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Ribosomal protein methyltransferases in the yeast *Saccharomyces cerevisiae*: Roles in ribosome biogenesis and translation

Qais Al-Hadid, Jonelle White, Steven Clarke*

Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, CA 90095, USA

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ABSTRACT

A significant percentage of the methyltransferasome in *Saccharomyces cerevisiae* and higher eukaryotes is devoted to methylation of the translational machinery. Methylation of the RNA components of the translational machinery has been studied extensively and is important for structure stability, ribosome biogenesis, and translational fidelity. However, the functional effects of ribosomal protein methylation by their cognate methyltransferases are still largely unknown. Previous work has shown that the ribosomal protein Rpl3 methyltransferase, histidine protein methyltransferase 1 (Hpm1), is important for ribosome biogenesis and translation elongation fidelity. In this study, yeast strains deficient in each of the ten ribosomal protein methyltransferases in *S. cerevisiae* were examined for potential defects in ribosome biogenesis and translation. Like Hpm1-deficient cells, loss of four of the nine other ribosomal protein methyltransferases resulted in defects in ribosomal subunit synthesis. All of the mutant strains exhibited resistance to the ribosome inhibitors anisomycin and/or cycloheximide in plate assays, but not in liquid culture. Translational fidelity assays measuring stop codon readthrough, amino acid misincorporation, and programmed −1 ribosomal frameshifting, revealed that eight of the ten enzymes are important for translation elongation fidelity and the remaining two are necessary for translation termination efficiency. Altogether, these results demonstrate that ribosomal protein methyltransferases in *S. cerevisiae* play important roles in ribosome biogenesis and translation.

1. Introduction

Translational components are modified by the addition of methyl groups in all domains of life. These modifications occur on ribosomal RNA (rRNA), transfer RNA (tRNA), messenger RNA (mRNA), translation factors, and ribosomal proteins [1–7]. More than half of the known methyltransferases in the yeast *Saccharomyces cerevisiae* modify these ribosomal components, suggesting that methylation of translational components is important for cellular function [8]. Methylation of the RNA components of the ribosome is important for ribosome synthesis, structure stability, and translational fidelity [3,4,9,10]. Similarly, methylation of elongation and release factors has been demonstrated to be important for translational fidelity and termination efficiency [5,11]. However, the functions of methyltransferases responsible for ribosomal protein methylation are not well characterized and little has been done to uncover their biological activities.

We previously showed that the yeast methyltransferase, Hpm1, plays an important role in ribosome biogenesis and translation [12,13]. Cells deficient in Hpm1 exhibited defects in 60S large ribosomal subunit synthesis and decreased translation elongation fidelity [12,13]. To determine if the nine other known ribosomal protein methyltransferases in *S. cerevisiae* are playing similar roles as Hpm1, we investigated the consequences of depleting each ribosomal protein methyltransferase on ribosome biogenesis and translation. Using a variety of assays, we show that the loss of each of these enzymes results in one or more alterations in ribosomal biogenesis (altered levels of ribosomal subunits), resistance to ribosome-binding antibiotics, readthrough of stop codons, amino acid misincorporation, and programmed −1 ribosomal frameshifting (−1 PRF). These results suggest that all the ribosomal protein methyltransferases in *S. cerevisiae* are necessary for ribosome biogenesis and accurate translation elongation or termination.

* Corresponding author. Dept. of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, 607 Charles E. Young Dr. E., Los Angeles, CA 90095-1569, USA.
E-mail address: clarke@mbi.ucla.edu (S. Clarke).

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2. Materials and methods

Strains and growth media – All strains used are in the BY4742 background (*MATα*, *his3Δ1*; *leu2Δ0*; *lys2Δ0*; *ura3Δ0*) obtained from the Open Biosystems yeast knockout collection (Thermo Scientific). All strains contain a kanamycin resistance marker in place of the open reading frame of each ribosomal protein methyltransferase gene. Yeast strains were grown in 1% yeast extract, 2% peptone, and 2% dextrose (YPD, Difco) or minimal synthetic defined media lacking uracil (SD −ura) containing 0.17% yeast nitrogen base without ammonium sulfate and amino acids (BD Biosciences), 0.077% complete supplement mixture without uracil (MP Biomedicals; 114511212), 0.5% ammonium sulfate, and 2% dextrose.

3. Results

3.1. Loss of yeast ribosomal protein methyltransferases results in abnormal ribosomal subunit levels and increased polysomes

In prior work, we showed that the protein histidine methyltransferase, Hpm1, in *S. cerevisiae*, is needed to promote normal ribosome biogenesis [12]. We sought to determine if loss of the other yeast ribosomal protein methyltransferases results in defects in ribosomal subunit levels and/or translation by polysome profile analysis. Lysates were prepared from wild type and each of the ten mutant strains and ribosomal components separated by sucrose density ultracentrifugation. We examined the levels of small (40S) and large (60S) ribosomal subunits, intact ribosomes (80S), and polyribosomes (polysomes) to indicate possible defects in ribosome biogenesis and/or translation. Like Hpm1-deficient cells, loss of Rkm1, Ntm1, Rmt1, or Rmt2 resulted in a deficit of 60S subunits (Fig. 1A). This defect in 60S biogenesis is highlighted by a significant decrease in the free 60S/free 40S subunit ratio in these strains, compared to wild type (Fig. 1B). Ntm1 was previously shown to be important for 60S biogenesis [14]. Loss of Rkm2, Rkm3, Rkm4, Rkm5, or Sfm1 had little or no impact on the levels of ribosomal components, suggesting they are not required for ribosomal subunit synthesis (Fig. 1A, 1B). Little or no change in the polysome/80S ratio was seen for the ten methyltransferase mutants, suggesting no significant change in translational output and cellular proliferation [15,16]. This result is consistent with similar growth of these mutant strains to the wild type strain in the absence of antibiotics on agar plates (Fig. 2) or in liquid medium (Fig. 3).

3.2. Cells deficient in ribosomal protein methyltransferases are resistant to the A-site and E-site ribosome-binding drugs, anisomycin and cycloheximide, respectively on agar plates

Next, we tested if the defects in ribosomal subunit biogenesis and/or the elevated levels of polysomes in the ribosomal protein methyltransferase mutants correlate with altered sensitivities to ribosome-binding drugs. Altered sensitivities to these drugs may indicate structural and/or functional distortions to the regions that these drugs bind. Drugs that bind to different functional centers of the ribosome were used including: anisomycin, paramomycin, and cycloheximide. Anisomycin binds to the A-site of the ribosome and acts as a competitive inhibitor of aminoacyl-tRNAs [17]. Paramomycin binds to the decoding center of the small subunit and induces translational errors [18]. Cycloheximide binds to the E-site of the large subunit and inhibits translation elongation [19]. Previously, we showed that Hpm1-deficient cells have enhanced resistance to cycloheximide in plate assays, suggesting alterations to the A and E-sites of the large ribosomal subunit of *hpm1Δ* cells (12). Remarkably, we were able to now show that all of the mutant strains demonstrated increased resistance to cycloheximide in plate assays with the exception of *rkm4Δ* (Fig. 2). Increased sensitivity of *rkm4Δ* strains to cycloheximide has previously been reported [20,21]. No differences in sensitivity of the mutants were seen on paramomycin-containing agar plates, compared to wild type (data not shown). In the presence of anisomycin, we also observed increased resistance in the ribosomal methyltransferase mutants with the exception of *hpm1Δ* cells (Fig. 2). The latter result is in accord with a previous study (10). The similar resistance phenotype of many of these strains to anisomycin and cycloheximide indicates a defect at a common functional step, likely in translation elongation or termination, rather than a common structural distortion at the A-site and E-site of the large subunit. We also measured growth rates in liquid culture of wild type and mutant strains in the presence or absence of cycloheximide (Fig. 3). Because the 500 ng/ml concentration used in the plate assays severely limited the growth of the cells in liquid culture, we used a lower concentration of 50 ng/ml that resulted in about a two-fold increase in doubling time. To our surprise, we found little or no differences in the growth rates of the wild type and mutant cells. Cells thus respond differently to the antibiotics in a colony on agar plates (Fig. 2) compared to individual cells in liquid media (Fig. 3).

3.3. Loss of ribosomal protein methyltransferases results in defects in the fidelity of translation elongation or termination

Previously, we demonstrated that loss of Hpm1 results in reduced fidelity in translation elongation [12]. To determine if the other ribosomal protein methyltransferases are important for translational fidelity, we performed assays measuring stop codon suppression, amino acid misincorporation, and programmed −1 ribosomal frameshifting (−1 PRF). These assays utilize dual-luciferase reporter genes: *Renilla* followed by firefly luciferase, separated by a linker region, under the control of a constitutive promoter. The amount of firefly luciferase luminescence correlates with translational errors in all three assays and the amount of *Renilla* luciferase luminescence is used to correct for differences in translation initiation and mRNA levels of the dual reporters. To measure stop codon suppression, vectors containing stop codons (UAA and UAG) in the linker region between the *Renilla* and firefly luciferase genes were used and the amount of reporter luminescence was measured. Increased readthrough of the stop codons, as a result of defects in elongation or termination, would result in increased firefly luciferase luminescence. Loss of each of the ten ribosomal protein methyltransferases resulted in increased readthrough of the UAA and UAG stop codons, compared to wild type (Fig. 4A, 4B). This result suggests that all ten ribosomal protein methyltransferases in *S. cerevisiae* are important for translation elongation or termination fidelity. To determine if the translational fidelity defects in all ten strains is occurring at the elongation or termination step, amino acid misincorporation was measured, which measures elongation fidelity defects. Amino acid misincorporation levels were determined using a dual-luciferase reporter vector with a point mutation in the firefly gene at a catalytically-important residue (K529) to a near-cognate asparagine residue [22]. This mutation renders firefly luciferase catalytically-deficient. High fidelity translation would result in incorporation of the asparagine residue at position 529 and synthesis of an inactive firefly luciferase. However, reduced translational accuracy would result in increased frequencies of near-cognate and non-cognate aminoacyl-tRNA accommodation and increases the chances of misincorporating the wild type lysine reside, resulting in the synthesis of an active firefly luciferase enzyme. Hence, reduced translation elongation accuracy would result in the production of more active firefly luciferase enzymes and as a consequence, greater firefly luciferase luminescence. All

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