5S rRNA binding proteins from the hyperthermophilic archaeon, *Pyrococcus furiosus*

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Abstract A determination was made of the nucleotide sequence of the 2719 bp region of a ribosomal protein gene cluster (PfeL32–PfeL19–PfL18–PfS5–PfL30) containing a 5S rRNA binding protein L18 homolog of hyperthermophilic archaea *Pyrococcus furiosus*. The organization of the archaeal ribosomal protein gene cluster is similar to that in the spc-operon of *Escherichia coli* (L6-L18-S5-L30-L15) but has two additional genes, namely those encoding PfeL32 and PfeL19, which were identified as extra proteins that are apparently not present in bacterial *E. coli*. Using an inducible expression system, *P. furiosus* mature PfL18 protein and a mutant PfL18 with the basic N-terminal amino acid region deleted were produced in large amounts in *E. coli* and Northwestern analysis showed the N-terminal region of PfL18, including the conserved arginine-rich region, to have a significant role in 5S rRNA–PfL18 interaction.

Key words: 5S rRNA; RNA-binding; Ribosomal protein; Archaea; *Pyrococcus furiosus*

1. Introduction

A study of the phylogenic tree based on rRNA or protein sequences shows that all known organisms are related, suggesting a common ancestor [1]. All organisms can be divided into three groups: eukarya, bacteria, and archaea. According to structural studies on ribosomal protein (rp), protein elongation factors, protein initiation factors and RNA polymerase, the archaea form a specific group that are apparently different in evolution, and most of the proteins of this group as gene products are much more analogous to those of eukarya [2–10]. Studies have indicated that 5S rRNA plays a key role in the structure and function of a ribosome [11]. The results of comparative structural studies suggest that there are significant differences between the secondary structures of free 5S rRNA from bacteria and eukaryotes [12]. Interestingly, archaeal 5S rRNA shows structural characteristics that are a mixture of eukaryotic, bacterial and unique traits [13]. Although there is far less structural information available, the secondary structure of intact 5S rRNA, when in the 5S rRNA–protein complex, appears to be quite similar in both bacteria and eukaryotes [14]. In *Escherichia coli*, the two proteins EcoL18 and EcoL25 appear to interact strongly with 5S rRNA, while a third protein, EcoL5, interacts weakly with 5S rRNA. In all eukaryotes examined to date, a single rp is found to be associated with 5S rRNA, for example the L1 protein from yeast [15].

In order to obtain a better understanding of the 5S rRNA–protein interaction in ribosomes, we attempted the cloning and sequencing of the 5S rRNA binding L18 protein family, and 5S rRNA–protein recognition analysis was conducted by using the recombinant Pf-L18 protein from the hyperthermophilic archaeon *Pyrococcus furiosus*.

2. Materials and methods

2.1. Bacterial strains, plasmids, and media

P. furiosus JCM 8422 was obtained from the Japan Collection of Microorganisms, RIKEN, Japan, Cells were grown at 98°C in a complex medium based on artificial seawater (Jamarine Laboratory, Osaka, Japan) supplemented with 0.5% yeast extract, 1% bacto-tryptone, and 1% soluble starch per l under strictly anaerobic conditions. Cloning, expression, and transcription of *P. furiosus* DNA was carried out by using the *E. coli* host/vector systems of JM109/pUC18 or pUC19, BL21(DE3)/pET15b (Novagen) and JM109/pGEM-3Z. *E. coli* strains were grown in yeast/tryptone medium at 37°C with 100 μg/ml ampicillin. A 0.25 kb DNA fragment including a $tRNA^{Arg}$ gene and promoter region amplified by PCR using *E. coli* genomic DNA was cloned into the *Hin*dIII/*Bam*HI restricted pACYC184 vector, referred to as pFU9872. Plasmid pSJS1240 bearing wild-type *E. coli* $tRNA^{Arg}$ and its $tRNA^{Ile}$ was a generous gift from Prof. S.H. Kim (University of California, CA, USA).

2.2. Preparation of P. furiosus genomic DNA and cell-free extract

Cells of *P. furiosus* (1.5 g) were resuspended in 20 ml TNE (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1 mM EDTA, 5% sucrose). 1.5 ml lysozyme (5 mg/ml), 2.5 ml 5% SDS and 0.5 ml proteinase K (10 mg/ml) was added, and the mixture was incubated for 1 h at 37°C. Proteins were extracted two times with phenol/chloroform/isoamyl alcohol. Nucleic acids were precipitated with ethanol and dissolved in TE (10 mM Tris–HCl, pH 7.5, 1 mM EDTA). The cell-free extract of *P. furiosus* cells was prepared by sonication in a 10 vol 50 mM Tris–HCl buffer, pH 7.4, containing 10 mM $MgCl_2$, 30 mM NH_4Cl, 6 mM ME, 0.1% Triton X-100. After centrifugation ($10\times000\times g$ for 15 min at 4°C) to remove the cell debris, the supernatant was subjected to SDS–polyacrylamide gel electrophoresis (PAGE).

2.3. Cloning and nucleotide sequence of five genes encoding rps

On the basis of the nucleotide sequences of the genes for the N-terminal part of rp PfL15 (GenBank AA164180) and the C-terminal part of rp PfL18 (GenBank AA164175), two oligonucleotide primers (P1: 5′-ATCCGTGAGTGTGACTGCCTCT-3′ and P2: 5′-TTTAA-AGGTGAGGGGGCGAG-3′) were designed and PCR amplifications

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Abbreviations: rp, ribosomal protein; PAGE, polyacrylamide gel electrophoresis

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were done to complete the nucleotide sequence of the entire gene encoding rps PfS5 and PfL30. On the basis of the nucleotide sequences of the genes for the C-terminal part of the conserved sequences of rp L6 from related archaeal species *Pyrococcus horikoshii* [16] and the N-terminal part of PfS5 determined in this work, two oligonucleotide primers (P3: 5′-GGTCAAACCGCTGCAAATATAGAGCAGGC-3′ and P4: 5′-CAAAGTGCTCGGGAAGCTTTTCAGGGTCTAACC-CT-3′) were designed and PCR amplifications were done to complete the nucleotide sequence of the entire gene encoding rps PfeL32, PfeL19 and PfL18. The DNA sequence was deposited to DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB040118.

2.4. Expression and purification of recombinant proteins

Recombinant *P. furiosus* wild-type rp PfL18 (PfL18-WT) was obtained by PCR amplification of the *P. furiosus* L18 gene and the subsequent cloning of this gene into the T7-polymerase-driven expression vector pET-15b (Novagen). For the PCR amplification of the L18 gene, two primers were designed. The upstream P5 primer (P5: 5′-CTTAGGCATATGGCTCACGGTCCTAGGTATAGAG-3′) contained an *Nde*I restriction site at the N-terminal methionine of PfL18 with a sequence ranging from 993 to 1017 on the coding strand and the downstream P6 primer (P6: 5′-TTGGGATCCTCATTCT-CTCGCCCCCTCACCTTC-3′) contained a *Bam*HI restriction site, immediately following the PfL18 stop codon with a sequence ranging from 1581 to 1601 on the non-coding strand. The resultant PCR product was digested with the enzymes *Nde*I and *Bam*HI and cloned in expression vector pET-15b digested with *Nde*I/*Bam*HI, resulting in plasmid pET-L18. For expression of recombinant rp PfL18, plasmid pET-L18 was transformed into *E. coli* BL21 (DE3) or *E. coli* BL21 (DE3) pLys, and was induced by addition of 1 mM IPTG for 3 h. Transfer of proteins from slab gels into a PVDF-membrane was performed by horizontal semi-dry blotting as described previously [10].

2.5. Construction of deletion of Arg-codon-changed mutants

A mutant deletion protein (PfL18-ΔB15), lacking the 15 N-terminal amino acids, was produced by expression of the PCR products, encoding amino acid positions 16–203 of PfL18-WT, and amplified from 1041 to 1601 on the coding strand using two oligonucleotide primers (P6 and P7;5′-CTTAAGGCATATGGGAAAGACAAACTATCG-TAAGCGTCTCAAG-3′). Codon-replacements were introduced into the PfL18 gene encoding arginine residues (AGA or AGG) at positions 12, 13, 14, 15, 22, and 24 of PfL18-WT by two-step PCR (PfL18-Arg/CGT). For the first step PCR, a 68 bp DNA fragment, encoding amino acid positions 1–23 of PfL18, was amplified with the oligonucleotides primers, P5 and P8 (5′-TTACGATAGTTTGT-CTTTCCTTCACGACGACGACGGAATG-3′), and a 564 bp DNA fragment, encoding amino acid positions 17–203, was amplified with P9 (5′-GGAAAGACAAACTATCGTAAGCGTCTCAAGCTCCTC-3′) and P6. For the second step PCR, a 612 bp DNA fragment modified in Arg-codons was amplified with P5 and P6 using the 68 bp and 564 bp DNA of the first step PCR products as templates. The purified PCR products were digested with both *Nde*I and *Bam*HI, then inserted between the *Nde*I and *Bam*HI sites in pET-15b, resulting in plasmids pET-L18 (Δ15) and pET-L18 (Arg/CGT).

2.6. In vitro synthesis of ^{32}P-labeled 5S rRNA of P. furiosus

A 120 bp DNA fragment, encoding the mature *P. furiosus* 5S rRNA studied in this work, was amplified with the oligonucleotides 5′-CCGGAATTCGGTACGGCGGCCATAGCGGG-3′ and 5′-CC-CAAGCTTTGGCCGGCGGCGTCCCCGGC-3′. These two primers were designed to create *Eco*RI and *Hin*dIII sites at the 5′ and 3′ ends of the resulting PCR product. The purified product was digested with both *Eco*RI and *Hin*dIII, then inserted between the *Eco*RI and *Hin*dIII in pGEM-3Z. 120 nucleotide 5S rRNA was produced in the presence of ^{32}P-UTP (Amersham; specific activity: 800 mCi/mol) using a in vitro runoff transcription kit supplied by Ambicon Co., Ltd. with the linearized pGEM-3Z.

2.7. Northwestern blot

The crude extract of *E. coli* BL21(DE3) cells bearing the expression plasmids was separated by 15% SDS–PAGE. Following electrophoresis, proteins were transferred to a PVDF-membrane (Bio-Rad) and Northwestern analysis were performed with ^{32}P-labeled 5S rRNA as described previously [10].

3. Results and discussion

3.1. Cloning of the genes encoding rp from P. furiosus

Sequencing of a 1.4 kb fragment amplified by PCR using primers determined from the partial sequence information of PfL18 and PfL15 genes (AA164189, AA164180) and a 1.8 kb fragment by PCR using the primer deduced from the conserved sequence of rp L6 allowed mapping of the positions of five complete neighboring ORFs of hyperthermophilic archaeon *P. furiosus* genomic DNA (PfeL32–PfeL19–PfL18–PfS5–PfL30) (Fig. 1). The PfeL32 and PfeL19 proteins deduced from the DNA sequence showed 39 and 34% sequence homology with the yeast rps eL32 and eL19, respectively, and no homology with bacterial rps. The proteins predicted from the PfL18, PfS5 and PfL30 genes show 34–40% sequence homology with the yeast rps L18, S5, and L30. Additionally, these three proteins were similar to eubacterial *E. coli* EL18, ES5 and EL30, although they showed lower similarity (10–25%) compared to those of eukaryotic rps. The organization of the archaeal rp gene cluster is similar to that in the spc-operon of *E. coli* (L6–L18–S5–L30–L15) but has two additional genes between the L6 and L18 genes, namely those encoding PfeL32 and PfeL19, which were identified as extra proteins that are apparently not present in *E. coli*.

3.2. Expression of the rp L18 gene

In order to prove the validity of the nucleotide sequence determined and obtain a better understanding of the 5S rRNA–protein interaction in ribosomes, the PfL18 gene (positions 993–1604), homologous to the EL18 gene encoding one of the 5S rRNA binding proteins in *E. coli* (Fig. 2), was expressed in *E. coli* under the control of a phage T7 promoter. Induction of the *E. coli* strains BL21 (DE3), transformed with the vector pET15b harboring the *P. furiosus* L18 coding region (pET-L18), resulted in the production of a protein with a molecular mass of 28 kDa as seen from *E. coli* crude extracts analyzed on SDS–PAGE stained with Coomassie blue (Fig. 3A, lane 1). The identity of the protein was confirmed by immunological cross-reactivity with a single antibody against a hexahistidine tag (Qiagen) (Fig. 3B). However, the level of expression of the PfL18 gene was significantly lower compared to that of the *E. coli* L18 gene (Fig. 6A, lanes 2 and 3). The steady state levels of charged tRNAs in *E. coli* are not equal

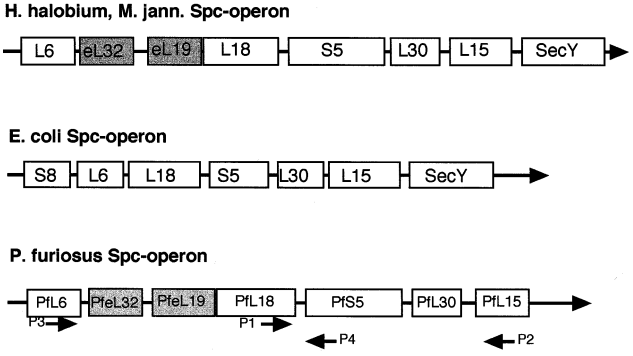

Fig. 1. Gene map of the region surrounding the PfL18 rp gene of *P. furiosus* and comparison of the gene arrangement of the rp gene cluster with those of *E. coli* [17] and archaea *Halobacterium halobium* (unpublished data) and *Methanococcus jannaschii* [18]. eL32, eL19, L18, S5 and L30 are the genes for the rp. Arrows indicate the position of the PCR primer.

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                    . ..        .  .       .. .
E.coli    1        MDKKSARIRRAT----RARRKLQELG-ATRLVVHRTPR 33
B.sub     1     MITKTSKNAARLKRHA----RVRAKLSGTAERPRLNVFRSYK 38
P.furi    1    MAHGPRYRVPFRRRREGKTNYRKRLKLLKSG-KPRLVVRKSLN 42
M.jann    1    MATGPTYRVKFRRRREAKTDYRKRLKLLLSR-KPRLVARRTLN 42
Yeast     8 KSSAYSSRFQTPFRRRREGKTDYYQRKRLV....KYRLVVRFTNK 58
Human     7 KNKAYFKRYQVKFRRRREGKTDYYARKRLV....KYRMIVRVTNR 57

              ..      .     .   ..          .   .   .
E.coli   34 HIYAQVI--APNGSEVLVAASTVEKAIAEQLKY-TGNKDAAAAVG 75
B.sub    39 HIYAQII--DDVNGVTLASAST--LDKDLNVES-TGDTSAATKVG 78
P.furi   43 HHIAQIIVYDPKGDRTLVSAHTRELIRDFGWKGHCGNTPSAYLLG 87
M.jann   43 HCIAQIVLYDEKGDKTVVSAHSRELIK-LGYKGHTGNLPSAYLTG 86
Yeast    59 DIICQIISSTITGDVVLAAAYSHELPR-YGITHGLTNWAAAYATG102
Human    58 DIICQIAYARIEGDMIVCARYAHELPK-YGVKVGLTNYAAAYCTG101

             .   .          . .     .   .     .   .
E.coli   76 KAVAERALEKGIKDVSFDRSGFQY-HGR-VQALADAAREAGLQF 117
B.sub    79 ELVAKRAAEKGISDVVFDRGGYLY-HGR-VKALADAAREAGLKF 120
P.furi   89 LLIGYKAKQAGIEEAILDIGLHPPVRGSSVFAVLKGAVDAGLNV 131
M.jann   87 YLLGKKALAKGYTEAVLDIGLHRATKGNAIFAILKGALDAGMEI 130
Yeast   103 LLIARRTLQ.......LDIGLQRTTTGARVFGALKGASDGGLYV 173
Human   102 LLLARRLLN.......LDAGLARTTTGNKVFGALKGAVDGGLSI 172


E.coli      ---------------------------------------------
B.sub       ---------------------------------------------
P.furi  132 PHSPEIFPDEYRIRGEHIAEYAKMLKEQDEEKFRRQFGGYLVKG 175
M.jann  131 PHGEEILPSEERIRGEHIKAYAEMLKEQDEERYKKQFSKYLEKG 174
Yeast   174 PHSENRFP....IFGGHVSQYMEELADDDEERFSELFKGYLADD 230
Human   173 PHSTKRFP....IMGQNVADYMRYLMEEDEDAYKKQFSQYIKNS 229


E.coli      ----------------------------          30(%)
B.sub       ----------------------------          31
P.furi  176 LDPEKLPEHFEEVKARIIEKFEGEGARE   203   100
M.jann  175 LEPEKLPEHFEEIKAKIDSMF         195    64
Yeast   231 IDADSL....EERAARVAAKIAALAGQQ   297    44
Human   230 VTPDMM....AQKKDRVAQKKASFLRAQ   290    38
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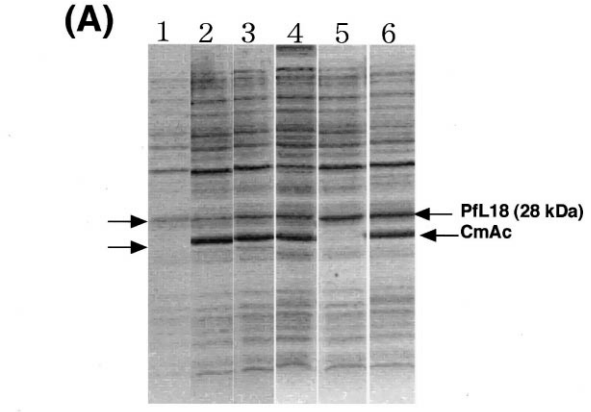
Fig. 2. Sequence similarity among the L18 rp family. Sequence similarity between *P. furiosus* L18, *E. coli* L18, *B. subtilis* L18, *M. jannaschii* L18, yeast L1, and human L18 is shown. Abbreviations and database accession numbers are as follows: *E. coli* (X01563); B. sub, *Bacillus subtilis* (L47971); M. jann, *M. jannaschii* (U67497); Yeast, *Saccharomyces cerevisiae* (M65056); Human, *Homo sapiens* (U14966). Residues which are identical amino acids among five or six species in all six organisms in the data set are marked on the top line with asterisks and printed in bold. Figures at the end of each alignment are percentage identities calculated for the N-terminal parts which are similar enough in all kingdoms between *P. furiosus* (positions 1–131) and the other sequence.

and the availability of these charged tRNAs relative to their demand dictate some of the rate limiting factors for gene expression [19]. The codon usage of the rp genes from this organism is skewed favoring A or T at the third position and the overproduction of *P. furiosus* proteins in *E. coli* can be problematic. In particular, two codon preferences for arginine (AGG and AGA) and one for isoleucine (AUA) in the PfL18 gene are very rare in *E. coli*. We tested if extra plasmid copies of the tRNA genes required for decoding AGG and AGA (arginine) and AUA (isoleucine) would increase the amount of PfL18 protein overproduced in *E. coli* BL21(DE3). Fig. 3 shows the expresion results of PfL18 protein in the presence or absence of arginyl tRNA (AGA and AGG codons) and isoleucyl tRNA (AUA codon) genes. Although the coexpression of the rare arginyl tRNA and isoleucyl tRNA genes can be helpful for the expression level of the PfL18 gene in *E. coli*, they did not dramatically enhance the production of the protein (Fig. 3C). In order to increase further the level of expression, we converted the arginine AGA and AGG tandem codons present in the N-terminal region of the PfL18 protein gene into the CGT codon used with high frequency in *E. coli*. The level produced by the PfL18-Arg/CGT in strain BL21(DE3) increased by more than three-fold over those produced by the PfL18-WT in the same strain. This indicates the importance of the arginine tandem codon usage in the N-terminal region for PfL18 gene expression. The expression level was also examined in strain BL21(DE3) pLysS and reached a level higher than that of BL21(DE3) (Fig. 3C). This result suggested that the pET-L18 plasmid might produce small amounts of recombinant rp PfL18 prior to IPTG induction with a toxic effect to *E. coli*.

3.3. Localization of a 5S rRNA binding region with PfL18

Comparison of the amino acid sequence of PfL18 with those of rp of other origins, including the 5S rRNA binding protein of *E. coli*, led to the observation of a highly conserved region (positions 8 to 39) of basic amino acid residues (Fig. 2). The preponderance of arginines and lysines in this region



(C)

lane	gene	Background host	amount of expressed protein
1	PfL18-WT	E. coli BL21(DE3)	50
2	PfL18-WT	E. coli BL21(DE3)-pFU9872	60
3	PfL18-WT	E. coli BL21(DE3)-pSJS1240	70
4	PfL18-WT	E. coli BL21(DE3)-pLys	100
5	PfL18-Arg/CGT	E. coli BL21(DE3)	168
6	PfL18-Arg/CGT	E. coli BL21(DE3)-pLys	185

Fig. 3. Analysis of PfL18 gene expression. A: *E. coli* bearing various plasmids (see C) were grown at 37°C to an A_{600} of 0.6. IPTG was added to a final concentration of 0.5 mM and growth was continued for 3 h. Total cell extracts were electrophoresed on 15% SDS–PAGE and stained with Coomassie blue. B: The same amounts of protein were applied for protein transfer in Western blots. The protein was semi-dry-blotted for 1 h at room temperature and probed with a 1:1000 dilution of anti-His Tag monoclonal antibody. The blot was developed with NBT and BCIP for 10 min. C: Expression level of PfL18-WT and mutant PfL18-Arg/CGT genes in *E. coli* hosts of different backgrounds (summarization of the data from Fig. 3A). The expresion of each gene was quantitated through staining density scanning using an NIH image (A). The position of PfL18 and CmAc (chloramphenicol acetyl-transferase) is marked by an arrow.

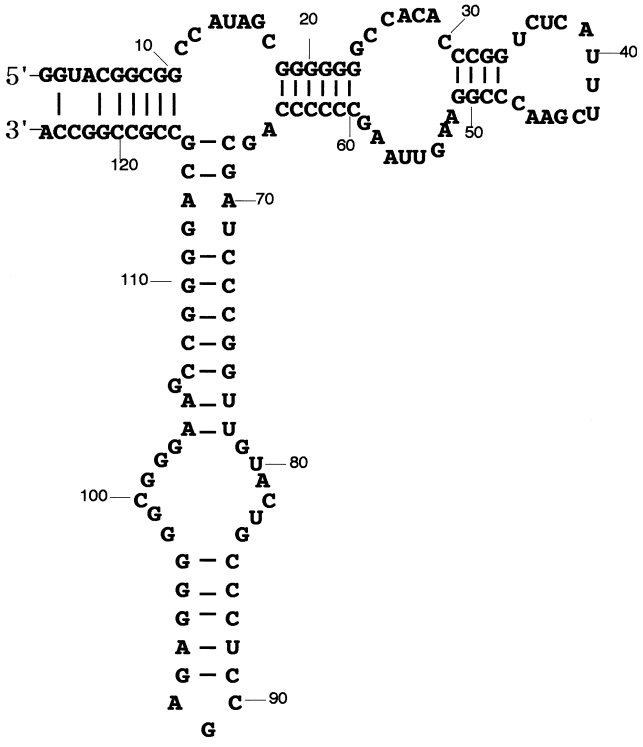
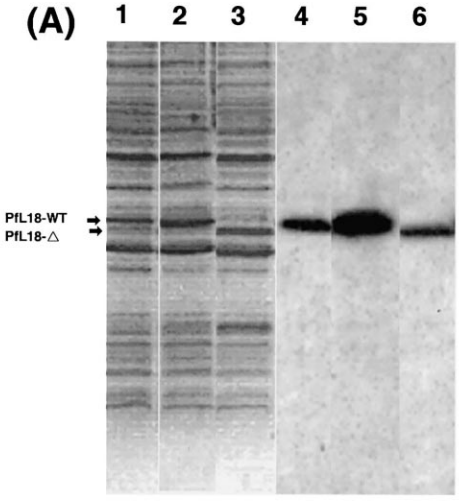


Fig. 4. Predicted secondary structure of 5S rRNA from *P. furiosus* as deduced from the DNA sequence.

suggests that the rRNA-binding domain of the protein PfL18 is related to the arginine-rich quality, that is characteristic of a family of RNA-binding proteins [20]. To test this, we generated radiolabeled 5S rRNA and examined its ability to bind to PfL18-WT and mutant PfL18 with N-terminal 15 amino acids deleted (PfL18-Δ15). We first determined the 5S rRNA sequence of *P. furiosus* from a PCR product amplified using the conserved 5′ and 3′ reversed primers of the related species *P. horikoshii* [16] and *Pyrococcus woesei* [21]. As indicated in Fig. 4, the secondary structure of this RNA was essentially similar to the typical secondary structure of known 5S rRNA. Using the radiolabeled 5S rRNA probe transcribed in vitro from a *Hin*dIII-linearized plasmid inserted into the PCR product, the size and specific intensity of PfL18 proteins binding to 5S rRNA were assessed by Northwestern blot analysis (Fig. 5). The PfL18-WT and mutant PfL18-Arg/CGT produced a protein of 28 kDa on SDS–PAGE, whereas mutant PfL18-Δ15 produced a protein of 26 kDa, which is slightly smaller (Fig. 5A, lanes 1–3). To determine the amount of protein produced and 5S rRNA binding, the intensities of the protein bands and autoradiogram density were quantitated by densitometric scanning and the values normalized to overexpressed PfL18-WT (Fig. 5B). When PfL18-Arg/CGT and PfuL18-Δ15 were expressed, these proteins accumulated to about two times the level of the wild-type (Fig. 5A, lanes 1–3). In contrast, the amount of 5S rRNA binding, visualized with Northwestern, for PfL18-Δ15 was significantly lower (16% in relative value) than that of PfL18-WT an dPfL18-Arg/CGT (100–105%) (Fig. 5A, lanes, 4–6). When the radiolabeled 7S SRP-RNA probe (as control) was used in the Northwestern analysis instead of the 5S rRNA probe, no band was observed (data not shown). These results indicated that PfL18 binds quite well to 5S rRNA as well as *E. coli* L18 protein, and the N-terminal Arg-rich region of PfL18 is strongly involved in 5S rRNA binding. However, when deletions of 15 N-terminal amino acids including the arginine rich sequence were made, the mutant partially maintained the ability to bind 5S rRNA. It appears that the other conserved regions of PfL18 excluding the 15 N-terminal amino acid region including 8RVPFRRRR15, such as 22RKRLK26 or 32KPRLVVRK39, are also required for 5S rRNA binding, despite low participation. The alignment of L18 homologs from phylogenetically diverse members of the kingdom bacteria revealed two conserved regions of the 26 amino acid C-terminal and an Arg- or Lys-rich 19 amino acid N-terminus [22]. From the chemical modification of *E. coli* L18, it had been suggested that the C-terminus conserved portion of L18 likely contains important determinants for the interaction with 5S rRNA [23,24]. It seems reasonable to suppose, therefore, that the L18–5S rRNA binding sites are more than a simple Arg-rich region of the N-terminus.

3.4. Characterization of 5S rRNA binding-proteins in whole cell extract

It is possible that the PfL18 5S rRNA binding detected in the assays might be part of a large complex of protein and



(B)

lane	gene	amount of expression	5s-rRNA bounded	Relative of 5S-rRNA bounded
1/4	PfL18 -WT	100	100	**100**
2/5	PfL18-Arg/CGT	185	195	**105**
3/6	PfL18-△15	204	32	**16**

Fig. 5. Analysis of 5S rRNA binding by a Northwestern blot. A: *E. coli* cell extract bearing the PfL18-WT gene (lanes 1 and 4) and the mutant gene (lanes 2, 3, 5, and 6) was separated on a 15% SDS–PAGE gel, transferred to a PVDF membrane, and incubated with ^{32}P-labeled 5S rRNA. Lanes 4–6 were incubated with ^{32}P-labeled 5S rRNA as described previously [10]. Lanes 1–3 are an image of the Coomassie-blue-stained gel, and lanes 4–6 are from an autoradiography of the gel. Two mutants in PfL18 were generated: a substitution of arginine AGG/AGA tandem codons to CGT(PfL18-Arg/CGT) (lanes 2 and 5), and a deletion of amino acids 1–15 (PfL18-Δ15) (lanes 3 and 5). B: Summarization of the data from Fig. 5A. The expression of PfL18 proteins and the amount of RNA-binding products were quantitated as arbitrary units from staining or autoradiogram density scanning, respectively, using an NIH image.

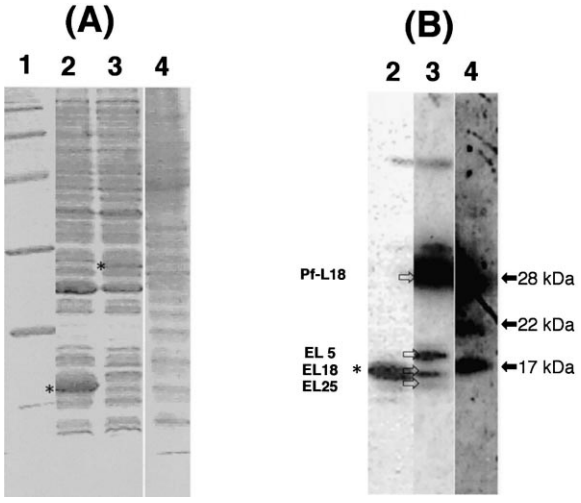


Fig. 6. 5S rRNA–protein binding in whole cell extracts. *E. coli* cell extract bearing the wild-type *E. coli* L18 gene (lane 2), the wild-type PfL18 gene (PfL18-WT) (lane 3), and *P. furiosus* whole cell extract (lane 4) was analyzed as described in Fig. 3. (A) is an image of the Coomassie-blue-stained gel, and (B) is from an autoradiography of the gel. The position of migration for several molecular weight markers is indicated (A, lane 1). The rp L18 of *E. coli* and expressed PfL18-WT is indicated by an asterisk (*) in lane 2 (A) and lane 3 (A), respectively. The positions of L5, L18 and L25 of *E. coli* are marked by white arrows (lane 3).

RNA interactions in ribosomes or that the 5S rRNA may contain binding sites for additional cytoplasmic proteins. In order to test this hypothesis, a *P. furiosus* whole cell extract was separated by SDS–PAGE and Northwestern blot analysis with ^{32}P-labeled 5S rRNA was carried out. Fig. 6 shows that two proteins with molecular mass of about 22 and 17 kDa bound with the 5S rRNA on the Northwestern blot except for PfL18 of the 28 kDa in *P. furiosus* (Fig. 6B, lane 4). The formation of a three protein 5S rRNA complex in *P. furiosus* is similar to that in bacterial *E. coli*, although the sizes of the proteins, 22 kDa and 17 kDa, differ from the molecular weight of the *E. coli* 5S rRNA binding proteins L5 (20 kDa), and L25 (11 kDa) detected in *E. coli* cell extract bearing the PfL18-WT gene using a *P. furiosus* 5S rRNA probe with autoradiography for a long time (Fig. 6B, lane 3). However, the three protein 5S rRNA complexes of archaeon *P. furiosus* are quite different from the one protein 5S rRNA complexes of eukaryotes, despite the higher sequence similarity of archaeon PfL18 to eukaryote yeast L1 rp than to eubacterial *E. coli* EL18 (Fig. 2). We cannot exclude the possibility that there are other cytoplasmic proteins besides rps as 5S rRNA binding protein. These results suggested that the archaeal specific protein 5S rRNA binding mechanism may participate in the structure and function of protein synthesis in a way different from those of eubacterial or eukaryotic systems.

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