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Tag-mediated Isolation of Yeast Mitochondrial Ribosome and Mass Spectrometric Identification of Its New Components

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SUMMARY

Mitochondrial ribosomal proteins (mrps) of the budding yeast, *Saccharomyces cerevisiae,* have genetically and biochemically been most extensively characterized. Yet, the list of the genes encoding individual mrps is still not complete and quite a few of the mrps are only predicted from their similarity to bacterial ribosomal proteins. We have constructed a yeast strain in which one of the small subunit proteins, termed Mrp4, was tagged with S-peptide and used it for affinity purification of mitochondrial ribosome. Mass spectrometric analysis of the isolated proteins detected most of the small subunit mrps which were previously identified or predicted and about half of the large subunit mrps. In addition, several proteins of unknown function were identified. To confirm their identity further, we added tags to these proteins and analyzed their localization in subcellular fractions. Thus, we have newly established Ymr158w (MrpS8), Ypl013c (MrpS16), Ymr188c (MrpS17) and Ygr165w (MrpS35) as small subunit mrps and Img1, Img2, Ydr116c (MrpL1), Ynl177c (MrpL22), Ynr022c (MrpL50) and Ypr100w (MrpL51) as large subunit mrps.

Keywords: mitochondrial ribosomal proteins; *Saccharomyces cerevisiae*; tag-assisted purification; mass-spectrometry

Abbreviations. Mitoribosome, mitochondrial ribosome; mrp, mitochondrial ribosomal protein; 2D, two-dimensional; AP, alkaline phosphatase

Enzyme. Lysyl endopeptidase (EC 3.4.21.50)

INTRODUCTION

The mitochondrial genome codes for only a small number of proteins that are translated on mitoribosome. Previous studies showed that mitoribosome contains more proteins than the bacterial counterpart [1-5]. This may indicate that some of the mrps have been recruited to compensate the reduced size of the mitoribosomal RNAs. It is likely at the same time that some of them might carry some hitherto unknown mitochondrial functions. It should be noted in this regard that the mitochondrial genome-encoded components of yeast respiratory chain require specific translational activators [6] and some of them interact with mitoribosome [7-10]. A large subunit protein, Rml2, was found to be involved in the utilization of oleate as a carbon source [11] and its mutation affected the activity of transcription factor Adr1 [12]. One of the small subunit proteins of yeast mitoribosome, YmS2 (Ppe1), has similarity to human protein phosphatase-methylesterase . Another small subunit protein Rsm23 is a member of the DAP3 family of mitochondrial apoptosis mediators [13]. Two large subunit proteins, MrpL31 and Ygl068w the latter of which is probably related to *E. coli* L7/L12 proteins, might be involved in cell cycle control [14]. Furthermore, MrpS18, Rsm10, and YmL6 proteins are not only essential for the function of mitochondria but also indispensable for cellular growth [15]. From these results it is conceivable that some mrps play a role in communication between mitochondria and other subcellular organelles including the nucleus and peroxisome.

Recent studies on the mammalian mitoribosomes showed that they also contain many proteins [16-19], despite the highly reduced size of their genomes. Interestingly, many of the proteins that do not resemble bacterial ribosomes appear to be unique in each organism. In order to investigate the functions of mitoribosome that are distinct from bacterial or cytoplasmic ribosomes, and to gain further insight into the evolution of mitochondrial

translation system, we have attempted to identify as many mrps and associated proteins as possible. For this purpose, we took advantage of the use of mass spectrometry and newly identified several yeast mrps as described below.

MATERIALS AND METHODS

Plasmid construction. pSH*Leu* plasmid (Fig. 1) was used to produce S-tagged mrps in yeast. It was based on the pDB*Leu* vector (GibcoBRL, Life Technologies) and constructed by inserting a 140 bp *Msc*I-*Nco*I fragment of plasmid pET-32a(+) (Novagen) encoding S-tag peptide into the *Msc*I-*Nco*I site of pDB*Leu*. Subsequently, the *Hin*dIII-*Sma*I fragment containing the *GAL4* DNA binding domain was replaced with the *Hin*dIII-*Sma*I fragment containing the multi-cloning site of pUC119 plasmid.

All plasmids containing the S-tagged mrp genes were constructed using pSH*Leu*. DNA fragments harboring respective genes without termination codon were amplified by PCR from the genomic DNA of RAY3A-D cells by using primers to add appropriate restriction sites. They were then inserted at the multi-cloning site of pSH*Le*u in frame.

For the disruption of *MRP4*, a 1.8 Kb DNA fragment containing the *MRP4* gene was PCR amplified and cloned into pUC119. Then, a 1.75 Kb *HIS3*-containing fragment was inserted at the *Bam*HI site within *MRP4* and the resultant plasmid, pUC-*mrp4::HIS3*, was used to replace the chromosomal *MRP4*. Similarly, pT7Blue-*ynr022c::HIS3* was constructed by inserting *HIS3* at the *Msc*1 site of *YNR022c* cloned on pT7Blue vector (Novagen).

Strains and media. Yeast strain Ray3Ãα (α type haploid of RAY3A-D *leu2/leu2, his3/his3, ura3/ura3, trp1/trp1*) was used to isolate mitoribosome. An *MRP4* disruptant was constructed by transforming RAY3A-D cells carrying plasmid pSH*Leu-MRP4* with linearized plasmid pUC-*mrp4::HIS3* and selecting histidine prototrophic recombinants. Haploid strain RAY3A-α (*mrp4::HIS3*/pSH*Leu-MRP4*) was obtained after sporulation. Derivatives of RAY3A-α with disrupted *MRPL50* were similarly constructed using plasmid pT7Blue-*mrpl50::HIS3*. Strains in which *MRPS8*, *MRPS16*, *MRPS35*, *MRPL1* or *MRPL51* was disrupted were

purchased from Research Genetics (Huntsvill, AL) either as haploid derivatives of BY4741 *mat* ***a*** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0* or heterozygous diploid derivatives of BY4743 *mat* ***a***/*α his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 ura3Δ0/ura3Δ0 MET15/met15Δ0 LYS2/lys2Δ0*. When necessary, haploid disruptants were isolated after sporulation. Disruption of the mrp gene in each of them was confirmed by PCR (data not shown). Growth media, culture conditions and genetic manipulations are essentially as described [20]. For the preparation of mitochondria, cells were grown in YPGE medium (2% Bacto-peptone, 1% yeast extract, 2% glycerol, 2% ethanol) until A_{600} of 5 was reached. *E. coli* strain XL-1 Blue {*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* $\bar{\lambda}$*Δ* (*lac-proAB*) F'[*proAB+ lacI*^{q} *lacZΔ*M15 Tn10(Tet^r)]} was used for plasmid propagation.

Purification of the complex containing S-tagged Mrp4. Mitochondria were purified from about 10 g wet-weight cells of strain RAY3A-α *mrp4::HIS3*/pSH*Leu-MRP4* essentially as described previously [21]. Mitochondria were suspended in Buffer F (350 mM NH_4Cl, 20 mM Mg-acetate, 1 mM EDTA, 2 mM β-mercaptoethanol, 20 mM Tris-HCl pH7.5) and lysed by adding 1/20 volume of 26% Triton X-100 and the debris was removed by centrifugation. The lysate was further purified by filtrating through Ultrafree-MC (0.65 μm pore size, Amicon Millipore), mixed with 50% S-agarose (Novagen) (60 μl/ml lysate) and incubated for 30 min at room temperature. Complexes containing S-tagged Mrp4 bound to S-agarose were washed four times with buffer F as recommended by the manufacturer, dissolved in loading buffer and subjected to SDS-PAGE.

Mass spectrometry. Proteins on SDS gels were visualized by the reverse staining method [22]. Proteins were reduced by incubating with 10 mM EDTA/10 mM DTT/100 mM

NH_4HCO_3 for 1 h at 50 ^oC and alkylated by treatment with 10 mM EDTA/40 mM iodoacetamide/100 mM NH_4HCO_3 for 30 min at room temperature. They were digested in gel with lysyl endopeptidase from *Achromobacter lyticus* (Wako Pure Chemical) in 100 mM Tris-HCl (pH8.9) for 15 h at 37 ^oC. Peptide fragments were extracted from and then concentrated *in vacuo*. After desalting with ZipTip (Millipore), peptide fragments were subjected to mass spectrometry. Mass spectra were recorded on a Micromass Q-Tof2 equipped with a nano-electrospray ionization source. Proteins were identified by peptide mass fingerprinting with the MASCOT program (Matrix Science) by searching against the NCBInr database.

Sucrose density gradient analysis of S-tagged mrps. Mitochondria obtained from the cells expressing the tagged mrp were lysed as described above and mitoribosomes were pelleted through 1.5 ml of 10% sucrose cushion by centrifugation in a Beckman 50Ti rotor at 40,000 rpm for 3 hours. Ribosomes were re-suspended in 0.5 ml of buffer F, layered on a sucrose gradient of 10 to 30% in buffer F and centrifuged in a Beckman VTi65.2 at 45,000rpm for 37 min at 4 ^oC. Fractions of about 0.12 ml were collected and their absorbance at 280 nm was measured. After diluting sucrose with the same volume of water, ribosomes were sedimented by adding two volumes of acetone. Proteins were dissolved in loading buffer and subjected to SDS-PAGE. S-tagged proteins were then detected by Western blotting with S-protein AP conjugate (Novagen) as suggested by the manufacturer.

RESULTS

Identification of yeast mrps by mass spectrometry. Previously, we have purified yeast mitoribosomal subunits by the standard sucrose density-gradient method and isolated their proteins by chromatography and two-dimensional gel electrophoresis [5]. The partial amino acid sequence of each protein was subsequently determined to clone the gene. However, the yeast mitoribosome, especially its small subunit, was unstable and we could not obtain enough amount of small subunit to purify each component. Therefore, to simplify the procedure of isolation and purification of mitoribosome, we constructed a plasmid containing the gene for Mrp4, one of the small subunit proteins, tagged with a peptide derived from ribonuclease S. This peptide of 15 amino acid residues (S-tag) interacts strongly with S-protein and forms an S-tag:S-protein complex with a K_d of 10^{-9}M, allowing easier purification and detection of the tagged protein. The resultant plasmid was then introduced into the yeast strain RAY3A-α *mrp4::HIS3* and complexes containing the S-tagged Mrp4 were isolated by affinity purification as described in Materials and Methods. The purified complex was subjected to SDS-PAGE and proteins were separated into fourteen fractions according to the molecular mass. Proteins in each fraction were analyzed by the peptide mass fingerprinting method using the MASCOT program.

As shown in Table 2, most of the small subunit mrps were detected in this way that have already been identified or predicted from the sequence similarity to prokaryotic ribosomal proteins. Some mrps of the large subunit were also detected, albeit to a limited extent. In addition, several proteins of unknown function such as Ygr165w and Ynr022c were detected.

Localization of newly identified proteins to mitoribosomal subunits. Subsequently, we examined whether the two proteins of unknown function mentioned above as well as Ymr158w, Ypl013c, Ymr188c, Ydr116c, Img1, Ynl177c, Ypr100w and Img2 are indeed yeast mrps and, if so, with which subunit they are associated. The latter eight proteins mentioned above have been related to bacterial ribosomal proteins S8, S16, S17, L1, L19, L22, and human mrps MRP-L43 and MRP-L49, respectively. The gene for each protein was cloned into the plasmid pSH*Leu* to attach an S-peptide tag as described in Materials and Methods and the resultant plasmid was introduced into RAY3A-□ cells by transformation. Subsequently, mitoribosomes were purified from the transformant and the subunits were separated by sucrose density gradient centrifugation. The proteins in fractions recovered were analyzed by SDS-PAGE followed by Western-blotting and each of the S-tagged proteins was detected.

As shown in Fig. 2, S-tagged Ypl013c, Ymr188c, Ygr165w proteins were detected in fractions of the small subunit, while S-tagged Ydr116c, Img1, Ynl177c, Img2, Ypr100w and Ynr022c proteins were localized in the large subunit. The molecular mass of the S-tagged proteins synthesized from the gene cloned on plasmid pSH*Leu* should be about 6 kDa larger than the authentic proteins. Apparent molecular mass data by SDS-PAGE for all mrps detected, however, were found to be about 10 kDa larger than expected. This was probably caused by the nature of S-tag, because all proteins were similarly affected, though we have no clear-cut explanation for the observed discrepancy.

In the case of Ymr158w, the S-tag signal in the ribosomal fractions was weak and unequivocal identification was not possible, although its localization to the mitochondrial fraction was certain (data not shown). We thought perhaps this was caused by the presence of untagged Ymr158w protein from the chromosomal gene that was more efficiently incorporated into the mitoribosome. Therefore, we introduced plasmid pSH*Leu-YMR158w*

into a derivative of strain BY4743 (*YMR158w*/*ymr158w::KAN*) and isolated a haploid strain harboring the disrupted gene on the chromosome and the S-tagged *YMR158w* on the plasmid. Using this strain we were then able to establish that Ymr158w was localized to the small subunit of mitoribosome. At the same time, we noticed that cells carrying only the tagged *YMR158w* gene grew poorly in YPGE medium, indicating that Ymr158w is essential for the mitochondrial function and the addition of S-tag to its C-terminus impaired its function.

Feature of newly identified mrps. The predicted amino acid sequences of Ymr158w, Ypl013c, Ymr188c and Ydr116c proteins clearly indicate their homologous relation with bacterial ribosomal proteins S8, S16, S17 and L1, respectively (Fig. 3, [18, 19]): Ymr188c has an extra sequence of about 150 amino acid residues at the C-terminus and is consequently three times as large as *E. coli* S17. Img1 and Ynl177c show similarity to L19 and L22 family proteins, respectively, although the degree of similarity is not high (Table 1, Fig. 3). Ypr100w and Img2 have no sequence similarity to bacterial ribosomal proteins, but recent analysis of bovine mrps by mass spectrometry in reference to human and mouse proteins predicted from the genome analysis data led to the discovery of proteins homologous to them [17, 18]. Subsequent analysis suggested the presence of Ypr100w homologues in other organisms such as *Drosophila melanogaster*, *Caenorhabditis elegans* and *Arabidopsis thaliana* (Table 1, [18]). Likewise, Img2 homologues were found in other organisms, although Img2 appears to be less conserved than Ypr100w (Table 1). In contrast, Ygr165w has no sequence similarity to any known ribosomal proteins. BLAST search, however, shows that the fission yeast *Schizosaccharomyces pombe* seems to possess a protein related to it. Similarly, no homologue of Ynr022c has been found yet. BLAST search shows a weak similarity to L9 of *Bacillus subtilis*, but it is not in the region conserved among the L9 family proteins (Fig 3), and we consider that Ynr022c is a novel protein unique to yeast mitoribosome.

Until now, we have named yeast mrps in the same way as we did with bacterial ribosomal proteins, namely according to their positions on the 2D-PAGE. However, mrps have been identified in various methods and not all proteins were actually corresponded to the spots on the 2D-gel. In addition, some of the proteins that are related to bacterial ribosomal proteins were named by including the bacterial protein names. It will therefore be necessary to rename all yeast mrps more systematically to avoid possible confusions. However, it will not be an easy task because the number of yeast mrps as well as that of *E. coli* ribosomal proteins may still increase [23] and the phylogenetic identity is not always clear due to the lack of data for mrps in other organisms. For these reasons, we simply name Ymr158w, Ypl013c, Ymr188c, Ydr116c and Ynl177c proteins to be MrpS8, MrpS16, MrpS17, MrpL1 and MrpL22, respectively, according to the protein families based on the ribosomal proteins of *E. coli*. Furthermore, we name Ygr165w, Ynr022c and Ypr100w proteins to be MrpS35, MrpL50 and MrpL51, respectively, since they are not related to bacterial ribosomal proteins and their assignment to protein spots on the 2D-PAGE [24] is not clear.

Functional characterization of novel mrps. Most of the yeast mrps have been shown to be essential for the mitochondrial function, that is, for growth on a non-fermentable sugar as a sole source of carbon (Table 2). We have examined the growth of cells in which the gene for the newly identified mrps was disrupted. As shown in Fig. 4A, disruptants of *MRPS8* (*YMR158w*), *MRPS16* (*YPL013c*), *MRPS35* (*YGR165w*) and *MRPL51* (*YPR100w*) failed to grow on YPGE medium and showed slow growth on YPD as in the case of disruptants of most other mrp genes.

In the case of *MRPS8*, it was indicated that the addition of a short peptide to the C-terminus caused poor growth in liquid YPGE medium as described already, although the effect of tagging was not clear on agar plates (Fig. 4B). Additionally, we constructed a strain

in which an HSV (Herpes Simplex Virus glycoprotein D) tag was attached to the C-terminus of MrpS8. A significant portion of the cells of the resultant strain was found to be respiration-deficient (data not shown). It was probable that the C-terminal modification of MrpS8 affected the mitoribosomal function. The bacterial homologue of MrpS8 is known to bind to 16S rRNA and the C-terminal region is important for this interaction [25, 26]. Therefore, the C-terminal portion of MrpS8 might be critical for its binding to rRNA in yeast mitochondria as well, despite that the amino acid sequence responsible for the binding in bacterial counterparts is not conserved in yeast MrpS8. The growth defect on YPGE was further exacerbated at a higher temperature. At 37 ^{o}C, cells with the HSV-tagged *MRPS8* failed to grow, and those carrying S-tagged *MRPS8* on the plasmid pSH*Leu-MRPS8* showed very poor growth (Fig. 4B). Disruptants of *MRPS8* were unable to grow at any temperatures on YPGE.

Disruptants of *MRPL1* showed reduced growth on YPGE which was recovered by the introduction of plasmid pSH*Leu-MRPL1*. The growth retardation was more pronounced at a lower temperature (Fig. 4C). This indicates that MrpL1 is not essential for the protein synthesis in mitochondria, just as the case of *E. coli* L1 [27]. It should be noted that all other mrps that were found dispensable for the mitochondrial function are not homologous to bacterial ribosomal proteins (Table 2). Therefore, MrpL1 is the first instance of yeast mrp that is homologous to a bacterial 'core ribosomal protein' and yet is dispensable. *MRPL50* has been found to be another example of dispensable mrp gene. The disruptant showed growth indistinguishable from its parental strain on both YPGE and YPD, which was also the case at different temperatures.

Disruption of *MRPS17* (*YMR188c*), *MRPL22* (*YNL177c*), *IMG1* and *IMG2* has previously been reported to render the mutant cells unable to grow on a non-fermentable carbon [28-30]. The loss of Img1 and Img2 was shown to destabilize the mitochondrial genome [29, 30]. It is well known that defects in mitochondrial protein synthesis lead to the

loss of mitochondrial genome. Recent analysis of mutants indicated that availability of isoleucine in the cell might be related to the stability of the mitochondrial genome [31], although the exact mechanism remains to be elucidated. In addition, a disruptant of *MRPL22* was reported to be defective in internalization of dye and α-factor [32]. In this connection, it should be noted that *MRPL4* disruptants showed poor growth on fermentable carbon sources with abnormal cell size and enlarged vacuoles in the stationary phase, although the mechanism which interrelates this protein and endocytosis is not known [33]. Perhaps, mitochondria and membranous subcellular organelles are somehow functionally related with each other.

DISCUSSION

The mass spectrometric analysis of the proteins associated with yeast mitoribosome isolated by affinity purification using the S-tag attached to Mrp4 protein led to the identification of 27 mrps of the small subunit and 22 of the large subunit (Table 2). The mrps thus identified include ten proteins that are either novel or only predicted before. This brings the total number of mrps identified to 31 of the small subunit and 46 of the large subunit (*MRPL7*, *MRPL38*, *MRPL10*, *MRPL24* and *MRPL17* produce two types of proteins [5]), which are in good agreement with the number estimated by 2D-PAGE analysis: namely, the mitoribosome of *S. cerevisiae* contains at least 34 and 49 proteins in the small and large subunit, respectively [5, 34]. From the structural similarity to known ribosomal proteins, *YNR036c* and*YNL081c* are likely to encode proteins of the small subunit, while *YGL068w*, *YDR115w* and *YPL183W-A* most probably encode those of the large subunit. However, their products were not detected as mrps in this work.

Saveanu and colleagues [13] used a similar strategy for the isolation of the small subunit of yeast mitoribosome and identified 12 new mrps. They used a “tandem affinity purification” tag that they claim to be suitable for the isolation of protein complexes under native conditions. However, their isolation conditions were suited for tag-antibody and tag-calmodulin interactions. In contrast, we performed affinity purification under the conditions suited for the isolation of active mitoribosome. This might have led to the identification of more mrps than Saveanu and colleagues, though we could not identify Rsm18, one of the new proteins found by them.

In addition to mrps, analysis of the tag-purified complex showed the existence of various yeast proteins of other functions (data not shown). One possible reason for this would be due to the method we used to isolate mitoribosomes. The tagged Mrp4 protein must be

synthesized on cytoplasmic ribosomes and then transported into mitochondria. Therefore, proteins such as Rpl6a, Rps19a and Mas6 were co-purified with mitoribosomes because of their association with the tagged Mrp4 protein during the course of these processes. The fact that proteins localized in mitochondrial inner membrane, such as Sdh2, Atp5, Qcr2, Pet9 and Ssc1, were detected would support the previous report that mitoribosomes are closely associated with inner membrane and a fraction of them remains within insoluble membrane fractions [35]. Indeed, in our previous examinations of mrps by 2D-PAGE, we reproducibly observed some faint protein spots that might have indicated the presence such proteins.

Another reason for the presence of various proteins other than mrps might indicate the possibility of their functional interaction with mitoribosome. In this connection, it should be noted that our mass spectrometric analysis identified Idh2, an NAD^+-dependent isocitrate dehydrogenase, and we were able to detect its loose but significant association with mitoribosomes in sucrose gradient centrifugation (data not shown). The NAD^+-dependent isocitrate dehydrogenase may bind to mRNA and regulate the translation in mitochondria [36]. Further analysis of the genes identified in our work might, therefore, reveal some new features of translation of genetic information in mitochondria.

As summarized in Table 2, proteins homologous to bacterial ribosomal proteins have been found in both yeast and mammalian mitoribosomes, although the degree of homology varies from one protein to another. The degree of differences between the yeast and mammalian mitoribosome is correlated with that of the differences in ribosomal RNA. Yeast MrpS8 protein, for example, has a weak but significant degree of similarity to *E. coli* S8 protein, and yeast 15S rRNA contains a hairpin structure, although much smaller in size, that corresponds to the *E. coli* S8 binding region. However, no protein corresponding to S8 was found in mammalian mitoribosome and mammalian 12S rRNA has no such hairpin structure (Cannone et al., http://www.rna.icmb.utexas.edu/). On the other hand, a protein homologous

to *E. coli* L24 was found in mammalian mitoribosome but so far its homologue has not been identified in yeast. It might be that the L24 homologue of yeast mitoribosome has so much deviated from bacterial L24 and is no longer discernible. Alternatively, the L24 homologue might have become dispensable in protein synthesis during the course of evolution as in the case of an *E. coli* mutant [27].

In both yeast and mammals, about a half of the mrps are unique to mitoribosome and only a small fraction of them are reasonably conserved. This is in a sharp contrast to the mrps similar to bacterial ribosomal proteins. The mitoribosome-specific proteins may have functions other than being involved in the translation in mitochondria. Otherwise, the various observed effects caused by the disruption of the mrp genes to the cellular growth under fermentable conditions cannot be explained.

To elucidate further the structure and function relationship as well as the evolution of ribosomes, it will be interesting and important to identify the molecular components of mitoribosomes in various organisms and investigate the differences among them. Perhaps, more clues with respect to parallel evolution of the structure and function of mitochondria as well as some related functions that are specific to individual organisms will be obtained.

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Legends to Figures:

Fig. 1. The structure of plasmid pSH*Leu*. Unique restriction sites within the multi-cloning site are underlined. The target sequences for thrombin and enterokinase cleavage, as well as His-tag and S-tag sequences are indicated.

Fig. 2. Subunit localization of newly identified mrps. Mitoribosomes with indicated S-tagged mrps were purified from yeast cells and subunits were separated by sucrose density gradient centrifugation. Proteins in each fraction were acetone-precipitated, separated by SDS-PAGE and analyzed by Western blotting. **A,** a typical profile of sucrose density gradient centrifugation. The 30S and 50S subunit peaks and the fractions analyzed in B are indicated. **B,** Western blot analysis of the respective mrps.

Fig. 3. Alignment of newly identified mrps in *S. cerevisiae* with related proteins from various organisms. Multiple alignment was performed with homologous proteins from *Schizosaccharomyces pombe*, *Synechococcus* sp. PCC 6301, *Bacillus subtilis*, *Escherichia coli*, *Homo sapiens*, *Drosophila melanogaster*, *Reclinomonas americana*, *Borrelia burgdorferi*, *Thermotoga maritim*a, *Caenorhabditis elegans*, *Synechocystis* sp., and *Staphylococcus aureus* by using CLUSTAL X [37]. For comparison, bacterial ribosomal proteins of L9 family were shown below the sequence alignment of Ynr022c (MrpL50) with its homologue. Boxes show degrees of sequence conservation with asterisks indicating the residues identical in all sequences.

Fig. 4. Growth of disruptants of newly identified mrps and of cells with tagged *MRPS8*. **A,** Strains with disrupted genes, *Δmrps8* (*Δymr158w*), *Δmrps16* (*Δypl013c*), *Δmrps35*

(*Δygr165w*), *Δmrpl1* (*Δydr116c*), *Δmrpl50*(*Δynr022c)* and *Δmrpl51* (*Δypr100w*) were streaked on YPD and YPGE plates and incubated at 30 ^{O}C. **B,** Strains with S-tagged *MRPS8* (*Δmrps8*/pSH*Leu-MRPS8*) and with HSV-tagged *MRPS8* (*Δmrps8*::*MRPS8-HSV*) were streaked on YPGE plates and incubated at either 30 ^{O}C or 37 ^{O}C. **C,** Disruptant of *MRPL1* (*Δmrpl1*) and its plasmid carrier (*Δmrpl1*/pSH*Leu-MRPL1*) were streaked on YPGE plates and incubated at either 23 ^{O}C or 30 ^{O}C. Strains harboring *Δmrps8* and *Δmrps16* deletions are α-type haploid derivatives of BY4743. Strains with *Δmrps35*, *Δmrpl1* and *Δmrpl51* deletions are derived from BY4741, and those with *Δmrpl50* and the HSV-tagged *MRPS8* from RAY3A-α. RAY3A-α and a haploid derivative with wild type mrps isolated from BY4743 (WT) were included as controls.

Table 1. Conservation of newly identified mrps in other organisms. Protein sequence similarity was searched by BLAST against the non-redundant database of NCBI. **n** indicates that no protein with significant similarity was identified. a), percent identity in the aligned region. b), probably belongs to the same protein family but the similarity was below the level of BLAST detection. c), BLAST search failed to find the corresponding *E. coli* ribosomal protein but found those of other bacteria. * L19 ribosomal protein from *Borrelia burgdorferi*. ** L22 ribosomal protein from *Thermotoga maritima*.

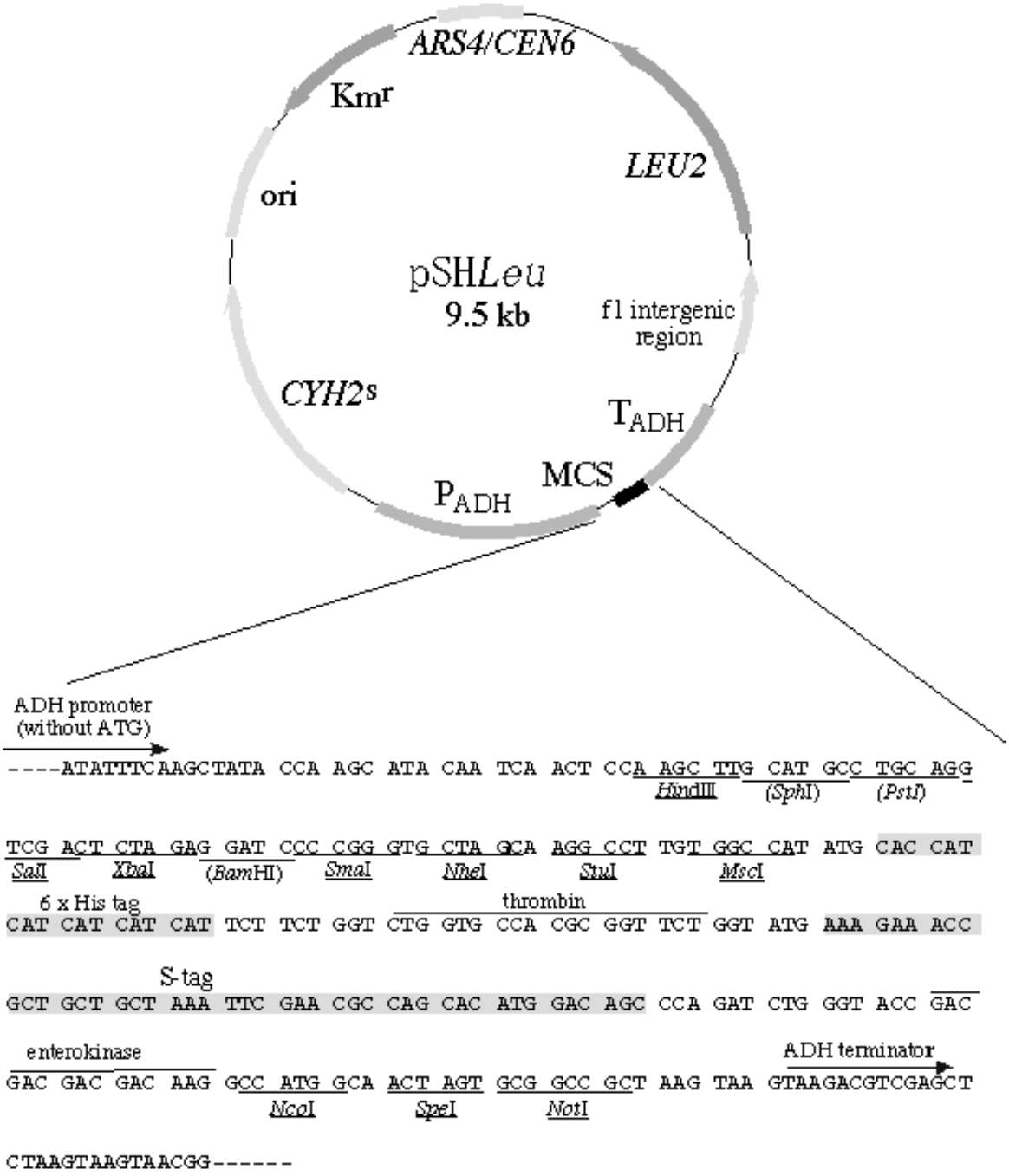
	E. coli			*S. pombe*			*D. melanogaster*			*C. elegans*			*Homo sapiens*		
	Identity (%) [a]	score	accession number	Identity (%) [a]	score	accession number	Identity (%) [a]	score	accession number	Identity (%) [a]	score	accession number	Identity (%) [a]	score	accession number
Ymr158w	22	42	AAC76331.1	40	294	CAA19274.1			**n**			**n**			**n**
Ypl013c	39	59	AAC75658.1	51	227	CAA17806.1	36	128	AAF58284.1	35	97	AAA81099.2	39	61	NP_057149.1
Ymr188c	35	34	AAC76336.1	36	108	CAB52616.1	-	-	AAF47177 [b]	-	-	AAB53829 [b]	35	37	NP_057053.1
Ygr165w			**n**	24	147	CAA18433.1			**n**			**n**			**n**
Ydr116c	24	62	AAC76958.1	29	221	CAB90309.1	27	32	AAF54214.1	24	32	T32555	24	37	AAH14356.1
Img1	(29) [c]	(37) [c]	NP_212833.1*	28	124	CAB52265.1	-	-	NP_524284 [b]			**n**	-	-	NP_055578 [b]
Ynl177c	(28) [c]	(40) [c]	NP_229295**	28	164	CAA20776.1	-	-	NP_523379 [b]	-	-	CAA21022.1 [b]	25	33	NP_054899.1
Ypr100w			**n**	47	317	CAB52039.1	33	149	AAF47047.1	28	133	CAB02765.1	25	42	NP_115488.1
Img2			**n**	35	130	CAA93160.1	34	36	AAF48212.1	-	-	CAA99906 [b]	36	46	NP_004918.1
Ynr022c			**n**			**n**			**n**			**n**			**n**

Table 2. Summary of yeast mrp genes. Genes newly identified or confirmed in this work are indicated in bold and those identified in this work by mass spectrometric analysis of the S-tag-complex are underlined. Orfs in italics are those predicted to be yeast mrps from sequence similarity to bacterial ribosomal proteins. Homologous ribosomal proteins of *Escherichia coli* (*E. c.*) and human mrps are listed. a). Protein names are taken from refs 16 and 17. b). "ypg-", "slow", "n" and "?" indicate, respectively, that the disruptant was unable to grow, grew slowly, showed no obvious growth defect on glycerol medium, or not examined. SGD indicates that the data were taken from the "Saccharomyces Genome Database". c). References not shown in Table II of ref. 13 or Table 1 of ref. 24 are listed.

ORF	Gene		**E. c.**	Human[a]	M(kD)	pI	Disruptant[b]	Reference[c]
(small subunit)								
			S1					
YHL004w	MRP4		S2	MRP-S2	44.2	8.86	ypg-	
			S3					
YNL137c	*NAM9*		S4		56.4	9.81	ypg-	
YBR251w	*MRPS5*		S5	MRP-S5	34.9	9.72	ypg-	
YKL003c	*MRP17*		S6	MRP-S6	15.0	9.99	ypg-	
YJR113c	*RSM7*		S7	MRP-S7	27.8	9.90	?	
YMR158W		*MRPS8*	S8		17.5		ypg-	This work
YBR146w	*MRPS9*		S9	MRP-S9	32.0	10.39	ypg-	
YDR041w	*RSM10*		S10	MRP-S10	23.4	9.41	lethal	
YNL306w	*MRPS18*		S11	MRP-S11	24.6	10.05	lethal	
YNR036c			S12	MRP-S12	16.9	11.23	ypg-	[38]
YNL081c			S13		16.1	10.59	slow	
YPR166c	*MRP2*		S14	MRP-S14	13.6	11.13	ypg-	
YDR337w	*MRPS28*		S15	MRP-S15	33.1	10.08	ypg-	
YPL013c		*MRPS16*	S16	MRP-S16	13.7	10.55	ypg-	This work
YMR188c	***MRPS17***		S17	MRP-S17	27.6	9.76	ypg-	This work, [28]
YER050c	*RSM18*		S18	MRP-S18(1-3)	23.5	10.63	ypg-	
YNR037c	*RSM19*		S19		10.3	10.68	ypg-	
			S20					
YBL090w	*MRP21*		S21	MRP-S21	20.4	10.68	ypg-	
YKL155c	*RSM22*				72.2	9.57	ypg-(SGD)	
YGL129c	*RSM23*			MRP-S29 (DAP3)	55.6	9.91	ypg-	
Q0140	*VAR1*				47.2	10.01	ypg-	
YHR075c	*PPE1(MRPS2)*				44.9	6.83	n	
YGR165w	*MRPS35*				39.6	10.01	ypg-	This work, [28]
YPL118w	*MRP51*				39.5	10.11	ypg-	
YGR084c	*MRP13*				39.0	9.58	n	
YDR175c	*RSM24*			MRP-S28	37.4	9.35	ypg-	
YDR347w	*MRP1*				36.7	9.23	ypg-	
YOR158w	*PET123*				36.0	9.97	ypg-	
YIL093c	*RSM25*			MRP-S23	30.5	6.01	ypg-(SGD)	

YJR101w	*RSM26*			30.2	8.99	ypg-	
YFR049w	*YMR31*		MRP-S36	13.7	9.69	n	[39]
YGR215w	*RSM27*			12.4	10.38	?	
YDL045w-A	*MRP10*			10.7	10.05	ypg-	
(large subunit)							
YDR116c	***MRPL1***	L1	MRP-L1	31.0	10.13	slow	This work
YEL050c	*RML2*	L2	MRP-L2	43.8	10.89	ypg-	[40]
YGR220c	*MRPL9*	L3	MRP-L3	29.8	10.33	ypg-	
YML025c	*YmL6*	L4	MRP-L4	32.0	9.75	lethal	
YDR237w	*MRPL7*	L5		33.1	9.84	?	
YHR147c	*MRPL6*	L6		23.9	10.06	ypg-	
YGL068w		L7/12	MRP-L7	20.7	9.38	lethal	[14]
		L9	MRP-L9				
YDL202w	*MRPL11*	L10	MRP-L10	28.5	9.86	ypg-	[41]
YNL185c	*MRPL19*	L11	MRP-L11	16.7	10.05	?	
YOR150w	*MRPL23*	L13	MRP-L13	18.5	10.27	ypg-	[42]
YKL170w	*MRPL38*	L14	MRP-L14	14.9	10.02	?	
YNL284c	*MRPL10*	L15	MRP-L15	36.4	10.52	?	
YBL038w	*MRPL16*	L16	MRP-L16	26.5	10.47	ypg-	
YJL063c	*MRPL8*	L17	MRP-L17	27.0	9.96	ypg-	
		L18	MRP-L18				
YCR046c	*IMG1*	L19	MRP-L19	19.4	10.51	ypg-	This work, [29]
		L20	MRP-L20				
YJL096w	*MRPL49*	L21	MRP-L21	25.4	10.74	ypg-	[43]
YNL177c	***MRPL22***	L22	MRP-L22	35.0	10.13	ypg-	This work, [28, 32]
YDR405w	*MRP20*	L23	MRP-L23	30.6	9.58	ypg-	
		L24	MRP-L24				
		L25					
YNL005c	*MRP7*	L27	MRP-L27	43.3	9.96	ypg-	
YMR193w	*MRPL24*	L28	MRP-L28	30.1	10.29	ypg-	[28]
		L29					
YMR286w	*MRPL33*	L30	MRP-L30	9.5	10.36	ypg-	
YBR122c	MRPL36	L31		22.2	9.73	?	[10]
YCR003w	*MRPL32*	L32	MRP-L32	21.5	10.01	?	
YML009c	*MRPL39*	L33	MRP-L33	8.0	10.91	?	
YDR115w		L34	MRP-L34	12.1	12.53	?	
		L35	MRP-L35				
YPL183W-A		L36	MRP-L36	10.7	11.33	?	
YMR024w	MRPL3		MRP-L44	44.0	9.58	?	
YDR322w	*MRPL35*		MRP-L38	42.8	9.64	?	
YLR439w	*MRPL4*		MRP-L47	37.0	7.51	ypg-	
YPL173w	MRPL40			33.8	9.62	?	
YNL252c	*MRPL17*		MRP-L46	32.2	9.19	ypg-	[43]

YKR006c	*MRPL13*		31.5	9.47	slow	
YLR312w-A	MRPL15		28.2	9.48	?	
YKR085c	*MRPL20*		22.4	10.31	ypg-	
YGR076c	*YMR26(MRPL25)*		18.6	10.19	ypg-	
YDR462w	*MRPL28*		17.4	10.64	?	
YBR282w	*MRPL27*	MRP-L41	16.5	10.27	ypg-	
YCR071C	*IMG2*	MRP-L49	16.4	10.02	ypg-	This work, [30]
YNR022C	**MRPL50**		16.3	8.90	n	This work
YPR100w	***MRPL51***	MRP-L43	16.1	10.62	ypg-	This work
YKL167c	*MRP49*		16.0	9.53	cs	
YKL138c	MRPL31		15.5	10.84	ypg-	[14]
YBR268w	*MRPL37*	MRP-L54	12.0	10.00	ypg-	[28]
YMR225c	*YMR44(MRPL44)*		11.5	9.72	?	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SalI XbaI (BamHI) SmaI NheI StuI MscI
6 x His tag
thrombin
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S-tag
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enterokinase
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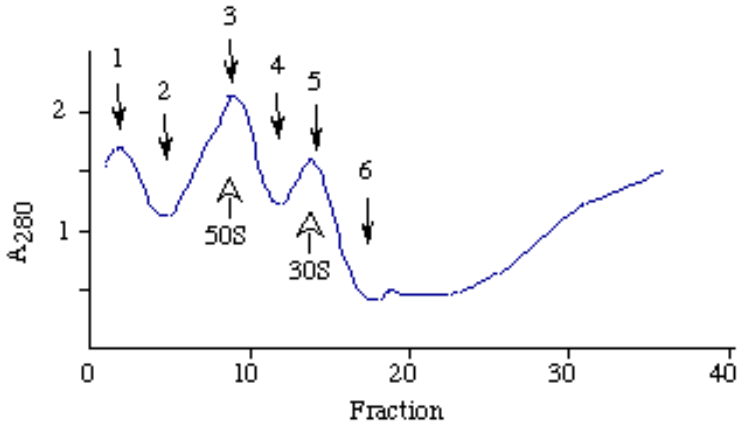
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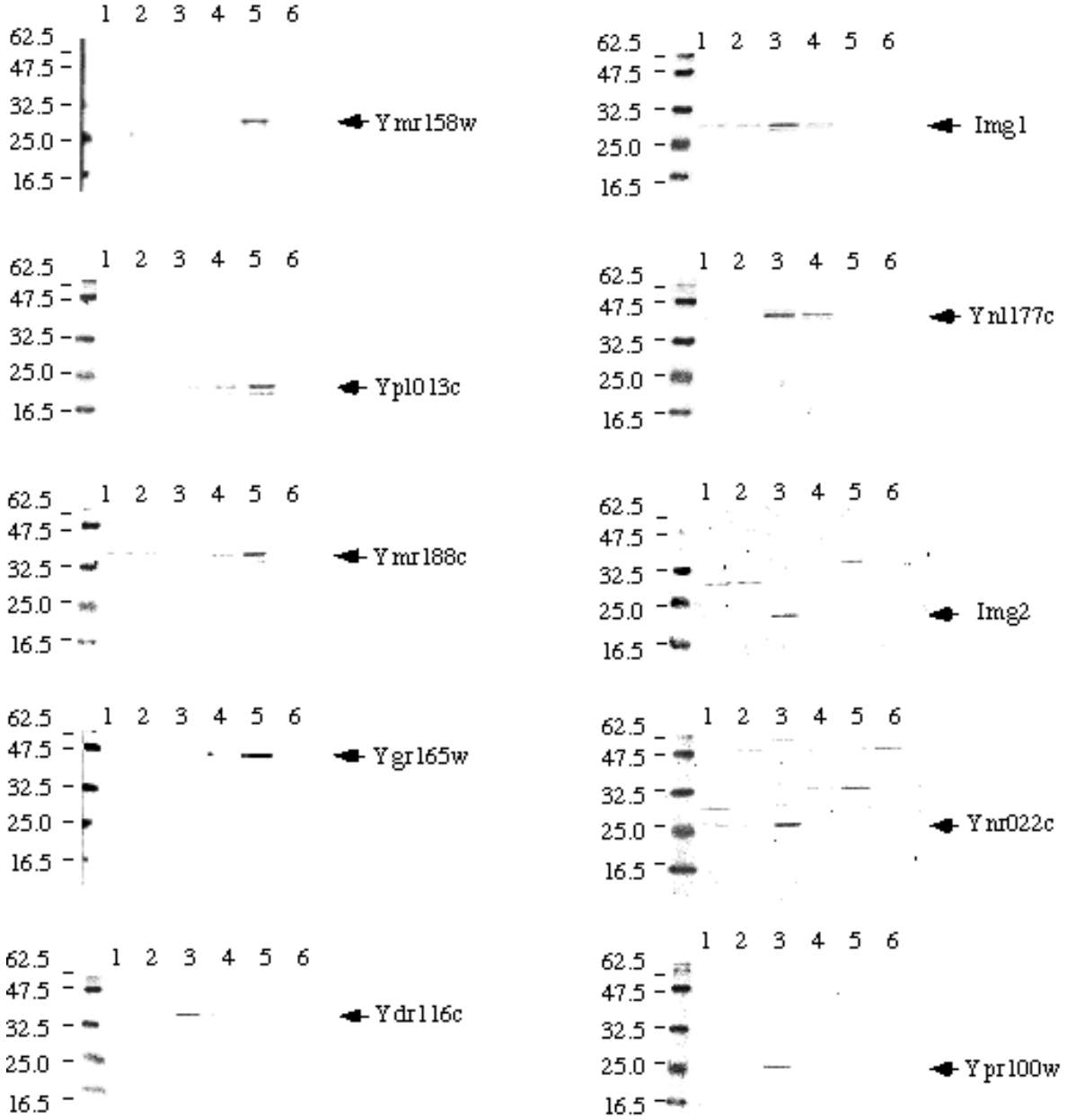
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S8 (Y mr158w)

```
S. cerevisiae   -MSLVKLANTGAHLGNCSKVRVALTSLPYTKLQLQFAYNLYQCGFLSSLGKGSTMGPDKD
S. pombe        ----MQLHYVFSHLGNSFRARKSLASVPNCNSVLELCAVLYHCGFLSSLCRGDIHGPDAL
Synechococcus   MAVNDPLADMLTRLRNASEARHATTVVPASRLARSLAEVLKRGFLADFE----------
B. subtilis     MVMTDPLADMLTRLRNANMVRHEKLELPASKLKRELAELLKRGFLRDME----------
E. coli         MSMQDPLADMLTRLRNGQAANKAAVTVPSSKLKVALANVLKHGFLEDFK----------
                      :    ::::*   ..      :*  .    :.  * .:**: ..:

S. cerevisiae   FVEVTPLNISTRRLWVGLKY--RDNKPVLSSCKLISKPNSRTHLPMEDVKKLCSGVTLRN
S. pombe        PTITTRLNVATRRLWVGLKY--FEGKPVLHYIRAVSKPSRKVNLTPSELLCFAKGRKVSF
Synechococcus   ---ESGLGVQR-HLVLSLKYKGKNCCPLLKALKRVSKPGLRVYSNRRDLPRVLGGIGLAI
B. subtilis     ---FVELSKQG-ILRVELKY-GCNNERMTGLKRISKPGLRVYAKSNEVPRVLNGLGLAI
E. coli         ---VEGLTKP--ELHLLKY--FQGKPVVESICRVSRPGLRLYKRKDFLPKVMAGLGLAV
                        :     : : ***   : : ::   : :*:*. ::     :: :.   *  :

S. cerevisiae   IKPLGPGELILVRAHNNIWDINPAISKKLGHVLCRVK
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Synechococcus   ISTSGG-----------LMTDRDARRQGVCGHVLCYVW
B. subtilis     ISTSGG-----------MLTDKPARAKQACGHVLAYVW
E. coli         VSTSGG-----------MMTDRAARQAGLCGHILCYVA
                :.  :            ::    *     .*::::. *
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L19 (Img1)

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S. cerevisiae   ---------MWSRNVRLLGSWTRSYMVPATKRKTIP----VYPPVQRIA-SSGIMKQVAL
S. pombe        -----MFNAKHFFNLGLGFQWLQKRGIGSLKRPYN-----FPKPVPGPK-HKLVLSLFEK
H. sapiens      MGLGRSFQAARTLLPPPASIACRVHAGPVRQQSTGPSEPGAFQPPPKPV-IVLKHRPVEP
D. melanogaster MNLSTRVMLNRLTQQCVYKRIVTFSTKTAEHVIENQEEQKKEAPPTTPTSPVNRKTIIPA
R. americana    ------------------------------------------------MLNLIQTLRM
B. burgdorferi  -------------------------------------------------MLLIRKIEA
E. coli         ------------------------------------------------MSNIIKQLEQ
                                                                     :    .

S. cerevisiae   SEIESLDPGAV--K-----RKLISKKNKDRLK-----------AGDVVRIVYDSS---KC
S. pombe        KCRSVLDEQSE--R-----FKMFHRSQPNRVR-----------PGAVLIVESYSKYPSKD
H. sapiens      ERRFLSPEFIP--RRGRTDPLKFQIERKDMLERRKVLHIPEFYVGSILRVTTADPY-ASG
D. melanogaster NYRFVYPEFLPDPKVEWRNLVREKLERLDMLDRRKQIDLPEFYVGSVLAVTSSDPH-AAG
R. americana    EATQ---KSR---------HKKTQIQ-----------------SGDILSLTTTQYK-NKK
B. burgdorferi  CNK----KNE---------AFVFN-------------------VGDTVRVVYKIIEGSNE
E. coli         EQM----KQD---------VPSFR-------------------PGDTVEVKVWVVEGSKK
                .                                            *  : :

S. cerevisiae   SYDTFVGYILSIDRKQLVQDASLILRNQIAKTAVEIRVPLFSPLIERLDLLTPHVSS--R
S. pombe        SVNRFAGYLLRIRHR--GPKSSLILRNVVMGVGVEYLLPIYSPCLKRLVVLKENGLSKRP
H. sapiens      KISCFLGICLQRSGR--GLGATFILRNVIEGCGVEICFELYNPRVQELCMVKLEKR----
D. melanogaster KTSRFVGICLNRDRC--GLRARFILRNVIDHCGMEVVYELYDPTILKLEVLRLEKR----
R. americana    RKQNLKGICLGIKKR--IGYTTLCLRNFIGGVSQEQSFILESPIINNLEIIGKIKG---N
B. burgdorferi  RLQSEGIVLSFQNK--GIGKTFIIRKISSGIGVEKIFPVYSPIIEKVEVLRR-GK---V
E. coli         RLQAEGVVLAIRNR--GLHSAFIVRKISNGEGVERVFQTHSPVVDSISVKRR-GA---V
                  . : *  :              : :*:     . *      .* :  : :

S. cerevisiae   QRNKHYYIRGTRLDVGDLEAGLRRKK-----------------------------------
S. pombe        RRAYLSYIRQPRFRLPPVESLVRKYIEQ-NQHKP--------------------------
H. sapiens      LDDSLLYIRDALPEYSTFDVNVKPVVQEPNQKVPVNELKVKMKPKPWSKRWERPNFNIKG
D. melanogaster LDDSLFYIRDALPEYSTFDENVEAEPLEEGAPVPVNDIKVVLRPRPWLERWERQNLRGVA
R. americana    TKAKKYYIRTKSPSENKV-----------------------------------------
B. burgdorferi  RRAKLYYVRN---RIGKAAMKIKERLTI-KKVKH---------------------------
E. coli         RKAKLYYIRE---RTGKAAR-IKERLN---------------------------------
                      *:*

S. cerevisiae   ------------------------------------------------------------
S. pombe        ------------------------------------------------------------
H. sapiens      IRFDLCLTEQQMKEAQKWNQPWLEFDMMREYDTSK-------IEAAIWKEIEASKRS---
D. melanogaster N-IDEYLKDKHRLSAAKVQKPWEKYDMMKDYRSSIPEEEQTEIFAEVHTELHALELQRKR
R. americana    ------------------------------------------------------------
B. burgdorferi  ------------------------------------------------------------
E. coli         ------------------------------------------------------------

S. cerevisiae   --------------
S. pombe        --------------
H. sapiens      --------------
D. melanogaster NKRKRTFIKPKQLA
R. americana    --------------
B. burgdorferi  --------------
E. coli         --------------
```

L 22 (Y nl177c)

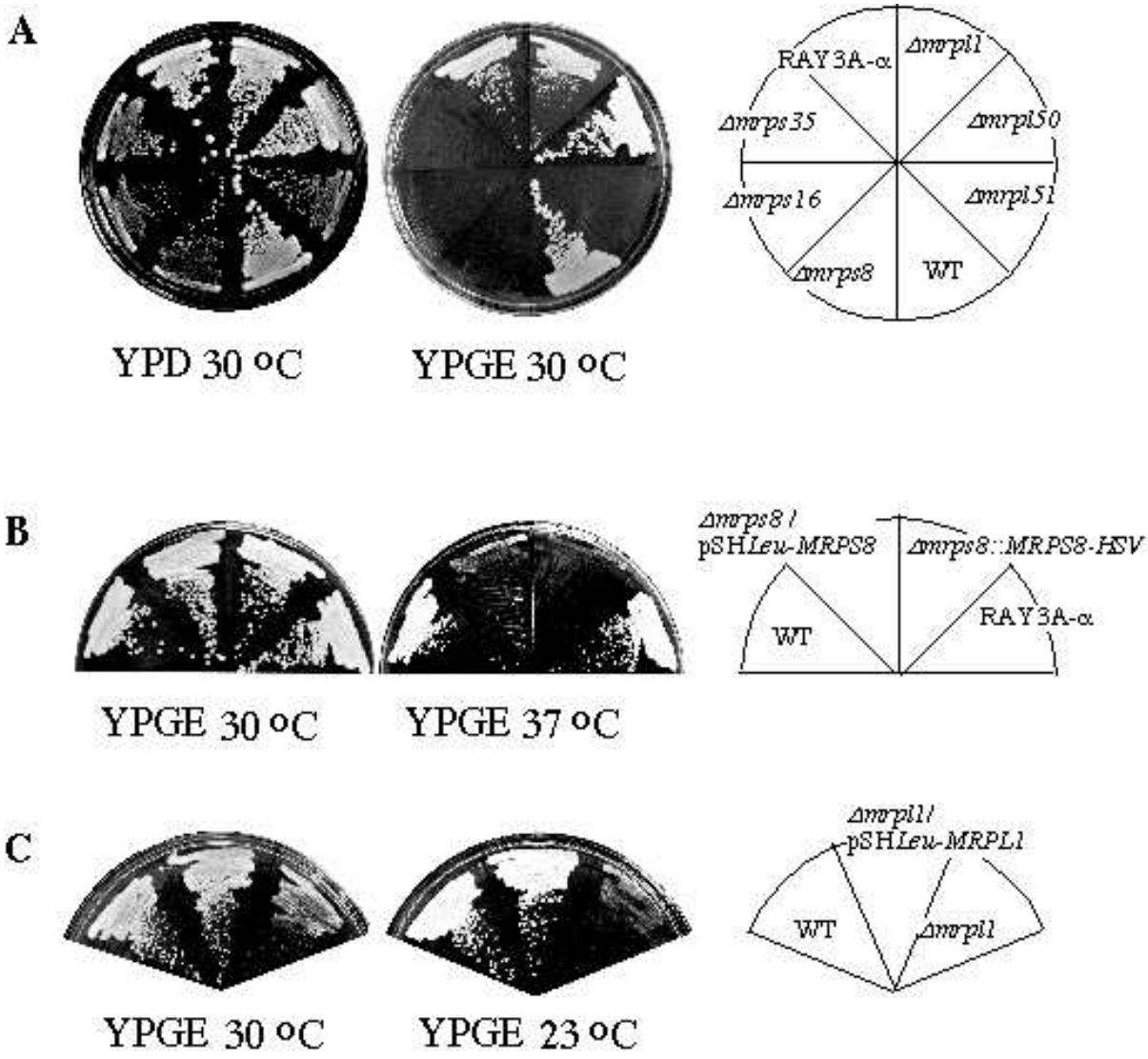
```
S. cerevisiae   MNFHTARISQVGVISRALLSSVSRRWIHVTPISLNNSGGSLFGSITENKPKEGKNRGDED
S. pombe        ----MKYINQFMKISKGFLVPSSTIGLNSKTYHYKFPLLSFVREFSNGSYLRESAQNPAN
H. sapiens      -------------MAAAVLGQLGALWIHNL-----------------------RSRGKLA
T. maritima     ------------------------------------------------------------
E. coli         ------------------------------------------------------------

S. cerevisiae   AGSFSNRLAIASDSSGEAPEVNRDSITIENDKLLQQHIISLQQPEQLASQSLLSPLKREI
S. pombe        LGSQKGSDIFSMLAN----Q------------------KDDSNRQQKEERVKERPRSRIS
H. sapiens      LGVLPQSYIHTS--------------------------ASLDISRKWEKKNKIVYPPQLP
T. maritima     ------------------------------------MKLQVPRNGLKRSLFHKKRKEL
E. coli         ------------------------------------------------------------

S. cerevisiae   YEANCKINGGFYKKDTIVKLPNSSERYKLKFTKREIEVLEPSVYAQSYRIKSSMKSATLL
S. pombe        FKK----------------QE----------------TMDPSYTLQSMVLRSSLKSVGAL
H. sapiens      GEP----------------RR------------------PAEIYHCRRQIKYSKDSMWYL
T. maritima     LSS----------------LP------------------KIEARAVAKYIRISPRSARAL
E. coli         ----------------------------------------METIAKHRHARSSAQSVRLV
                                                          .        : *  *   :

S. cerevisiae   LRLLGGLDWMKALSQCHFSNKKIAREVAELLDKGVK-DGCKLGLKP-EDLMSCIWTGSD
S. pombe        CRQLAHKPFYHALLQMKMSDKKISKYLATALVSARENAVREAGLDE-STLMVDCIWVG-K
H. sapiens      AKLLRGMSLDCALAQLEFNDKKGAKILKEVLLEAQDMAVRDHNVEFRSNLMLAESTSG-R
T. maritima     ANTLRGKSVEEAFQILAFSPKKAARIMEKVLKSAVANAENNFGLSV-ENLMVSECYVN-D
E. colii        ADLLRGKKVSCALDILTYTNKKAAVLMKKVLESAIANAEHNDGADI-DDLKMTKIFVD-E
                   :    . .*:     . ** :  :   * ..      .. .  .  . * : :   .

S. cerevisiae   GFWRKRVEFKARTRIGIISHPYIHVRCILRT------KGVTKRRLAYEAHLKEQKRAPWV
S. pombee       AKYLKKLITMGRGGRAIERSPRVRVTVVLRD-----ERALLRDLQRRQQRLERKK--VWT
H. sapiens      GQCLKRLRYHGRGRFGIMEKVYCHYFVKLVEGPPPPPEPPKTAVAHAKEYIQQFAAGPSF
T. maritima     GPRMKRLWPRGRGRADIIQKRMSHITVVVRD------RSREDEYRKALEELQKKISSEE-
E. coli         GPSMKRLMPRAKGRADRILKRTSHITVVVSD------R----------------------
                .   *::   .:           :    :        .

S. cerevisiae   QLGDKPIRGVTGGVYKW--------
S. pombe        PLPNRPIYLKSNFFTC---------
H. sapiens      TLYDEEIQTPQCIYFAIYFLKINKN
T. maritima     -------------------------
E. coli         -------------------------
```

I mg2

```
S. cerevisiae   ------------------------------------------------------------M
S. pombe        ------------------------------------------------------------
D. melanogaste  ----------------------------MATSAKLMLGSGLCQKLSQLTRQIHMQPALM
C. elegans      MIKIANSNKRAKKTEIDSLKADIDLYLDEHASDDDKMLGSSLRIFSPRAVSRLSFSTTSV
H. sapiens      ----------------------------------MAAT---MF--RATLRG--WRTGV

S. cerevisiae   ISSCVTRCFGRGKCLPGP-ATASIYQTIRCISTNSNKAAEAPIRPKLELVKMH-----EL
S. pombe        ----------------------------------------MRSSLKPVLSNLRFN-------
D. melanogaster SSFRSSREVQELDKYPEV-EVVANPPE----WRFVERLLPAKTVPQPVEKPKY--PSGWQ
C. elegans      EKAEKLWENPWKHALPEKSRSLATVEEAPIDWSYVERLMPIEVVPNVPEHEKYPTPSGWT
H. sapiens      QRGCGLRLLSQTQGPPDYPRFVESVDE----YQFVERLLPATRIPDPPKHEHYPTPSGWQ
                                                                 *   .

S. cerevisiae   IGNNNFGKKTYYVERSRTGNLPMYS---AYKNGGN------KITTEIRKIEGDVIQLRND
S. pombe        S-TIASESLRFHVSRTPSKNLPMYL---DYKQRGT------KILTIIRKIHGDSNALRLR
D. melanogaster PQKEDGSELGMHVARTKNHMVPMYL---HTRFRGQ------RMITVVRRVQGDIWTLEKD
C. elegans      PPTEAAKTHCYMIRRHDHILPLYLERKRDLLNEKTLDFDYVELVTIRNVDGDIFACEND
H. sapiens      PPRDPPPNLPMFVRRSRMHNLPMY----KDITHGN------RCMTVIRKVEGDIWALCKD
                          :.: *     :*:*                     :. :*.:.**    .

S. cerevisiae   LQECLPF---IPKKSWSVVMQSKKTILKGNAVEAVKRVLTKKF-
S. pombe        LISTLKM---S-PKDVYVNKLTNQVLKGNHIVTVREWLQDCGF
D. melanogaster LRAVVEQSRNGKVCATRINELSGQLHHGDHVDVLRDYLKEKGF
C. elegans      LRSYLEE-HLGHSIASHVDELKGRLKLKGAPRVLIEQRHYSKGF
H. sapiens      VEDFLSP-LLGKTPVTCVNEVTGTLRLKGYFDQELKAWLIEKGF
                :   :            :   .  : ::*     :.  : .:
```

M rpL 50 (Y nr022c) / L 9

```
S. cerevisiae                           -MLHCTQVCLSALTKRTHRVKVQVLKDFPRFQLYKG
B. subtilis                             MKVIFLQDVKGKGKKG--EVKNVADGYAHNFLIKKG
                                                :  *   .  .*   .**  .     .* : **

B. subtilis     ------------------------MKVIFLQDVKGKGKKG--EVKNVADGYANFLIKKG
S. aureus       ------------------------MKVIFTQDVKGKGKKG--EVKEVPVGYANNFLIKKN
T. maritima     ------------------------MKVILLRDVPKIGKKG--EIKEVSDGYANYLIRRG
Synechocystis   MEVCELLHKLNKLHIGKITVMAKRMKVLNETINKLGFTG--ILVEVAPGYANYLIRKG
E. coli         ------------------------MKVILLDKVANLGSLG--IQVNVKAGYANFLMRQG
                                        ::*::   :   *  *  :  :*  ***.*:*: :.

S. cerevisiae   QVANMKFSLVRNYLHNPNGAKYILSEPHDINT-PLLKQYQTL-PAKLPPLHQCLSKRHI
B. subtilis     IAVEANASNISALNGCKGKPKKEAIAEIEQAK-SLKETIEKL-TVELSAKSGEGGRLFGS
                 ..:.:.* :     : :  *     * :  .-.* :  :.*- .:*. .  : .: . :

B. subtilis     LAVEANASNISALNGCKQKEKKEAIAEIEQAK-SLKETIEKL-TVELSAKSGEGGRLFGS
S. aureus       YAVEATPGNLKQLPLQKKRAKCERQQEIEDAK-ALKETLSNI-EVEVSAKTGEGGKLFGS
T. maritima     FAKEYTEGLERAIKHEKEIEKRKKERPREESE-KILKELKKR-THVVKVKAGEGGKIFGA
Synechocysti    LGVVATPGILRQVEQRRLKELERLKAEKDAAE-ARKVALETIGRFVLKKCVGEAEAIFGT
E. coli         KAVPATKKNIEFFERR-AELEAKLAEVLAAANARAEKINALETVTLASKAGDEGKLFGS
                 .  .      .: .:    .    *  :        :.      :  : *:   :**:

S. cerevisia    EVCKNWELRKESVFGHKKHHKPKEEKKG-LLDSGITIEEVKI-PGLII----------
B. subtilis     VTSKCITEQLQKDHNIKVDKRKLELPLG-IRALGYTNVPVKLHPEVCAVLKVHVKEEA
                ..*::  : :. .. * :::  *  .* :   * *   **: * ::

B. subtilis     VTSKCITEQLQKDHNIKVDKRKLELPDG-IRALGYTNVPVKLHPEVQAVLKVHVKEEA
S. aureus       VSTKQIAEALKAQHDIKIDKRKMDLPNG-IHSLGYTNVPVKLDKEVEGTIRVHTVEQ-
T. maritima     VIAATVAEEISKTTGLKLDKRWFKLDKP-IKELGEYSLEVSLPGGVKDTIKIRVEREE
Synechocystis   VITQEVALAVEAATNGSLDRRGISLPD--IHKTGFYGAQIKLHPEVIATVEVQVAPL-
E. coli         IGIRDIADAVTAA-GVEVAKSEVRLPNGVLRTTGEHEVSFQVHSEVFAKMIMNVVAE-
                : :  ::: :    . .: :  . * .  ::  *  .  ..:   *   : :..
```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pSHLeu-MRPS8
Δmrps8::MRPS8-HSV
WT
RAY3A-α
YPGE 30 °C
YPGE 37 °C
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pSHLeu-MRPL1
WT
Δmrpl1
YPGE 30 °C
YPGE 23 °C