METHYLATION OF RIBOSOMAL PROTEIN L42 REGULATES RIBOSOMAL FUNCTION AND STRESS-ADAPTED CELL GROWTH

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Lysine methylation is one of the most common protein modifications. Although lysine methylation of histones has been extensively studied and linked to gene regulation, that of non-histone proteins remains incompletely understood. Here, we show a novel regulatory role of ribosomal protein methylation. Using an in vitro methyltransferase assay, we found that S. pombe Set13, a SET domain protein encoded by SPAC688.14, specifically methylates lysine-55 of ribosomal protein L42 (Rpl42). Mass spectrometric analysis revealed that endogenous Rpl42 is monomethylated at lysine-55 in wild-type S. pombe cells and that the methylation is lost in ∆set13 mutant cells. ∆set13 and Rpl42-methylation-deficient mutant S. pombe cells showed higher cycloheximide sensitivity and defects in stress-responsive growth control compared with wild type. Genetic analyses suggested that the abnormal growth phenotype was distinct from the conserved stress-responsive pathway that modulates translation initiation. Furthermore, the Rpl42 methylation-deficient mutant cells showed a reduced ability to survive after entering stationary phase. These results suggest that Rpl42 methylation plays direct roles in ribosomal function and cell-proliferation control independently of the general stress-response pathway. Post-translational modifications regulate protein structure and function, and one of the most common is lysine methylation (1). While lysine methylation does not change the overall charge of the side chain, it can influence a protein’s activity by providing a novel interface for its interactions with other molecules. Most protein lysine methylations, with a few exceptions, are catalyzed by the SET domain-containing protein (SET protein) family (2). The SET domain was originally identified in three Drosophila proteins, Su(var)-3-9, Enhancer of zest, and Trithorax, and was later demonstrated to be the responsible domain for histone lysine methyltransferase function (3). The characterization of these enzymes has revealed the roles of histone lysine methylation in many different biological processes, including higher-order chromatin assembly and transcriptional regulation (4). Recent studies have shown that histone lysine methylation is directly reversed by several histone demethylase families (5).

Lysine methylation has also been identified in non-histone proteins that include Rubisco in plants (6), Cytochrome c in yeast (7), mammalian TAF10 and p53 (8-10), and ribosomal proteins in a diverse range of species (11). The methylation of ribosomal proteins has been observed in both prokaryotes and eukaryotes. In the budding yeast Saccharomyces cerevisiae, a combination of in vivo labeling and direct mass spectrometric analysis of the ribosomal proteins revealed that six of them, Rpl1, Rpl3, Rpl12, Rpl23, Rpl42, and Rpl43, are post-translationally methylated (12,13). By analyzing the methylation state of S. cerevisiae mutant strains with deletions in candidate SET domain-containing genes, two SET proteins, Rkm1 and Rkm2, were identified as specific methyltransferases responsible for the dimethylation of Lys-105 and Lys-109 in Rpl23, and the trimethylation of Lys-3 in Rpl12 (14,15), respectively. A recent study further demonstrated that the monomethylation at Lys-40 and Lys-55 in Rpl42 is dependent on two other SET proteins, the Ybr030w gene product and Set7, respectively (16), although the direct enzymatic activity of these proteins has yet to be demonstrated. Several mass
spectrometric studies have also identified methyl modifications on ribosomal proteins in plants and mammals (17-19). While ribosomal protein methylation appears to be conserved among different organisms, the physiological roles of these lysine methylations remain to be fully elucidated.

The ribosome plays a central role in a cell’s adaptation to environmental stress, as a checkpoint for sensing shifts in temperature and nutrient levels (20,21). Global translation is reduced in response to these cellular stresses by triggering the phosphorylation of the eukaryotic initiation factor 2α (eIF2α) (22,23). This prevents the formation of the eIF2-methionine-initiator tRNA (Met-tRNA<sub>Met</sub>-GTP ternary complex and thus blocks translational initiation. The stress-induced attenuation of global translation is often accompanied by the selective translation of proteins that are required for cell survival under stress. A downshift in temperature, one of the most common environmental changes for microbial life, induces the expression of genes encoding a number of ribosomal proteins and proteins involved in ribosome biogenesis and assembly (24). Thus, it has been suggested that cells remodel the translational machinery and secondary structure of RNA for cold growth. Interestingly, ribosomal protein biogenesis and the stress-responsive signaling pathway are also linked with the life span in both yeast and <i>C. elegans</i> (25-28).

In the fission yeast Schizosaccharomyces pombe (S. pombe), 13 SET proteins have been identified in the genome, four of which (Set1, Set2, Chr4, and Set9) are histone lysine methyltransferases and involved in transcriptional regulation (29-32). Using an <i>in vitro</i> methyltransferase assay and genome-wide screen for methylated proteins, we previously demonstrated that Set5, Set10, and Set11 are specific methyltransferases for EF1α, Rpl23, and Rpl12, respectively (33,34). However, the roles played by other SET proteins in cellular processes and their physiological substrates remain unresolved.

In this study, we show that <i>S. pombe</i> Set13, a SET protein encoded by SPAC688.14, is a specific methyltransferase responsible for monomethylation at lysine-55 in Rpl42. We further demonstrate that this methyl modification is highly conserved from yeast to humans. Rpl42-methylation-deficient mutant <i>S. pombe</i> cells showed defects in stress-adapted growth control and reduced survival potential. Notably, Rpl42-methylation-mediated stress adaptation occurred independently of the general stress-response pathway. These results suggested that this ribosomal protein methylation is involved in global ribosomal function and cellular growth control.

**EXPERIMENTAL PROCEDURES**

<i>Strains and media–</i> The strains used in this study are listed in Supplemental Table S4. All of the yeast strains were grown at 30°C or the indicated temperature in YEA (0.5% yeast extract, 3% glucose, 75 µg/ml adenine) or minimal medium (SD or EMM) supplemented with amino acids for auxotrophic markers and antibiotics. The deletion and tagging of endogenous genes were conducted using a PCR-based gene-targeting protocol (35). The deletion mutants for the set13<sup>+</sup>, gcn5<sup>+</sup>, or gcn2<sup>+</sup> gene were made by replacing the gene with a kan<sup>R</sup> or ura4<sup>+</sup> marker gene. The integrated ura4<sup>+</sup> gene was then removed by homologous recombination of the flanking TEF terminator sequences to obtain cells lacking it. To obtain the <i>rpl42<sup>K55R</sup></i> and <i>rpl42<sup>P56Q</sup></i> mutant strains, the <i>rpl42<sup>+</sup></i> coding sequence was first cloned into pCRII-TOPO (Invitrogen), and each mutation was introduced by site-directed mutagenesis. After insertion of the ura4<sup>+</sup> marker gene, the resultant plasmids were digested with MfeI and used to transform cells. Cells in which the plasmid was introduced into the original <i>rpl42</i><sup>+</sup> locus were isolated as ura4<sup>+</sup>-expressing cells. The mutant <i>S. pombe</i> strains that lost the wild-type <i>rpl42</i><sup>+</sup> allele and ura4<sup>+</sup> gene by internal homologous recombination were isolated using counter-selective media containing 5-fluoroorotic acid (FOA).

To obtain <i>S. pombe</i> cells expressing EGFP-fused Set13, the set13<sup>+</sup> coding sequence was cloned into pREP1-EGFP, a pREP1 derivative containing the EGFP-coding sequence (36), and cells transformed with the resulting plasmid were isolated with minimal medium lacking leucine. To express EGFP-fused Rpl42, the <i>rpl42</i><sup>+</sup> coding
sequence previously isolated as part of the ORFeome project (37) was transferred to a pDUAL-GFH1c vector (38) by the “LR” recombination reaction. The resultant plasmid, pDUAL-GFH1-rpl42, was digested with NotI and introduced into the leu1 locus of wild-type or Δset13 cells. The transformed cells were selected on SD lacking leucine.

Expression and purification of recombinant proteins—To produce recombinant Set13 or Rpl42 proteins in E. coli, the coding sequence for set13+ or rpl42+ was amplified by PCR and cloned into pRSET (Invitrogen) for Set13, or pGEX6P-3 (GE Healthcare) or pTriEX-4 Hygro (Novagen) for Rpl42. To produce mutant Rpl42 proteins, the above plasmids were subjected to site-directed mutagenesis. Each expression vector was introduced into E. coli BL21 (DE3), and protein expression was induced by adding 1 mM isopropyl-b-D-thiogalactopyranoside. The culture was incubated for 2 h more at 37 °C before harvesting, and the cells were then lysed by sonication (for His-Set13 and GST-Rpl42, -Rpl42-N, -Rpl42-M, and -Rpl42-C) or with buffer containing guanidine hydrochloride (for Rpl42-His and its derivatives). The expressed proteins were purified using TALON metal affinity resin (Invitrogen) or Glutathione Sepharose (GE Healthcare), according to the manufacturer’s instructions. The eluted materials were dialyzed against phosphate-buffered saline (PBS) or PBS with 10% glycerol, divided into aliquots, and stored at -80°C before use.

Antibodies—Anti-Rpl42 rabbit polyclonal antibodies were raised and affinity purified using recombinant Rpl42-His. The purified antibodies were used for western blot analyses. Other antibodies used in this study were: anti-eIF2α [pS52] (Invitrogen, 44728G) and anti-Tubulin (kindly provided by K. Gull).

In vitro methyltransferase assay—S. pombe nuclear extracts of wild-type and Δset13 cells were prepared as described previously (39). The in vitro methyltransferase assay and the chromatographic fractionation of S. pombe nuclear extracts were performed as described previously (34).

Analysis of methylated peptide by nano-liquid chromatography tandem mass spectrometry (LC-MS/MS)—The LC-MS/MS analysis was performed as described previously (34).

Preparation of ribosomes from HEK293T cells—To prepare ribosomes from human cells, HEK293T cells were grown to ~80-90% confluence in 100-mm dishes, washed once with cold PBS, and harvested using a rubber scraper. The cells were pelleted, resuspended in 2 volumes of homogenization buffer (10 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 10 mM KCl, 1 mM dithiothreitol), and lysed with a Dounce homogenizer. The lysate was freed of cell debris by centrifugation at 20,000 x g for 10 min at 4°C. The supernatant was layered at a 1:1 ratio (v/v) over a sucrose cushion buffer (50 mM Tris-HCl, pH 7.5, 5 mM MgCl2, 25 mM KCl, 2 M sucrose) and centrifuged at 100,000 x g for 24 hr at 4°C. The ribosome-enriched pellet was resuspended in homogenization buffer, and the proteins were resolved on 8-13% SDS-polyacrylamide gel electrophoresis (PAGE) gels.

Microscopy analysis—To analyze the localization of EGFP-fused Set13, wild-type S. pombe cells harboring the pREP1-EGFP-set13 plasmid were grown to early-log phase in liquid medium and washed twice with deionized H2O, and the DNA was visualized by incubation with 1 µg/ml Hoechst 33342. To analyze the localization of GFP-fused Rpl42, wild-type and Δset13 mutant S. pombe cells that had integrated the pDUAL-GFH1-rpl42 construct were grown and treated as described above. Microscopic images were captured on a Zeiss Axioplan 2 imaging microscope and an ORCA-ER camera (Hamamatsu).

Spotting assay—Wild-type and mutant cells were grown in YEA medium. Five-fold serial dilutions were made from cultures of 1×10⁷ cells/ml and spotted onto plates with YEA alone or YEA containing antibiotics of the indicated concentrations. To analyze the growth rate under different stress conditions, the spotted YEA plates were incubated at 30°C for 2-4 days, 38°C for 2-4 days, or 15°C for 19 days.

Two-dimensional electrophoretic analysis of proteins—The two-dimensional gel analysis of methylated proteins was performed as described previously (34).

Polysome analysis—S. pombe cells were grown in 100 ml YEA medium to mid-log phase (optical density at 595 nm = 0.8) and harvested
immediately following the addition of cycloheximide (100 µg/ml). The pelleted cells were washed with CH-cell-wash buffer (20 mM Tris-HCl [pH 7.5], 50 mM KCl, 10 mM MgCl₂, 1 mM DTT, 100 µg/ml cycloheximide, and 200 µg/ml Heparin), suspended in 0.5 ml of CH-cell-lysis buffer (CH-cell-wash buffer supplemented with proteinase inhibitor cocktail [Complete™ EDTA-free; Roche Applied Science]), and lysed with Multi-Beads Shocker (Yasui Kikai). An aliquot of the cleared lysate was overlaid on top of a 5-45% (w/v) sucrose gradient containing 20 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM MgCl₂, 1 mM DTT, 100 µg/ml cycloheximide, and proteinase inhibitor cocktail, and centrifuged for 2 h at 36,000 rpm (222,000 x g max) at 4°C in a Beckman SW41TI rotor. The gradients were then fractionated using a Piston Gradient Fractionator (Biocomp). Polysome profiles were generated by continuous absorbance measurement at 254 nm using a UV monitor (Econo UV-monitor, BioRad) connected to a chart recorder.

qRT-PCR analysis of cold-induced genes—Yeast cells were grown until they reached 1-2 × 10⁷ cells/ml, and then 15 ml of the yeast culture was collected for a time 0 reference. The cells were harvested by centrifugation, flash-frozen in liquid nitrogen, and stored at -80°C until RNA preparation. The remaining cultured cells were cold-shocked at 15°C in a precooled water bath and were further aerobically cultured at 15°C. Cells were collected at 1, 4, 8, 24, and 30 h after the cold shock by centrifugation. The harvested cells were also flash-frozen and stored as described above. The total RNA was prepared by a hot phenol method (40). Real-time PCR was performed using the Applied Biosystems 7300 Real-Time PCR system. The following components were mixed on ice: 10 µl of 2× one-step SYBR RT-PCR Buffer III, 0.4 µl of ROX reference Dye, 0.8 µl of PrimeScript RT enzyme Mix II, 8 µM of each primer, 50 ng of template RNA, and RNase-free distilled H₂O to a total volume of 20 µl. The RT reaction (cDNA synthesis) was carried out at 42°C for 5 min. The reaction mixture was then incubated at 95°C for 10 s to inactivate the enzyme and denature the RNA/cDNA hybrid. The DNA amplification by PCR was next performed for 40 cycles, each cycle consisting of denaturation at 95°C for 5 s, primer annealing and extension at 60°C for 31 s. The PCR product was subjected to dissociation conditions to confirm that it consisted of a single component. The relative mRNA amount was calculated and normalized to the amount of act1.

Survival in stationary phase—Yeast strains were streaked on YEA and grown for 5 days at 30°C. From these plates a preculture was inoculated on YEA and allowed to grow it until it reached 1-2 × 10⁷ cells/ml. This preculture was then used to inoculate a 100-ml culture. This culture was grown until the end of the exponential phase, when the OD₅₉₅ stopped increasing, and the cells had reached their maximum density. At this point, we started monitoring the cell survival by measuring the ability of individual yeast cells/organisms to form a colony (colony forming units [CFUs]). The cultures were serially diluted to reach a 1: 2×10⁴ dilution in YEA, and 100 µl of this dilution was plated in triplicate onto YPD plates. After 5-7 days, the total number of colonies was counted, with this number representing 100% survival and day 0 of the curve. The subsequent measurements were taken every day.

RESULTS

S. pombe Set13 is an active methyltransferase that modifies ribosomal protein L42—Thirteen SET domain-containing proteins (SET proteins) have been identified in the S. pombe genome (Supplemental Table S1). Using an in vitro methyltransferase (MTase) assay, we previously demonstrated that one of these SET proteins, Set11, specifically methylates ribosomal protein L11 (34). Here we applied the same approach to investigate the cellular function of Set13, a previously uncharacterized SET protein encoded by SPAC688.14.

We prepared a recombinant His-tagged full-length Set13 (His-Set13) (Fig. 1A), and incubated it with acid-extracted nuclear extracts prepared from wild-type or Δset13 S. pombe cells in the presence of ³H-labeled methyl donor ([³H] AdoMet). Although specific methylation signals were not detected in the assay using an extract from wild-type cells, a strongly methylated band with a molecular mass of ~15 kDa on SDS-PAGE was detected in the assay using the Δset13 cell
extract (Fig. 1B, p15me). This result suggested that Set13 is an active methyltransferase and that p15 is one of Set13’s physiological targets. As was the case for Set11 (34), the methylation site(s) may have been modified already by endogenous Set13 in the wild-type cells.

To identify the target protein(s) of Set13, we separated the nuclear extracts of Δset13 mutant cells by reverse-phase chromatography, and the eluted proteins were tested in the in vitro MTase assay. As shown in Figure 1C, the target protein(s) was eluted in several fractions, with a peak at fraction 17. The protein band showing the same elution profile in the chromatography (indicated by an arrowhead) was excised from the gel and subjected to LC-MS/MS analysis. In parallel with this chromatographic approach, we separated the methylated product(s) by two-dimensional (2D) acetic acid-urea-Triton X-100 (AUT) and acetic acid-urea-cetyltrimethylammonium bromide (AUC) gel analysis (34). After 2D separation, one discrete signal was detected in the autoradiograph (Supplemental Fig. S1). The protein spot corresponding to this signal was excised and subjected to LC-MS/MS analysis. From both the chromatographic (Fig. 1C) and 2D gel (Supplemental Fig. S1) approaches, we obtained a series of peptides that matched perfectly with the deduced amino acid sequence of the S. pombe ribosomal large subunit protein L42 (Rpl42) (Fig. 1D, indicated by underlines). Rpl42 is highly conserved from yeast to humans (Fig. 1D), and its structural homologue was also identified in Haloarcula (41).

Set13 modifies recombinant Rpl42 in vitro– To confirm that Rpl42 is a physiological substrate for Set13, an in vitro MTase assay was performed using recombinant full-length Rpl42 (GST-Rpl42-Full) (Fig. 2A). The full-length GST-Rpl42 was clearly methylated by Set13 (Fig. 2B), indicating that Rpl42 is indeed a substrate for Set13. To determine the methylation site(s) on Rpl42, we separated Rpl42 into three parts: N, N-terminus; M, middle part; and C, C-terminus, and GST-fusion proteins containing each part (Fig. 2A) were used in the MTase assay. Set13 selectively methylated the GST-fusion protein containing the middle part of Rpl42 (Fig. 2B, GST-Rpl42-M), suggesting that the methylated residue(s) resides in amino acids 36-72 of Rpl42.

To identify the site of Rpl42 methylation, we introduced a series of alanine substitutions for the candidate lysine residues in Rpl42-M-His, either alone or in combination (Fig. 2C), and used these mutant proteins in the in vitro MTase assay. While the K60-67A mutation decreased the Set13 activity, the K40-55A mutation completely abolished it (Fig. 2D, Rpl42-M-HisK40-55A). Further detailed mapping revealed that the single alanine substitution of lysine 55 (K55A) clearly blocked the Set13 MTase activity (Fig. 2D, rRpl42-M-HisK55A). We also confirmed that the same K55A mutation in full-length Rpl42 (rRpl42-His) abolished Set13’s activity (Fig. 2E). Together, these results indicate that Rpl42 is a physiological substrate of Set13 and that lysine 55 of Rpl42 is the candidate target residue for Set13. The reduced activity seen with the K60-67A mutant (Fig. 2D) may have been caused by poor substrate recognition by Set13, since these mutations could have altered the local structure of the protein.

Determination of the methylation sites of Rpl42 by LC-MS/MS – To determine the in vivo methylation site of Rpl42, the endogenous Rpl42 in wild-type or Δset13 cells was isolated by reverse-phase chromatography (as shown in Fig. 1C) and analyzed using LC-MS/MS. While the overall elution profiles of the digested Rpl42 peptides in the nano-LC spectra were superimposable, the representative masses of several eluted peptides were different between the wild-type and Δset13 cells (Fig. 3A,B, indicated by asterisks). The experimental mass of the faster-eluted peptides obtained from a linear ion-trap TOF system was 1532.04 (MH+) for wild-type cells and 1517.99 (MH+) for Δset13 cells (Supplemental Table S2, NanoFrontierLD) and the mass difference was 14.05 Da, which corresponds to the mass of one methyl group. A similar mass difference was observed for a slower-eluted fragment at 8.12 min.

MS/MS analysis revealed that the amino acid sequence of both the faster- and slower-eluted fragments matched the 47-60 residues of Rpl42, and that the slower-eluted peptide was a deaminated derivative of the faster-eluted peptide (Fig. 3C,D, and Supplemental Table 2). Importantly, the peptide in wild-type cells was monomethylated at lysine 55 (Fig. 3C), whereas
the corresponding residue in Δset13 cells was unmodified (Fig. 3D). This result is consistent with our in vitro MTase assay results (Fig. 2E), and suggests that Set13 is a specific MTase that monomethylates lysine 55 of Rpl42.

By analyzing the MS/MS results, we were able to identify additional methylated peptides from the Rpl42 of wild-type cells (Supplemental Table S3). We found, however, that these modifications were present in the Δset13 cells, and that the corresponding unmethylated peptides were frequently detected in both wild-type and Δset13 cells. Thus, it is unlikely that these additional lysine residues are the physiological targets of Set13.

Rpl42 methylation at lysine 55 is conserved from yeast to humans— Rpl42 is evolutionarily conserved among eukaryotes, and the methylation at lysine 55 has also been identified in other species, including budding yeast (16) and plant (19). To obtain further evidence for the importance of the Rpl42 methylation, we determined whether the methyl modification is present on human Rpl36a, the homologue of S. pombe Rpl42 (Fig. 1D).

Ribosomes were purified from HEK293T cell extracts by centrifugation, and the associated proteins were resolved by SDS-PAGE (Fig. 4A,B). Human Rpl36a, detected by an anti-SpRpl42 antibody (Fig. 4B, indicated by an arrowhead), was excised and subjected to the LC-MS/MS analysis. Mass spectrometric analysis revealed that human Rpl36a was also monomethylated at lysine 53, which corresponds to lysine 55 of S. pombe Rpl42 (Fig. 4C). The high conservation of Rpl42 methylation suggests that it has an important role in the ribosomal function.

Location of Rpl42 and lysine 55 on the large ribosomal subunit— To gain insight into the role of Rpl42 methylation, we examined the structure and relative position of Rpl42 on the large ribosomal subunit. Since structural information on the S. pombe ribosome is not yet available, we used the previously characterized cryo-EM structure of the S. cerevisiae 80S ribosome (42) to visualize the three-dimensional structure and relative location of Rpl42 in the large ribosomal subunit (Fig. 5A,B). Rpl42 has a large loop extension and is positioned between the central- and L1-protuberances of the large ribosomal subunit. Within Rpl42, lysine 55 is located in this loop extension and lies close to the E site (Fig. 5A,B, indicated by yellow).

In Haloarcula marismortui, the L44e protein, the structural homolog of Rpl42, is located at the same position in the large ribosomal subunit (41) and interacts through its loop extension with an RNA oligonucleotide that mimics the CCA end of deacylated tRNA bound to the E site (43). Lysine 51, one of the L44e residues that make specific contact with the C75 of CCA (Fig. 5C, indicated by pink arrowheads), corresponds to lysine 55 of Rpl42. Thus, Rpl42 methylation may play a role in the recognition of deacylated tRNA bound to the E site, although this possibility needs to be clarified by further detailed structural analyses.

Set13 predominantly localizes to the nucleus— To gain further insight into the function of the Set13 methyltransferase, we examined its localization by expressing it as an EGFP-fusion protein. EGFP-Set13 predominantly localized to the nucleus, including both the nucleolus and the other DAPI-dense nuclear hemisphere (Fig. 5D) (37), suggesting that Set13 modifies Rpl42 in the nucleus, presumably prior to ribosome assembly. Since the localization of Rpl42-GFP was the same for wild-type and Δset13 cells (Fig. 5E), it is likely that the assembly of Rpl42 into the ribosome is independent of its methylation.

Rpl42 methylation-defective mutants show cycloheximide sensitivity— Cycloheximide is a potent protein synthesis inhibitor that blocks translational elongation by interfering with the peptidyl transferase activity of the 60S ribosome. In several organisms, mutations in the large ribosomal subunit lead to a recessive cycloheximide-resistance phenotype (44). One of these mutations maps to proline 56 of Rpl42 (45), which is close to lysine 55 (Fig. 5A, indicated in blue). This result prompted us to investigate the role of Set13 and Rpl42 methylation in cycloheximide sensitivity.

The Δset13 mutant cells were viable and showed no noticeable growth defects under normal culture conditions (e.g. see Fig. 6B, 0 µg/ml CYH). However, the Δset13 cells displayed higher cycloheximide sensitivity than wild-type cells (Fig. 6B, 10 and 20 µg/ml CYH). The increased sensitivity was not attributable to a change in the Rpl42 protein level, which was
comparable between wild-type and ∆set13 cells (Fig. 6A). To rule out the possibility that other Set13 substrates indirectly affected the cycloheximide sensitivity, we introduced two amino acid substitutions (K55R and P56Q) into the rpl42+ gene, and examined the cycloheximide sensitivity. As previously observed (45), the rpl42<sup>P56Q</sup> mutation conferred a strong resistance to cycloheximide (Fig. 6A,B). In contrast, the rpl42<sup>K55R</sup> mutant cells were even more sensitive to cycloheximide than the ∆set13 cells, although the Rpl42 protein level was the same (Fig. 6A,B). Taken together, these results demonstrated that Set13 and Rpl42 methylation play a direct role in cycloheximide sensitivity, which is tightly linked to ribosomal function. The rpl42<sup>K55R</sup> cells' greater sensitivity to cycloheximide was presumably owing to the imperfect mimicry of the lysine residue by arginine.

To gain further insight into the roles of Set13 and the methylation of Rpl42 in ribosomal function, we examined whether the ∆set13 and rpl42 mutant cells were sensitive to other ribosome-targeting translational inhibitors, including anisomycin, paromomycin, G418, and hygromycin (46-48). The ∆set13 and rpl42 mutant cells showed no clear sensitivity to these translational inhibitors, and only the rpl42<sup>K55R</sup> cells showed a weak resistance to hygromycin (Supplemental Fig. S2). These results suggested that methylated Rpl42 contributes to particular step(s) in the peptidyl transferase reaction rather than affecting overall ribosomal function. Considering its location and a previous observation that cycloheximide arrests the ribosome when the first deacylated tRNA reaches the E-site (49), it is likely that cycloheximide blocks elongation by interacting with the E-site when it contains deacylated tRNA, and that Rpl42 methylation-defective mutant cells affect the binding or affinity of cycloheximide.

Rpl42 methylation-defective mutants show abnormal cell growth under various environmental stresses— We next examined whether Set13 and Rpl42 methylation are involved in other cellular processes that are linked to ribosomal function. As described above, Rpl42 methylation-defective mutant cells showed no obvious growth defects under normal culture conditions (Fig. 6B). This finding was confirmed by a polysome analysis that showed similar levels of 40S, 60S, and 80S ribosomes, and comparable profiles of polyribosomal components (Supplemental Fig. 3) in the methylation-defective and wild-type cells. We noticed, however, that unlike the wild-type cells, the ∆set13 and the rpl42<sup>K55R</sup> mutant cells showed robust growth at low temperature (Fig. 6C, 15°C). In addition, these mutant cells showed resistance, to different degrees, to other stress conditions such as high temperature (Fig. 6C, 38°C), high salt concentration (Fig. 6D), and glucose starvation (Fig. 6E). The rpl42<sup>K55R</sup> cells showed stronger effects compared with the ∆set13 cells. The cycloheximide-resistant rpl42<sup>P56Q</sup> mutant cells also displayed abnormal responses to these environmental stresses. Interestingly, the rpl42<sup>P56Q</sup> mutant cells showed the opposite phenotype for high temperature (Fig. 6C, 38°C) and glucose starvation (Fig. 6E) to that observed for the ∆set13 and rpl42<sup>K55R</sup> cells. Together, these results demonstrated that Set13 and Rpl42 methylation are required for proper growth control under various stress conditions, and suggest that the E-site configuration determined by Rpl42 may control ribosomal function under these environmental stresses.

Cold adaptation of Rpl42 methylation-defective mutants is independent of the general stress-response pathways— Among several stress conditions that we examined, the Rpl42 methylation-defective mutant cells displayed the greatest differences under the low-temperature condition (15°C) (Fig. 6C). While this temperature is far below that of laboratory culture conditions (~30°C), it commonly occurs in the natural world, and therefore the phenotype appears to be linked with important cell-survival responses. To investigate the role of Rpl42 methylation further, we focused on this cold-adaptive phenotype.

Cells first grown at 30°C were shifted to 15°C, and the cellular growth after the temperature shift was monitored (Fig. 7A). Upon the shift to the low temperature (Fig. 7A, 0 hr), the growth rate of the wild-type cells immediately slowed, although they continued to grow. While the ∆set13 and rpl42 mutant cells showed a similar growth arrest at the time of the temperature shift, their growth, following adaptation, was clearly different from
that of wild-type cells (Fig. 7A). That is, the \( \Delta \text{set13} \) and \( rpl42 \) mutant cells showed a normal response to the temperature shift, but grew at an abnormal rate under cold-adapted conditions. The expression profiles of known cold-induced genes (Supplemental Fig. S4) supported the notion that the initial cold responses functioned properly in these mutant cells.

The \( rpl42^{K55R} \) mutant cells showed a higher growth rate than the wild-type cells, consistent with the spotting assay (Fig. 6C). In contrast, the \( \Delta \text{set13} \) and \( rpl42^{P56Q} \) mutant cells showed a lower growth rate (Fig. 7A). For \( \Delta \text{set13} \), this was seemingly in contrast to the results of the spotting assay (Fig. 6C). We think, however, that these mutants may simply have needed a longer time to recover from the cold-induced growth arrest, because once they recovered, their cold-adapted growth was faster than that of wild-type cells.

Translational control is tightly linked to stress responses. Many different types of stress reduce global translation by triggering the phosphorylation of eIF2\( \alpha \), which is mediated by stress-activated protein kinases, Gen2 and Hri2, in fission yeast (50). To investigate the relationship between Rpl42 methylation and the global stress-response pathway, we combined \( \Delta \text{gcn2} \) with the \( \Delta \text{set13} \) or \( rpl42 \) mutations and examined the growth of these double-mutant cells under stress conditions (Fig. 7B). Although the \( \Delta \text{gcn2} \) mutation itself caused a weak growth defect or resistance under the stress conditions, it caused little or no change in the cold-adapted growth of \( \Delta \text{set13} \) or \( rpl42 \) mutant cells. The same was true for \( \Delta \text{hri2} \) mutant cells (data not shown). In addition, cold stress did not trigger the phosphorylation of eIF2\( \alpha \) (Fig. 7C), whereas heat-shock stress did induce the phosphorylation. These results suggested that the cold-adapted growth of Rpl42 methylation-deficient cells was not coupled to the eIF2\( \alpha \)-mediated stress-response pathway.

We also explored potential genetic links between the \( \Delta \text{set13} \) mutation and other stress-response pathways such as TOR (51) and the stress-activated protein kinase (SAPK) (52). However, we did not find any correlations with the cold-adapted growth of Rpl42 methylation-deficient cells (data not shown). Therefore, it is likely that the cold-adapted growth of \( \Delta \text{set13} \) and \( rpl42^{K55R} \) mutant cells was caused, at least in part, by an altered physical property of ribosomes rather than by defects in the stress-response pathways.

\textit{Rpl42 methylation is linked to chronological aging—} The \( \Delta \text{set13} \) and \( rpl42^{K55R} \) mutant cells appeared to have a growth advantage under several stress conditions, while the global stress-response pathway was maintained. However, it is possible that proper growth suppression under such stress conditions is beneficial to the survival of the population. Consistent with this idea, we frequently observed that these mutant cells recovered poorly after prolonged storage in the laboratory refrigerator (data not shown). Thus, to determine the potential involvement of Rpl42 methylation in population survival, we examined cell viability after the cells entered the stationary phase.

The viability of individual yeast cells decreases with the time they spend in stationary phase, a phenomenon known as chronological aging (53,54). To study the role of Rpl42 methylation in this process, the \( \Delta \text{set13} \) and \( rpl42 \) mutant cells were grown until the stationary phase, and their survival rate was determined by counting the colony-forming units (CFUs). Under our experimental conditions using rich culture medium, 99.9% of the wild-type cell died after 9 days (Fig. 8A). Interestingly, the \( \Delta \text{set13} \) and \( rpl42^{K55R} \) mutant cells exhibited roughly half the life span of wild-type cells, with the \( rpl42^{K55R} \) mutant cells showing the more severe phenotype (Fig. 8A,B). In contrast, the cycloheximide-resistant \( rpl42^{P56Q} \) mutant cells exhibited a longer life span than wild-type cells (Fig. 8A). The survival potential appeared to correlate with cycloheximide sensitivity. Together, these results suggested that the Set13 activity and Rpl42 methylation correlate with the chronological lifespan.

\textbf{DISCUSSION}

Using an \textit{in vitro} methyltransferase assay, we demonstrated that fission yeast Set13 is a methyltransferase for lysine 55 of the ribosomal protein Rpl42. Since under our assay conditions we could not detect any other proteins that were efficiently modified by Set13 (Fig. 1B), it is most likely that Rpl42 is a specific substrate for Set13.
Although its direct enzymatic activity has yet to be examined, the budding yeast SET7 gene was recently demonstrated to be required for the monomethylation at lysine 55 of Rpl42ab (16). In addition, we found that human Rpl36a/Rpl42 is also monomethylated at the corresponding lysine residue (Fig. 4). Together with a mass spectrometric analysis of plant ribosome proteins (19), these findings indicate that Rpl42/Rpl36a methylation and its responsible enzyme are highly conserved among a wide range of eukaryotic species.

In *S. cerevisiae*, Rpl42 is also monomethylated at lysine 40, and this methylation is dependent on the Ybr030w gene product (16). Although we could not obtain concrete evidence for a corresponding methylation in *S. pombe* Rpl42 or human Rpl36a (data not shown), a combination of methylation modifications at different residues on Rpl42 may modulate its function in certain species. Most of the methyl modifications on histones appear to be enzymatically reversed by a family of demethylases (5). It remains an open question whether a member of the demethylase family can target the methyl modifications on ribosomal proteins.

Because Rpl42 prepared from wild-type cells was not a good substrate for Set13 in vitro (Fig. 1B), it is likely that Rpl42 is predominantly methylated at lysine 55 in wild-type cells (16). In addition, Rpl42 is tightly associated with, or rather embedded in, the ribosomal RNA of the 60S subunit (Fig. 5B). Rpl42 methylation appears to occur during the ribosomal assembly process, as supported by the nuclear localization of Set13 (Fig. 5D), and, once Rpl42 is assembled into the 60S subunit, its methylation might be stably maintained and important for ribosomal function.

From the cryo-EM structure of the *S. cerevisiae* 80S ribosome (42), Rpl42 was determined to lie close to the E-site (Fig. 5B). In addition, the x-ray crystal structure of complexes between the *Haloarcula marismortui* 60S subunit and E-site substrates revealed that deacylated tRNA makes specific contacts with the loop extension of the L44e protein, the structural homolog of Rpl42, and the residues responsible for this interaction lie close to the region containing lysine 55 and proline 56 of Rpl42 (43) (Fig. 5C). Together, these observations support the possibility that the loop extension of Rpl42 and the methylation at lysine 55 play a critical role in an interaction with the deacylated tRNA positioned at the E-site. Intriguingly, human L36a-like, which is closely related to Rpl36a/Rpl42, has been demonstrated to make contact with the CCA end of P-site-bound tRNA (55). Therefore, it is also possible that Rpl42 plays a role in the recognition of tRNA positioned at the P-site.

The function of the E-site has been extensively studied for the *E. coli* ribosome. A specific interaction between the 3’ end of deacylated tRNA with the E-site is required for an efficient translocation reaction (56). Furthermore, the mutation of a highly conserved residue in the 23S rRNA that interacts with the 3’ end of deacylated tRNA at the E-site leads to a translocation defect and promotes frameshifting and misreading at stop codons in vivo (57). According to these observations, we examined the efficiency of frameshifting events in the *Δset13* and *rpl42* mutant cells. However, we could not obtain direct evidence for an effect on frameshifting (data not shown). The role of E-site may be regulated differently in the eukaryotic ribosome.

The *Δset13* and *rpl42* mutant cells showed enhanced cycloheximide sensitivity, and in contrast, the *rpl42* mutant cells showed enhanced resistance to cycloheximide. Although the exact mechanism and position of this antibiotic’s action has yet to be determined, these two residues and the methyl modification may affect the binding affinity of cycloheximide for the 60S ribosome. This is quite consistent with a previous observation that cycloheximide arrests the ribosome when the first deacylated tRNA reaches the E-site (49).

It is noteworthy that the *Δset13* and *rpl42* mutant cells also showed defects in stress-adapted growth control. Under stress or starvation conditions, translation initiation is blocked by eIF2α phosphorylation and eIF4F disassembly (22,23). In the present study, we demonstrated that the defects of the stress-induced growth control of *Δset13* and *rpl42* mutant cells were distinct from the signaling pathways that control translation initiation.

Another intriguing finding was that the stress-adapted growth defect observed in these
mutant cells appeared to be correlated, at least to some extent, with the cycloheximide sensitivity and chronological life span. As described above, a simple explanation for the cycloheximide sensitivity is that the loss of methylation or amino acid substitution at lysine 55 changes the structural conformation of Rpl42 in a way that affects the binding affinity of cycloheximide to the ribosome. However, this idea does not fully explain our observation that the cycloheximide sensitivity correlates with stress-adapted cell growth control.

It is conceivable that cycloheximide treatment mimics the condition of ribosomes under some sort of stress and that the methylation of Rpl42 at lysine 55 fine-tunes the ribosomal function rather than affects the ribosome’s affinity for cycloheximide. The loss of the methylation may lead to a defect in this precision machinery that results in a defect in the stress-responsive growth control. An alternative is that the E-site configuration determined by Rpl42 functions as an intrinsic sensor of environmental conditions and modulates ribosomal function. In this scenario, the \( \Delta set13 \) and \( rpl42 \) mutant cells would be defective in their ability to sense environmental conditions or to change the growth rate to the appropriate level.

Some yeast species possess a variant Rpl42 that confers cycloheximide resistance on the cell (58). It is possible that cells adapt to stress by preparing several subtypes of ribosomes that provide distinct responses to environmental stresses. It is interesting to imagine that a minor fraction of ribosomes that contains unmodified Rpl42 plays a role under stress conditions and affects cellular processes such as cancer development in humans (59). Further studies are necessary to elucidate how Rpl42’s methylation regulates stress-adapted cell growth.

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FOOTNOTES

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FIGURE LEGENDS

Figure 1. *S. pombe* Set13 specifically methylates a protein in nuclear lysates. (A) N-terminal His-tagged Set13 (His-Set13) was expressed in *E. coli* and purified by metal affinity chromatography. The eluted protein was resolved by 5-20% SDS-PAGE and visualized by Coomassie staining. His-Set13 is indicated by an arrowhead. (B) *In vitro* MTase assay using *S. pombe* nuclear extracts. *S. pombe* nuclear extracts prepared from wild-type or ∆set13 cells were resolved by 5-20% SDS-PAGE and visualized by Coomassie staining (left). These extracts were incubated with His-Set13 and [3H] AdoMet. The proteins were resolved by 15% SDS-PAGE, and labeled proteins were detected by autoradiography (right). The positions of size markers and labeled proteins are indicated, respectively, to the left and right of each image. (C) Fractionation of Set13 substrate(s) by reverse-phase chromatography. Nuclear extracts prepared from ∆set13 cells were fractionated by reverse-phase chromatography. The proteins in each fraction were then concentrated and subjected to the *in vitro* MTase assay using His-Set13. After being resolved by 15% SDS-PAGE, the [3H]-labeled proteins were detected by autoradiography (top), and the total proteins were detected by Coomassie staining (bottom). Protein(s) showing a similar migration profile to the [3H]-labeled band is indicated by an arrowhead. (D) The amino acid sequences of *S. pombe* Rpl42 (SpRpl42), *S. cerevisiae* Rpl42 (ScRpl42), human Rpl36a (HsRpl36a), and *Mus musculus* Rpl44 (MmRpl44) aligned by the ClustalW 1.83 program. Identical amino acids are heavily shaded, and conserved amino acids are shown with light shading. The positions of peptide fragments identified in the LC-MS/MS analysis are indicated by black lines under the alignment.

Figure 2. Recombinant Rpl42 is methylated in vitro by His-Set13. (A) Schematic drawing of full-length and three fragments of Rpl42: the N terminus (GST-Rpl42-N), the middle part (GST-Rpl42-M), and the C terminus of the protein (GST-Rpl42-C). The position of each lysine residue is indicated by a K. The amino acid numbers are also shown. (B) *In vitro* MTase assay using recombinant Rpl42. GST-Rpl42-Full, GST-Rpl42-N, GST-Rpl42-M, and GST-Rpl42-C were incubated with His-Set13 and
[3H]AdoMet. The proteins were resolved by 15% SDS-PAGE and visualized by Coomassie staining (right). Proteins methylated by Set13 were detected by autoradiography (left). (C) Schematic representation of the middle part of Rpl42 (Rpl42-M-His) and lysine candidates for methylation. These eight lysine residues were replaced by non-methylatable alanine either alone or in combination. A, alanine. (D, E) In vitro MTase assay using recombinant alanine-substituted mutants of Rpl42. Alanine-substituted mutants of Rpl42-M-His (D) and Rpl42-His (E) were incubated with His-Set13 and [3H]AdoMet. The proteins were resolved by 15% SDS-PAGE and visualized by Coomassie staining (D, bottom). Proteins methylated by Set13 were detected by autoradiography (D, upper; E).

Figure 3. LC-MS/MS analysis of Rpl42 derived from wild-type and ∆set13 S. pombe. Rpl42 was isolated from wild-type or ∆set13 mutant cells by reverse-phase chromatography, digested with trypsin, and analyzed using a quadrupole ion trap mass spectrometer (Finnigan LTQ; Thermo Fisher Scientific). (A, B) Base peak, ion chromatogram for a 12-min separation of the digested Rpl42 peptides from the wild-type (A) and ∆set13 strains (B). The elution time (upper) and representative m/z (lower, underlined) of the eluted peptides are indicated at the top of each peak. The peaks for the peptide fragment spanning residues 47-60 are indicated by an asterisk. (C) MS/MS spectra of the peptide fragment spanning residues 47-60 from the wild-type (C) and ∆set13 strains (D) are shown. The observed y and b ions and fragment map are shown.

Figure 4. Human Rpl36a is methylated at lysine 53 in vivo. (A) Procedure for preparing ribosomes and polysomes from HEK293T cells. (B) Detection of human Rpl36a using an anti-SpRpl42 antibody. Proteins were resolved by 8-16% SDS-PAGE and visualized by Coomassie staining (left). Rpl36a detected by the anti-SpRpl42 antibody is shown (right). The protein showing a similar migration as the western blot signal is indicated by an arrowhead. (C) LC-MS/MS analysis of human Rpl36a. Rpl36a excised from an SDS-PAGE gel was digested with trypsin, and the digested peptides were subjected to LC-MS/MS analysis. The MS/MS spectrum of the peptide fragment spanning residues 45-57 is shown. The observed y and b ions and fragment map are shown.

Figure 5. Overview of Rpl42 on the large subunit of the ribosome. (A) Structure of S. cerevisiae Rpl42 (42). Blue, Proline 56 of Rpl42; Yellow, Lysine 55 of Rpl42. (B) Surface view of the large subunit of the S. cerevisiae ribosome (PDB file 1S1i) (42). Pink, Rpl42; Aqua, 5.8S/25S ribosomal RNA; Blue, 5S ribosomal RNA; Yellow, Lysine 55 of Rpl42. The locations of the E-site, P-site, and A-site are indicated. The visible proteins are identified. (C) Amino acid sequences of S. pombe Rpl42 and H. marismortui L44e aligned by the ClustalW 2 program. Identical amino acids are in red, and conserved amino acids are in blue. Pink arrowheads indicate the main residues that interact with the 3'-CCA end of tRNA. Yellow arrowhead indicates the residue that is methylated in S. pombe Rpl42. Blue arrowhead indicates the residue whose substitution confers resistance to cycloheximide. (D) EGFP-fused Set13 predominantly localized to the nucleus. EGFP-fused Set13 was expressed from the nmt1 promoter in wild-type cells. The cells were stained with Hoechst33342 (DNA). A merged image of the EGFP-Set11 and DNA (Merged), and differential interference contrast (DIC) image are also shown. (E) GFP-fused Rpl42 mainly localized to the cytoplasm and the nucleolus. Rpl42-GFP was expressed from the nmt1 promoter in wild-type or ∆set13 cells. The DIC image is also shown.

Figure 6. Rpl42 methylation-defective mutants show abnormal cell growth under environmental stresses. (A) Protein levels of Rpl42 in the ∆set13, rpl42K55R, and rpl42P56Q mutant cells. Exponentially growing cultures of wild-type, ∆set13, rpl42K55R, and rpl42P56Q cells in YEA were harvested. The total cell lysates prepared from these strains were resolved on a 12.5% SDS-PAGE gel. Rpl42 was detected by western blotting with an anti-SpRpl42 antibody (right). As a loading control, the Amido-black stained blot is shown (left). (B) The ∆set13 and the rpl42K55R mutant cells showed a greater sensitivity to cycloheximide. Five-fold dilutions of wild-type, ∆set13, and rpl42K55R cells were plated onto YEA alone.
or YEA containing different doses of cycloheximide for 3 days (top). Five-fold dilutions of wild-type and rpl42^{P56Q} mutant cells were plated onto YEA alone or YEA containing different doses of cycloheximide for 2 days (bottom). (C) The set13-null and rpl42^{K55R} mutant cells showed resistance against cold stress. Five-fold dilutions of wild-type, Δset13, rpl42^{K55R}, and rpl42^{P56Q} cells were plated onto YEA at 30 °C, 38 °C, or 15 °C for 2 days, 3 days, or 19 days, respectively. (D,E) The rpl42^{K55R} mutant cells showed resistance against environmental stresses. Five-fold dilutions of each strain were plated onto YEA alone, YEA containing different doses of NaCl (D), YEA containing 3% glycerol instead of glucose, or YEA containing 0.01% glucose plus 3% glycerol (E), for 3 days.

Figure 7. Cold-adapted growth of Rpl42 methylation-defective mutants is independent of general stress-response pathways. (A) Effect of cold shock on cell growth. Wild-type (blue), Δset13 (red), rpl42^{K55R} (orange), and rpl42^{P56Q} (green) cells were grown until the mid-log phase in YEA medium at 30 °C. The cells were then shifted to 15°C at time 0. Cell growth was monitored by measuring OD 595. (B) No genetic interaction between gcn2 and methylation-defective mutants was observed. Five-fold dilutions of each strain were plated onto YEA containing 0.25 M NaCl for 4 days or YEA alone at 30°C, 38°C, or 15°C for 2 days, 3 days, or 19 days, respectively. (C) Methylation-deficient mutation did not affect eIF2α phosphorylation in response to cold stress or heat shock. Exponentially growing cultures of wild-type, Δset13, rpl42^{K55R}, rpl42^{P56Q}, Δgcn5, and Δgcn2 cells growing in YEA were subjected to cold stress at 15 °C or heat shock at 48°C for the times indicated. The total cell lysates prepared from the indicated strains were resolved on an 11% SDS-PAGE gel. The level of eIF2α phosphorylation was analyzed by immunoblotting using an antibody that specifically recognizes phosphorylated eIF2α (top). The western blot signal of tubulin was used as a loading control (bottom).

Figure 8. Rpl42 methylation and chronological life span. (A) The survival of wild-type, Δset13, rpl42^{K55R}, and rpl42^{P56Q} in stationary phase was evaluated by counting the CFUs (colony-forming units). The Y-axis is shown in logarithmic scale. (B) Wild-type and mutant cells on the indicated days were observed under a light microscope. Arrowheads indicate abnormal or dead cells.
B

GST-Rpl42

| GST-Rpl42 | GST-Rpl42 |
|-----------|-----------|
| (kDa)     | (kDa)     |
| GST       | GST       |
| Full (1-106) | Full (1-106) |
| N (1-35)  | N (1-35)  |
| M (36-72) | M (36-72) |
| C (73-106)| C (73-106)|

C

Rpl42-M-His

| Rpl42-M-His |
|-------------|
| (kDa)       |
| GST         |
| Full (1-106) |
| M (36-72)   |
| K40-55A     |
| K60-67A     |
| K40A        |
| K46A        |
| K55A        |

D

Rpl42-M-His

| Rpl42-M-His |
|-------------|
| (kDa)       |
| GST         |
| Full (1-106) |
| M (36-72)   |
| K40-55A     |
| K60-67A     |
| K40A        |
| K46A        |
| K55A        |

E

rRpl42-His

| rRpl42-His |
|------------|
| (kDa)      |
| GST        |
| Full (1-106) |
| M (36-72)  |
| K55A       |
| K55A       |
| Substrate  |
| rSet13     |
| +          |
| -          |
| +          |
| -          |
| +          |
| +          |

Autoradiograph

Coomassie

Downloaded from http://www.jbc.org/ on March 23, 2020
A

Human HEK 293T cells

Homogenize with Dounce homogenizer

20,000g x 10 min

Supernatant

Layer over 2 M sucrose cushion buffer

100,000g x 24 hr

Pellet (Ribosomes and polysomes)

B

Purified ribosomes

(kDa)

Amido black

Anti-SpRpl42

(kDa)

C

Rpl36a in human 293T cells

Precursory ion: 727.18 m/z (2+)

CH₃

Shirai et al. Figure 4
Figure 5

**A**  
*S. cerevisiae* Rpl42

**B**  
*S. cerevisiae* ribosome 60S subunit

**C**

| Protein | Sequence |
|---------|----------|
| Hm L44e | L44e     |
| Sp Rpl42| Rpl42    |
| Hm L44e | 1 -MQMPRRFTYCP--HCNEHQHEVEKVRSGRQTMKWIDRQEREFGNDSIGNDGKFSKVF 56 |
| Sp Rpl42| 1 MVNIKPTRKTYCPGKNCRKhHTVRQTVYKKGPDSKLAQKRKYRDRKQSGFGQGQTK-FPVH 59 |
| Hm L44e | 57 GGDKPTKTDLKYYYYGGCGC--KALR--EGWRAGRLEFQE------ 92 |
| Sp Rpl42| 60 KKAHTTKVVLRECVSCKYKQLVLKCRKHFELGGEKKTGQAIF 106 |

**D**

Wild type  
EGFP-Set13  DNA  Merged  DIC

**E**

Wild type  
DIC  Ppl42-GFP

∆set13
Shirai et al. Figure 6

A

Wild type
Δset13
rpl42^K55R

S. pombe extract

(kDa)

Amido black

Anti-SpRpl42

B

Wild type
Δset13
rpl42^K55R

Wild type
rpl42^P56Q

0
10
20
(μg CYH/ ml)

3 days
2 days

C

Wild type
Δset13
rpl42^K55R
rpl42^P56Q

30
15
38
(°C)

D

Wild type
Δset13
rpl42^K55R
rpl42^P56Q

Control
+ 0.1 M NaCl
+ 0.25 M NaCl

(3% Glucose)

E

Wild type
Δset13
rpl42^K55R
rpl42^P56Q

0.01% Glucose
+ 3% Glycerol
3% Glycerol
Shirai et al. Figure 7

A

![Graph showing OD (log) vs. time (h) for different strains and conditions.]

B

![Images of gel blots showing expression levels of different strains and conditions.]

C

![Table and blot showing expression levels of eIF2α and Tubulin under heat and cold shocks.]
A

Survival (%)

Time (day)

Wild type

Δset13

rpl42\textsuperscript{K55R}

rpl42\textsuperscript{P56Q}

B

|          | wild type | Δset13 | rpl42\textsuperscript{K55R} | rpl42\textsuperscript{P56Q} |
|----------|-----------|--------|-----------------------------|-----------------------------|
| 0 day    | ![Image](wild type_0_day.png) | ![Image](Δset13_0_day.png) | ![Image](rpl42\textsuperscript{K55R}_0_day.png) | ![Image](rpl42\textsuperscript{P56Q}_0_day.png) |
| 3 days   | ![Image](wild type_3_days.png) | ![Image](Δset13_3_days.png) | ![Image](rpl42\textsuperscript{K55R}_3_days.png) | ![Image](rpl42\textsuperscript{P56Q}_3_days.png) |
Methylation of ribosomal protein L42 regulates ribosomal function and stress-adapted cell growth
Atsuko Shirai, Mahito Sadaie, Kaori Shinmyozu and Jun-ichi Nakayama

J. Biol. Chem. published online May 5, 2010

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