.

RESULTS

The YLR137W Gene Is Required for the Modification of the Yeast Ribosomal Protein Rpl1ab—In an effort to identify new protein methyltransferases, yeast strains with deletions of known and putative methyltransferase genes were screened for loss of ribosomal protein methylation. Ribosomal proteins from large subunits of wild-type and 37 yeast deletion strains (supplemental Table S1) were isolated as previously described (19) and analyzed for their intact mass by mass spectrometry after HPLC separation (9). For the Rpl1ab protein, a mass of 24,410.4 Da was detected in the wild-type strain, corresponding to its predicted mass after the loss of the initiator methionine residue (30), N-terminal acetylation (30), and the addition of a methyl group (14 Da) (Fig. 1). This mass is consistent with previous observations (18). An identical mass at a similar HPLC retention time was observed in 36 of the 37 putative methyltransferase gene deletion strains. In the case of the YLR137W deletion strain, however, the 24,410-Da species was not observed, and a new species was detected at 24,396 Da, corresponding to the loss of the methyl group (Fig. 1). The YLR137W gene product was originally identified as a putative S-adenosylmethionine-dependent methyltransferase by sequence analysis (31) and was recently classified as a member of the seven-β-strand methyltransferase superfamily (32). These results suggest that the YLR137W gene product is required for Rpl1ab methylation and that it may directly catalyze the transfer of the methyl group from S-adenosylmethionine to the ribosomal protein.

The Large Ribosomal Protein Rpl1ab Is Modified in a Region Containing Lysine 46—To localize the site of the 14-Da modification within Rpl1ab, intact mass top-down mass spectrometry analysis was performed. Unbiased assignment of post-translational modifications across the entire polypeptide chain is possible using this approach, making it ideal for the assignment
Methylylsine Formation in Yeast Ribosomal Protein L1

FIGURE 1. Intact mass mass reconstruction of the large ribosomal protein Rpl1ab from wild-type (BY4742) and ΔYLR137W/rkm5 deletion strains. Large ribosomal proteins were isolated from wild-type (BY4742) and the ΔYLR137W/rkm5 deletion strains using high salt sucrose gradients as described under “Experimental Procedures.” The proteins were separated from the rRNA and further fractionated using reverse phase HPLC. The resulting effluent was coupled to an electrospray mass spectrometer (QSTAR Elite), and the intact mass of each protein was measured. The resulting MS spectra of the Rpl1ab proteins were deconvoluted to yield the average mass of the protein. The loss of 14 Da, corresponding to the loss of a methylation event, is indicated.

FIGURE 2. Localization of the modification site on the Rpl1ab ribosomal protein by intact fragmentation. Rpl1ab was isolated from wild-type (BY4742) yeast strains as described under “Experimental Procedures.” Rpl1ab was subjected to top-down analysis where the intact protein was fragmented using collisionally activated dissociation producing “b” and “y” type cleavages (shown by upper and lower hash marks) or electron capture dissociation producing “c” and “z” type cleavages (shown by upper and lower horizontal lines). The site of the +14 Da modification is indicated by the gray box, localizing the site of methylation to lysine 46 or arginine 47. All y and z fragments terminating before arginine 47 were found to not contain an additional 14 Da mass; all of these fragments terminating before lysine 46 contained an additional 14 Da mass. A similar site of modification was observed in both lysine 46 and arginine 47 (Fig. 2). Similar analysis of Rpl1ab purified from the ΔYLR137W deletion strain demonstrated the absence of any 14 Da modification within the polypeptide chain (data not shown).

FIGURE 3. Rpl1ab contains a monomethyllysine residue. Wild-type (BY4742) cells were labeled in vivo with [3H]AdoMet as described previously (19), and large subunit ribosomal proteins were prepared by acetic acid and ethanol extraction as described (9). The Rpl1ab protein was purified using reverse phase HPLC (9), placed in a 6 × 50-mm glass vial, and dried down by vacuum centrifugation. Acid hydrolysis was then carried out by adding 50 μl of 6 N HCl to the vial and 200 μl of 6 N HCl to the reaction vial assembly (Eldex Laboratories, Napa, CA; catalog number 1163). The assembly was heated for 20 h in vacuo at 110 °C using a Waters Pico-Tag vapor phase apparatus. Residual HCl was removed by vacuum centrifugation, and the free amino acids were resuspended in 50 μl of water and 500 μl of pH 2.2 sodium citrate buffer (0.2 m Na+). Standards of methylated amino acids purchased from Sigma included e-N-monomethyllysine hydrochloride (M6004), asymmetric N, N'-dimethylarginine dihydrochloride (D4268), symmetric N,N'-dimethyllysine (M7033), and ω-N,N'-dimethyl-arginine acetate (M7033). 3-Methyl-histidine (or γ-methyl-γ-histidine) was purchased from Aldrich (67520). After the addition of 1.0 μmol of each standard, fractionation was performed on a column of PA-35 sulfonated polystyrene beads (0.9-cm inner diameter × 10-cm column height; 6–12-μm bead diameter; Benson Polymeric Inc., Reno, NV). The column was equilibrated and eluted at 55 °C with pH 5.27 sodium citrate buffer (0.35 m Na+) at 1 ml/min; regeneration was performed by elution with 0.2 N NaOH for 25 min. One-minute fractions were collected. The elution positions of the standards were identified by a ninhydrin assay (dashed line). Briefly, 100 μl of each column fraction was mixed with 600 μl of water and 300 μl of a solution of 20 mg/ml ninhydrin and 3 mg/ml hydridinantin in a solvent of 75% (v/v) dimethyl sulfoxide and 25% (v/v) 4 μl pH 4.2 lithium acetate buffer. The mixture was then heated at 100 °C for 15 min, and the absorbance was measured at 570 nm. Radioactivity (solid line) was determined by mixing 900 μl of the fraction with 400 μl of water and 10 ml of fluor (Safety Solve, Reagents Products International) followed by counting for three 5-min periods on a Beckman LS6500 instrument with a measured tritium efficiency of 55%. In this chromatography system, δ-monomethylarginine elutes near the position of symmetric dimethylarginine (31). The slightly earlier elution of the radioactivity compared with the monomethyllysine standard is consistent with the isotopic separation of the δ-H-methylated versus the 3H-methylated species (34–38).

of post-translational modifications (33). Rpl1ab from yeast wild-type (BY4742) and the ΔYLR137W gene deletion strains was isolated as described above by reverse phase HPLC. Purified wild-type Rpl1ab was directly infused on a hybrid linear ion trap Fourier transform ion cyclotron resonance mass spectrometer (Thermo Scientific LTQ FT Ultra) and fragmented using collisionally activated dissociation. This process localized the site of modification between lysine 46 and arginine 47 (Fig. 2). Similar analysis of Rpl1ab purified from the ΔYLR137W deletion strain demonstrated the absence of any 14 Da modification within the polypeptide chain (data not shown).

Rpl1ab Is Monomethylated at Lysine 46 by an S-Adenosylmethionine-dependent Methyltransferase—Wild-type (BY4742) yeast was in vivo labeled with [3H]AdoMet as described previously (29), and the large ribosomal proteins were isolated as described above. HPLC-purified Rpl1ab was acid hydrolyzed, and the products were separated using high resolution cation exchange chromatography (Fig. 3) and thin layer chromatography (Fig. 4). In Fig. 3, we show that the major 3H-methylated product elutes just slightly before a nonlabeled standard of e-monomethyllysine. No radioactivity co-eluted with the ω-monomethylarginine or δ-monomethylarginine standards.

4 The abbreviations used are: [3H]AdoMet, S-adenosyl-l-[methyl-3H]methionine; [13C]AdoMet, S-adenosyl-l-[methyl-13C]methionine.

JOURNAL OF BIOLOGICAL CHEMISTRY 18407

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It has been shown that tritium-labeled amino acids and similar compounds can be partially resolved from their unlabeled hydrogen forms with high resolution cation exchange chromatography (34–38), suggesting that the slight difference in retention times of the labeled amino acid and the unlabeled standard observed in Fig. 3 might be a result of an isotope effect. To confirm this, we also analyzed the labeled acid hydrolysates by thin layer chromatography (Fig. 4). Here, the radioactivity was found to migrate slightly slower than the ε-monomethyllysine standard. We considered the possibility that methylation might occur on the peptide bond nitrogen atom of the lysine residue rather than the side chain nitrogen. However, we found that a standard of N-α-methyllysine is clearly resolved from ε-monomethyllysine. These results provide strong evidence that Rpl1ab is monomethylated at a lysine 46 by an S-adenosylmethionine-dependent methyltransferase and suggest an isotope effect for the migration of ε-monomethyllysine in thin layer chromatography and high resolution cation exchange chromatography.

Modification of an ~24-kDa Large Ribosomal Protein by Purified YLR137W/Rkm5—To determine whether the YLR137W gene product might directly methylate the Rpl1ab protein, a recombinant His-tagged form of YLR137W was expressed in E. coli and purified for in vitro methylation assays. When intact 80 S ribosomal subunits isolated from the ΔYLR137W deletion strain were incubated with the His-tagged YLR137W protein and [3H]AdoMet and size-fractionated by SDS-polyacrylamide gel electrophoresis, we were able to detect methylation of a ~24-kDa polypeptide species, which corresponds to the size of Rpl1ab (Fig. 5). This methylation activity was only observed with intact 80 S ribosomes isolated from the ΔYLR137W deletion strain; this methylation was not observed in wild-type 80 S ribosomes (Fig. 5). These results suggest that YLR137W is a methyltransferase capable of recognizing and methylating Rpl1ab on the ribosome. The lack of in vitro methylation of Rpl1ab in ribosomes prepared from wild-type cells (Fig. 5) reflects the stoichiometric methylation of Rpl1ab (Fig. 1); there is no unmethylated polypeptide available as a substrate in these cells.
Recombinantly Expressed YLR137W/Rkm5 Produces Monomethyllysine—We next examined the specific amino acid residue methylated by His-tagged YLR137W/Rkm5. His-YLR137W/Rkm5 was incubated with intact 80 S ribosomes derived from the ΔYLR137W/rkm5 deletion strain in the presence of radiolabeled [3H]AdoMet or [14C]AdoMet. Methylated proteins were acid hydrolyzed and analyzed by thin layer chromatography (Fig. 6). The reaction with [3H]AdoMet produced a product that migrated less rapidly than the ε-monomethyllysine standard (Fig. 6, upper panel), similar to the migration pattern observed in the in vivo methylation experiment in Fig. 4. When a [14C]AdoMet substrate was used in the in vitro reaction with His-tagged YLR137W/Rkm5; however, the [14C]-product co-migrated with the unlabeled ε-monomethyllysine standard (Fig. 6, lower panel), showing that Rpl1ab contains a ε-monomethyllysine residue. These results demonstrate that 3H-isotope effects can occur in thin layer chromatography as well as in high resolution cation exchange chromatography (34–38).

Purified YLR137W/Rkm5 Catalyzes the Methylation of a Synthetic Peptide with the Rpl1ab Sequence from Residues 38 to 53—To ask whether the purified YLR137W gene product was sufficient to catalyze the methylation reaction or whether it required other factors present on the ribosome, as well as to determine whether tertiary structure is needed for the methylation reaction, we incubated the recombinant protein with a short synthetic peptide including the unmodified target lysine residue. When incubated with [3H]AdoMet and enzyme, radioactivity was found to elute with the peptide when the reaction products were separated by HPLC (Fig. 7A). In a control reaction without peptide, no radioactivity was seen. However, in a control reaction without enzyme (peptide only), some radioactivity was also detected at the elution position of the peptide. Nevertheless, when peptide-containing HPLC fractions were acid hydrolyzed and analyzed by high resolution cation exchange chromatography, radioactivity eluting with a monomethyllysine standard was only found in the samples incubated with both peptide and enzyme (Fig. 7B). Importantly, these results provide direct evidence that the YLR137W gene product has by itself methyltransferase activity and is capable of recognizing a short sequence derived from Rpl1ab. We now propose that the YLR137W gene be given the standard name RKM5 for ribosomal lysine (K) ethyltransferase 5.

Rkm5 Orthologs Are Limited to Fungal Species—A BLAST search revealed proteins with sequence similarity to YLR137W/Rkm5 in a number of fungal species, but not in proteins from other organisms (Fig. 8). The closest nonfungal species identified in a BLAST search is the Arabidopsis thaliana protein NP_973791.1 (57 of 282 identities, expect value = 0.003). This Arabidopsis protein matches more closely to the yeast YNL024C putative methyltransferase and to the yeast N-terminal Xaa-Pro-Lys methyltransferase, indicating that the Rpl1ab modification may be unique to fungi. We note, however, that the amino acid sequence adjacent to the methylated lysine 46 residue (DKRF) in S. cerevisiae Rpl1ab is highly conserved in the L1 ribosomal protein family in eukaryotic cells, including protozoans, fungi, plants, invertebrates, and vertebrates. This suggests that this region of Rpl1ab might have a critical role in

![FIGURE 6. Recombinant His-YLR137W/Rkm5 produces monomethyllysine.](image-url)
Methyllysine Formation in Yeast Ribosomal Protein L1

**FIGURE 7.** Monomethylation of a lysine residue in a synthetic peptide derived from Rpl1ab by recombinant His-YLR137W/Rkm5. A, a 16-amino acid synthetic peptide (KNYDPQRDKRFSGSLK) was prepared by Biosynthesis, Inc. (Lewisville, TX) corresponding to residues 38–53 of Rpl1ab. Peptide (60 μg) was incubated with (squares) or without (triangles) recombinant His-YLR137W/Rkm5 (60 μg), 1 μM [3H]AdoMet, 100 mM NaCl, 100 mM Na2HPO4 at pH 7 as described in Fig. 6 for 16 h at 30 °C in a final volume of 200 μL. An additional incubation with enzyme but without peptide was also prepared (circles, solid line). The reactions were terminated by adding 20 μL of 10% trifluoroacetic acid. After centrifugation at 20,000 × g for 5 min at room temperature, the supernatant was fractionated by HPLC using a 150-mm, 2-mm column maintained at 50 °C (Polymer Laboratories, Amherst, MA). The column was maintained at 50 °C and initially equilibrated in 95% solvent A (0.1% trifluoroacetic acid in water) and 5% solvent B (0.1% trifluoroacetic acid in acetonitrile). The peptides were then acid hydrolyzed, dried as in Fig. 3, and then mixed with 50 μl of water, 150 μl of pH 2.2 citrate buffer, and a standard of 10 μl of 0.2 M e-methyllysine standard. Amino acid fractionation was performed as described in Fig. 3 except with a buffer of pH 5.84 sodium citrate (0.2 M Na+). The elution position of the monomethyllysine standard was identified by a ninhydrin assay (dashed line). Briefly, 30 μl of each column fraction was mixed with 200 μl of water and 100 μl of a solution of 20 mg/ml ninhydrin and 3 mg/ml hydriindan in a solvent of 75% (v/v) dimethyl sulfoxide and 25% (v/v) 4 M pH 4.2 lithium acetate buffer. The mixture was then heated at 100 °C for 15 min, and the absorbance was measured at 570 nm using a SpectraMax M5 microplate reader. Radioactivity (solid lines) was determined by mixing 400 μl of sample with 10 ml of fluid and counting as described for A. The values are shown normalized to the total volume for each fraction and for the portion of the HPLC fractions hydrolyzed. The slightly earlier elution of the radioactivity compared with the standard is consistent with the isotopic separation of the 3H-methylated versus the 1H-methylated species (34–38).

The Rpl1ab protein in yeast is a member of the highly conserved L1 ribosomal protein family. These proteins form the L1 protuberance, an extension from the large ribosomal subunit that is suggested to facilitate the exit of the tRNA from the E site (22). Although there is no high resolution structure of the yeast ribosome, it is possible to estimate the three-dimensional site of the lysine 46 modification by studying the known structures of bacterial (23) and archaeal (21) L1 proteins in complex with rRNA. Although the lysine 46 residue of yeast Rpl1ab is not generally conserved in prokaryotes, its position has been established between the first and second β-strands, away from the RNA binding interfaces (21, 23). This suggests that the side chain of lysine 46 of Rpl1ab may be accessible on the surface of the ribosome, in contrast to the methylated lysine residues of yeast Rpl23ab (19) and Rpl42ab (9), as well as the methylated histidine residue of Rpl3 (13) that appear to interact directly with rRNA. Interestingly, the lysine 46 residue and its surrounding sequence is well conserved in the mammalian L1 ortholog, Rpl10A. It is not clear whether the mammalian protein might also be subject to methylation.

The Rkm5 methyltransferase joins a growing family of enzymes of the seven-β-strand class (32, 39) that catalyze protein lysine methylation. Until recently, nearly all of the protein lysine methyltransferases were of the SET domain class, with the key exception being the Dot1 enzyme that was responsible for the trimethylation of Lys-79 in histone H3 (25, 26). Others have now identified seven-β-strand enzymes that catalyze the trimethylation and dimethylation of lysine residues in calmodulin (27) and elongation factor 1A (17), respectively. Additionally, evidence has been presented that PrmA, the seven-β-strand methyltransferase responsible for the N-terminal methylation of prokaryotic ribosomal protein L11, also catalyzes trimethylation of lysine residues at two sites within the polypeptide (28). We ana...
lyzed the amino acid sequences surrounding the methylated lysine residue in each of these proteins and did not identify any common sequence motifs that might serve as recognition sequences for seven-/H9252-strand protein lysine methyltransferases (data not shown). It is clear, however, that protein lysine methylation is catalyzed by members of both the SET domain and seven-/H9252-strand methyltransferase superfamilies.

Interestingly, protein lysine methylation appears to be more common in fungal species than in mammalian species, at least for ribosomal proteins of the large subunit (Table 1). Notably, there are no mammalian orthologs of any of the five yeast ribosomal large subunit protein lysine methyltransferases described to date (Table 1). Moreover, none of the human orthologs of the methylated yeast Rpl1ab, Rpl12ab, Rpl23ab, and S. pombe (diaminohydroxyphosphoribosylamino-pyrimidine deaminase, P87241; expect value, 4 \times 10^{-10})

The closest human homolog is the FAM86B2 protein (UniProt P0C5J1) with an expect value of 0.78. The S. pombe sequence contains a hypothetical pyrimidine deaminase domain in the C-terminal region adjacent to the aligned sequence. Light gray shading indicates sites where three residues are identical, medium gray shading indicates sites where four residues are identical, and dark gray shading indicates sites where all of the residues are identical.

**FIGURE 8.** *S. cerevisiae* YLR137W/RKM5 homologs are present in fungi. BLAST searches were performed against the yeast YLR137W/RKM5 sequence to identify homologs in other organisms and ClustalW was used to align sequences. Signature methyltransferase motifs common to all class I protein methyltransferases are boxed. The UniProt IDentification designation, corresponding protein name if given, and BLAST expect value for each sequence identified are as follows: *Aspergillus fumigatus* (Q4X1H7; expect value, 10^{-8}), *Neurospora crassa* (Q7RZ91; expect value, 7 \times 10^{-10}), *Candida albicans* (C4YJI9; expect value, 2 \times 10^{-18}), *S. cerevisiae* (YLR137W/RKM5, Q12367), and *S. pombe* (diaminohydroxyphosphoribosylamino-pyrimidine deaminase, P87241; expect value, 4 \times 10^{-10}). The closest human homolog is the FAM86B2 protein (UniProt P0C5J1) with an expect value of 0.78. The S. pombe sequence contains a hypothetical pyrimidine deaminase domain in the C-terminal region adjacent to the aligned sequence. Light gray shading indicates sites where three residues are identical, medium gray shading indicates sites where four residues are identical, and dark gray shading indicates sites where all of the residues are identical.
TABLE 1

Protein lysine methyltransferases in the yeast Saccharomyces cerevisiae have few mammalian orthologs

| Methyltransferase gene product | Methyltransferase superfamily | Substrate and methylated products^a | Methyltransferase ortholog in mammals^c | Ref. |
|--------------------------------|-------------------------------|-----------------------------------|----------------------------------------|------|
| Rkm1 (YPL208W)                | SET domain                    | Rpl23ab                           | No                                     | 7    |
| Rkm2 (YDR198C)                | SET domain                    | Rpl12ab                           | No                                     | 19   |
| Rkm3 (YBR030W)                | SET domain                    | Rpl42ab                           | No                                     | 9    |
| Rkm4 (YDR257C)                | SET domain                    | Rpl42ab                           | No                                     | 9    |
| Rkm5 (YLR137W)                | Seven-ß-strand                | Rpl1ab                            | No                                     | This study |
| Efml (YHL039W)                | SET domain                    | eEF1A                             | Yes                                    | 17   |
| See1 (YIL064W)                | Seven-ß-strand                | eEF1A                             | Yes (human METTL10)^e                  | 17   |
| Ctm1 (YHR109W)                | SET domain                    | Cytochrome c trimethyllysine 77^f | No                                     | 40   |
| Dot1 (YDR440W)                | Seven-ß-strand                | Histone H3                        | Yes (human DOT1)^f                     | 25, 26 |

^a Residue numbering based on mature protein (loss of initiator methionine) except for Rpl42ab and eEF1A.
^b Sites of methylation suggested based on location of known monomethylated sites in yeast eEF1A (6, 17).
^c Site of methylation suggested based on location of known dimethylated site in yeast eEF1A (6, 17).
^d Methylation site in isoform 1; site at lysine 81 in isoform 2.
^e 38% amino acid sequence identity over 182 residues.
^f 28% amino acid sequence identity over 297 residues.

The role of protein lysine methylation of ribosomal proteins is unclear. Some information is available from the phenotype of yeast cells lacking specific protein lysine methyltransferases. Yeast cells lacking the YLR137W/Rkm5 methyltransferase are viable and do not yet have a clear phenotype. When grown in a competition with a parent strain in minimal medium, the YLR137W knock-out shows a slight growth advantage (relative fitness score is 1.003) (46). In both S. cerevisiae and S. pombe, cells lacking methylation at lysine 55 of Rpl42 are more sensitive to cycloheximide (9, 11), and S. pombe mutants have survival defects in stationary phase (11). The growth defect in S. pombe resulting from the overexpression of the enzyme responsible for lysine 3 methylation of Rpl12, as well as the nucleolar localization of the enzyme, suggests a role for methylation in ribosomal assembly. More generally, protein lysine methylation has been associated with increases in protein stability. The chemical introduction of e-dimethyllysine residues in bovine trypsin results in decreased autolysis (47) and in enhanced surface contacts in crystal structures (48). The thermal stability of ß-glycosidase from the thermophilic archaean Sulfolobus solfataricus is dependent upon the methylation of five of the 23 lysine residues (49). It has also been suggested that lysine side chain methylation generally increases the stability of proteins, exemplified by the extensive modification of several proteins in the thermophilic archaean Thermoproteus tenax, where 52 methylated lysine residues were detected in 30 different proteins (50). Taken together, these results suggest that ribosomal protein lysine methylation may play multiple roles and may act to allow optimization of ribosomal function to allow organisms to thrive in a wide range of growth environments.

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