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ARTICLE *in* BIOCHIMIE · DECEMBER 1999

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In vitro assembly of yeast 5S rRNA and a fusion protein containing ribosomal protein L5 and maltose binding protein

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(Received 9 April 1999; accepted 24 September 1999)

Abstract — Binding of yeast ribosomal protein L5 with 5S rRNA has long been considered a promising model for studying molecular mechanisms of protein-RNA interactions. However, in vitro assembly of a ribonucleoprotein (RNP) complex from purified yeast ribosomal protein L5 (also known as L1, L1a, or YL3) and 5S rRNA proved to be difficult, thus limiting the utility of this model. In the present report, we present data on the successful in vitro assembly of a RNP complex using a fusion (MBP-L5) protein consisting of the yeast ribosomal protein L5 fused to the carboxyl terminus of the *E. coli* maltose-binding protein (MBP). We demonstrated that: 1) the MBP-L5 protein binds yeast 5S rRNA but not 5.8S rRNA in vitro; 2) the MBP protein itself does not bind yeast 5S rRNA; 3) formation of the RNP complex is proportional to the concentration of MBP-L5 protein and 5S rRNA; and 4) the MBP moiety of the fusion protein in the RNP complex can be removed with factor Xa. The electrophoretic mobility of the resultant RNP complex is indistinguishable from that of L5-5S rRNA complex isolated from the ribosome. Using this new experimental approach, we further showed that the RNA binding capability of a mutant L5 protein is decreased by 60% compared to the wild-type protein. Additionally, the mutant RNP complex migrates slower than the wild-type RNP complex suggesting that the mutant RNP complex has a less compact conformation. The finding provides a probable explanation for an earlier observation that the 60S ribosomal subunit containing the mutant protein is unstable. © Société française de biochimie et biologie moléculaire/Éditions scientifiques et médicales Elsevier SAS

RNA-protein interaction / ribosomal protein / 5S rRNA / yeast / fusion protein

1. Introduction

Yeast ribosomal protein L5 (also known as L1, L1a and YL3) [1] binds 5S rRNA forming a ribonucleoprotein (RNP) complex which is a part of the large (60S) ribosomal subunit. The RNP complex is essential for 60S subunit assembly. Insufficient supply of the L5 protein blocks the synthesis of the 60S ribosome subunit and causes 5S rRNA instability. On the other hand, the RNP complex remains stable and accumulates in the nucleus even when the assembly of 60S subunit is disrupted [2]. Recent publications [3, 4] show that the RNP complex may be essential for the association of the large and the small subunit to form the 80S ribosome. Thus, studying the structure-function relationship of the components in this complex is important for our understanding of ribosome function and has been the subject of numerous studies. The RNP complex also appears to be a convenient model for studying the fundamentals of RNA-protein binding.

Knowledge about the physical state of the 5S rRNA molecule and the L5 protein within the RNP complex have been obtained from studies using different chemical and enzymatic modifications of the RNP complex [5-9]. The 5S rRNA and L5 protein binding sites have been partly mapped and characterized [8-10]. Nevertheless, the structural and chemical nature of the RNA-protein interactions in the RNP complex is still far from clear.

Attempts to assemble the yeast RNP complex in vitro directly from 5S rRNA and L5 protein have met with limited success. Supposedly, conformational changes of the L5 protein after dissociation from 5S rRNA led to its eventual insolubility at neutral pH, making it unsuitable for reassociation with the RNA molecule [5, 9]. Unlike the yeast protein, the *Xenopus* L5 protein is more soluble, allowing successful reassembly of the *Xenopus* RNP complex from homologous components [11].

A system for reconstitution of the yeast RNP in vitro was reported by Yeh and Lee [12]. This system involves in vitro expression of the L5 protein from its cloned gene in the presence of exogenous yeast 5S rRNA. The RNP complexes formed in vitro are structurally similar to the RNP complexes formed in vivo. Using site-directed mutagenesis, the authors examined the role of individual amino acids in the L5 protein in its interaction with 5S rRNA, and the effect of mutations on ribosome assembly and stability [13, 14]. However, RNP assembly using this system has its limitations, e.g., the yield of RNP complexes

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is low and the formation of RNP complexes can only be studied under conditions suitable for L5 expression.

In the present study, we tested the hypothesis that L5 protein expressed as a fusion protein with the *E. coli* maltose binding protein (MBP) would appear in a conformation suitable for binding with 5S rRNA. We provide data on the successful assembly of the yeast RNP complex in vitro using a fusion form of the L5 protein.

2. Materials and methods

2.1. Materials and buffers

Expression vector pMAL-c2, restriction enzymes, factor Xa, anti-MBP rabbit serum, and amylose resin were obtained from New England Biolabs (Beverly, USA). Double-strand DNA sequencing kit and chemiluminescent reagents for Western blot analysis were from Amersham Pharmacia Biotech (Piscataway, USA). Geneclean II kit for DNA purification was purchased from Bio 101 (Vista, USA). Protease inhibitors cocktail tablets, Complete™, were supplied by Boehringer Mannhein (Indianapolis, USA). Isopropyl-β-D-thiogalactopyranoside (IPTG) was from Sigma (St. Louis, USA). Nitrocellulose membrane was from Schleicher & Schuell (Keene, USA). Anti-HA epitope 12CA5 mouse monoclonal antibodies were obtained from Berkeley Antibody Company (Richmond, USA). Goat anti-mouse and goat anti-rabbit horseradish peroxidase conjugates were provided by Promega (Madison, USA) and Bio Rad (Hercules, USA), respectively. RNase T1 was purchased from Sankyo Company (Tokyo, Japan).

Buffer 1: 10 mM Tris, pH 8.0, 200 mM NaCl, 5% glycerol, 1 mM EDTA, and 1 mM DTT. Buffer 2: 25 mM Tris, pH 8.3, 192 mM glycine, 0.1% SDS, and 20% methanol. Buffer 3: 20 mM Tris, pH 7.6, 137 mM NaCl, and 0.05% Tween-20. Buffer 4: 10 mM Tris, pH 7.4, 10 mM EDTA, and 0.5% SDS.

2.2. Cloning ribosomal protein L5 gene into pMAL-c2 expression vector

Yeast ribosomal protein L5 gene was subcloned into pMAL-c2. Briefly, the pL5-119 plasmid containing the ribosomal protein L5 (*RPL5*) gene of *Saccharomyces cererevisiae* as described previously [13] was used as a template. The L5 coding region together with extensions containing the *Bam*HI and *Xho*I recognition site at the 5'- and 3'-terminus, respectively, was generated by PCR using (5')CATAGGATCCATGGCTTTCCAAAAAGAC(3') as the 5' primer and (5')CATACTCGAGTCATTGT TGACCAGCCAAAGC(3') as the 3' primer. The resultant 0.9 kb fragment was digested with *Bam*HI and *Xho*I, resolved on a 0.8% agarose gel, and purified using the Geneclean kit. The purified DNA fragment was ligated into the pMAL-c2 which was pre-treated with *Bam*HI and *Sal*I. The recombinant DNA, designated pMAL-c2-L5, was used to transform *E.coli* DH5αF'. The orientation and sequence of the *RPL5* gene in the plasmid were verified by restriction mapping with *Eco*RV and double-strand DNA sequencing.

2.3. MBP-L5 fusion protein expression and purification

The MBP-L5 fusion protein was expressed in *E. coli* DH5αF' transformed with pMAL-c2-L5 following the procedure described by Scripture and Huber [15] with the following modifications. Protein expression was induced with 100 μM IPTG when the *E. coli* culture reached an OD_{600} = 0.40–0.45. After 2.5 h of incubation at 30 °C, cells were harvested. All subsequent steps were conducted at 4 °C. Cells were suspended in cold buffer 1 with protease inhibitors (1 tablet/10 mL) and lysed using French press (16 000 psi, six cycles). The cell extract was cleared by centrifugation at 35 000 *g* for 30 min, diluted 1:5 (v/v) with buffer 1, and loaded onto an amylose column (1 mL) at a flow rate of 35 mL/h. The column was washed with 60 mL of buffer 1 and the fusion protein was eluted with 10 mM maltose in buffer 1. Protein concentration was determined by the method of Bradford using bovine serum albumin as a standard. The yield of the MBP-L5 protein averaged between 700 and 800 μg/L of culture. Purity of the fusion protein was verified on a 10% SDS polyacrylamide gel and was judged to be 90–95% pure by silver staining. The purified protein was stored at –20 °C for subsequent analyses.

2.4. Proteolytic cleavage of the MBP-L5 fusion protein and the MBP-L5-RNA complex

Cleavage of MBP-L5 protein by factor Xa was performed in buffer 1 at 4 °C (1 μg factor Xa/20 μg MBP-L5/50 μL buffer) for 0.5, 1, 3, and 19 h. The effectiveness of the digestion was monitored by Western analysis or silver staining of SDS polyacrylamide gels. Pre-formed RNP complexes were treated with factor Xa under identical conditions and analyzed by electrophoresis on non-denaturing gels.

2.5. 5S and 5.8S rRNA preparation

RNAs were prepared from *Saccharomyces cerevisiae* cells (*Dal* strain) harvested at mid-log phase. Briefly, cells were re-suspended in buffer 4 and repeatedly extracted with re-distilled phenol. The aqueous layers were re-extracted with phenol and the RNAs were precipitated with 2.5 vol of cold (–20 °C) ethanol. 5S and 5.8S rRNA were purified on a Sephacryl S-200 column as described earlier [12]. The concentrations of rRNAs were determined spectrophotometrically at 260 nm.

2.6. RNA binding assay

Fusion protein (30–130 pmol) in buffer 1 and rRNA (0.3–30 pmol) in DEPC-treated water were incubated in a

ratio of 3:2 (v/v) in a final volume of 25 μL for 1 h at 25 °C. The reaction mixture was loaded onto an 8% non-denaturing polyacrylamide gel or was stored at 4 °C until further analysis. Gels were run at room temperature at 60 V with circulation of 1X TBE buffer, stained with ethidium bromide (EtBr) and silver. Alternatively, Western analysis was used to detect proteins. The intensity of the RNA or RNP complex bands in EtBr-stained gels was captured with a CCD camera and analyzed using the Image-Pro Plus 3.0 program (Media Cybernetics, Silver Spring, USA) or the AlphaImager™ digital imaging system and the Alpha Ease™ software (Alpha Innotech Corp., San Leandro, USA).

2.7. RNase T1 digestion of RNP complex

RNP complexes were formed as described above. Half of the sample was treated with RNase T1 (4 units/25 μL reaction) for 30 min at 37 °C; the other half was used as a control and was incubated under similar conditions except without RNase. Both samples were immediately loaded onto an 8% non-denaturing gel. RNP complexes were detected under an UV light first and by silver staining.

2.8. Immunodetection of proteins

Proteins resolved on a SDS polyacrylamide gel (10%) were blotted onto a nitrocellulose membrane for 15 h at 30V in buffer 2. The membrane was preblocked for 1 h in buffer 3 with 5% powdered milk. For L5 protein detection, the primary antibody (anti-HA epitope 12CA5 mouse monoclonal antibody) was diluted 1:5000 in buffer 3 with 1% powdered milk and incubated with the membrane for 1 h. The membrane was washed four times (5 min each) with buffer 3 alone. The secondary antibody (anti-mouse IgG horseradish peroxidase conjugate) was diluted 1:7500 in buffer 3 with 1% powdered milk and incubated with the membrane for 1 h. The membrane was washed three times (5 min each) with buffer 3, once with buffer 3 without Tween 20, and finally incubated with ECL detection reagents for 1 min. Proteins were detected by autoradiography. The MBP protein was identified using anti-MBP rabbit serum as the primary antibody (1:7500 dilution). Goat anti-rabbit IgG horseradish peroxidase conjugate was used as the secondary antibody (1:10 000 dilution).

3. Results and discussion

Previous attempts to study the binding of yeast ribosomal protein L5 (formerly known as L1, L1a, YL3) with 5S rRNA in vitro have been impeded by the failure to form RNA-protein complexes with purified protein and RNA. The recent development of an in vitro coupled transcription-translation system [12, 13] expressing the protein from its cloned gene in the presence of exogenous yeast 5S rRNA has been useful, but its utility is limited. In the present study, we established an in vitro system for studying the specific interaction between purified protein and RNA. The system involves expressing the ribosomal protein as a soluble fusion protein. Yeast gene *RPL5* (formerly *RPL1*), which encodes the HA-tagged L5 ribosomal protein, was inserted into the expression vector, pMAL-c2, downstream from the coding sequence of the maltose binding protein (MBP). The plasmid was used to transform *E. coli*. Expression of the fusion protein in *E. coli* was controlled by the P*tac* promoter which was induced with IPTG. Both soluble and insoluble forms of the MBP-L5 chimeric protein were detected as a 76 kDa species in lysates of transformed cells. Production of the fusion protein was time-dependent *(figure 1)*. The protein

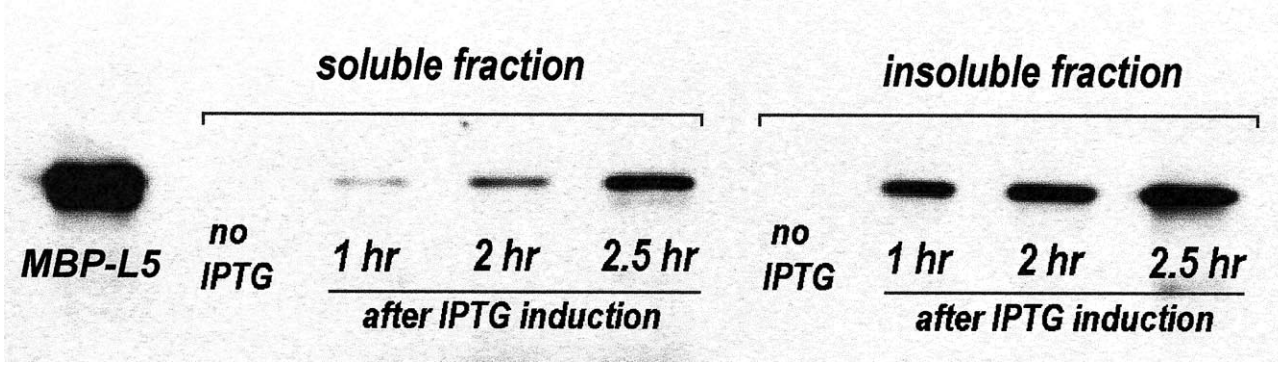

Figure 1. Expression of MBP-L5 fusion protein by *E. coli* as a function of IPTG induction. The *E. coli* culture transformed with pMAl-cL5 was treated with IPTG. At 1, 2, and 2.5 h after IPTG induction, cells were collected and lysed. After low speed centrifugation, both the supernatant fraction and the pellet fraction were resolved on SDS polyacrylamide gels. Proteins were probed with anti-HA antibodies.

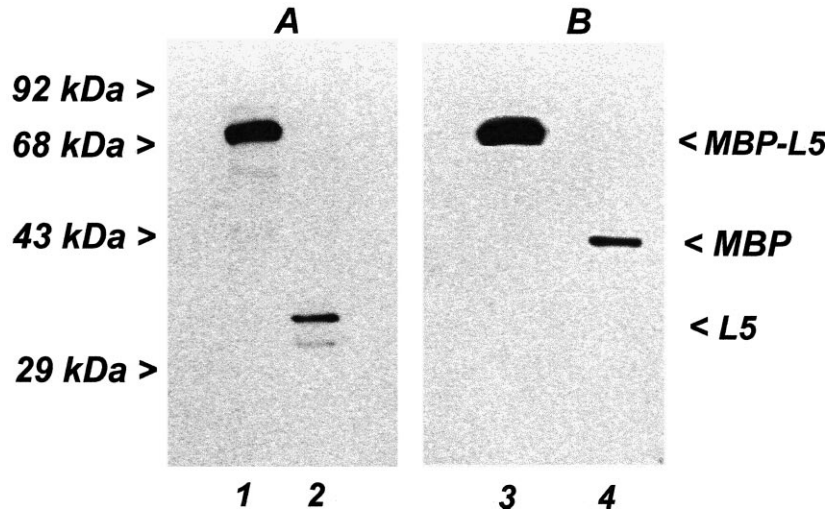

Figure 2. Western analyses of MBP-L5 fusion protein and its factor Xa cleavage products. MBP-L5 fusion protein was purified on amylose column and was treated with factor Xa digestion overnight at 4 °C. The products were analyzed on a 10% SDS polyacrylamide gel and Western blotting using anti-HA (**A**) or anti-MBP (**B**) antibodies. Lanes 1 and 3 are MBP-L5 fusion protein. Lanes 2 and 4 are factor Xa digested products. Molecular mass standards are indicated on the left and locations of the MBP-L5 fusion protein, MBP protein, and L5 protein are indicated on the right.

was reactive towards both anti-HA and anti-MBP antibodies, indicating that the 76 kDa species contains both L5 and MBP.

The soluble form of the protein was purified by affinity chromatography and was used for all subsequent experiments described here. As shown in *figure 2*, cleavage of the fusion protein with factor Xa protease generated two polypeptides with molecular masses of about 34 kDa and 42 kDa, which corresponded to those of L5 and MBP, respectively [15, 16]. The former polypeptide reacted with anti-HA antibodies and the latter with anti-MBP antibodies, confirming the identity of the two polypeptides.

The fusion protein was tested for its capability to bind 5S rRNA. A mixture of 5S rRNA and the MBP-L5 protein was incubated for 1 h and loaded onto a non-denaturing gel. *Figure 3* shows that ethidium bromide staining of the gel revealed the presence of a new band, with a slower mobility than free 5S rRNA. Presence of the MBP-L5 protein in the new, putative RNP band was confirmed by silver staining and Western blot analysis using anti-HA antibodies *(figure 3)*. The free MBP-L5 protein barely entered the gel and, at times, remained at the interface. The diffused, faster-moving bands, just ahead of the fRNP bands (*figure 3*, lanes 2 and 3) appeared to be a minor contaminant of the protein preparation. The diffused, minor band in *figure 3C* (lane 2) appeared to be an artifact in Western blot analyses with the HA antibody, perhaps due to contamination of the protein preparations with cross-reactive material. However, these minor components did not appear to interfere with 5S rRNA binding. The 5S rRNA-MBP-L5 fusion protein complex is referred to as 'fusion RNP' (fRNP). This fRNP band was completely abolished by RNase T1 treatment *(figure 4A)*, providing additional evidence for the presence of RNA in this band. *Figure 4B* also shows that the MBP-L5 fusion protein did not bind yeast 5.8S rRNA, indicating that the RNP complex is specific for 5S rRNA. A slower migrating 5.8S rRNA band was apparent (*figure 4B*, lane 3). It has been previously reported that 5.8S rRNA could exist in two conformations separable on gels. Several minor, faster migrating bands were detected also (*figure 4B*, lane 4). Although the nature of which is unclear at present, they might be degraded products of the 5.8S rRNA. However, a significant concentration of intact 5.8S rRNA was present in the reaction mixture to allow detection of RNP complexes should they be formed. Moreover, that no RNP complex was detected when purified MBP was added to the RNA binding assay eliminated the possibility that the MBP moiety of the fusion protein provided the binding site for 5S rRNA (data not shown).

To determine whether the MBP moiety of the fusion protein-RNA complex could be removed yielding an intact 5S rRNA-protein complex, the fusion protein-RNA complex was subjected to digestion with factor Xa for varying times (0.5 h, 1 h, 3 h, 19 h). The reaction mixtures were loaded onto an 8% non-denaturing gel. Staining of the gel with ethidium or silver revealed the presence of a new RNP band in those samples treated with factor Xa (*figure 5*, lanes 4–7). The electrophoretic mobility of this new RNP band matched that of the native RNP isolated

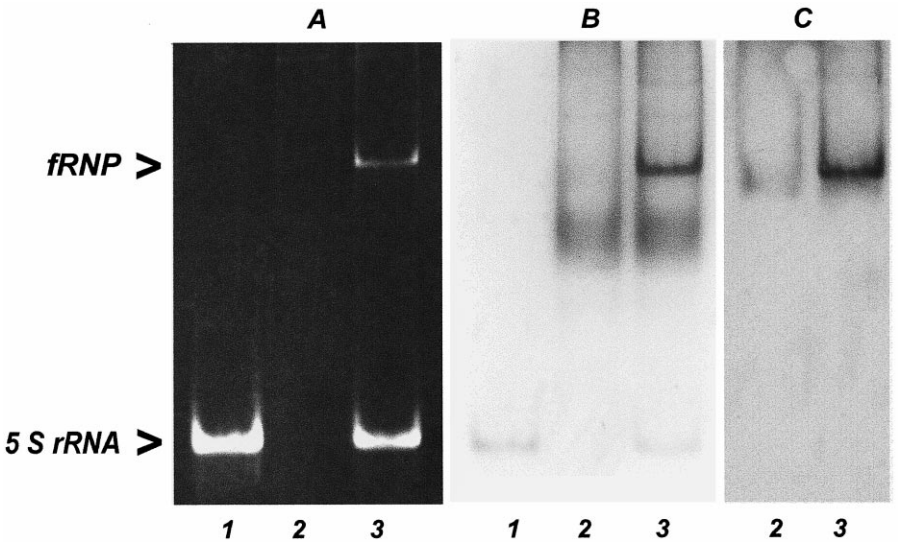

Figure 3. Formation of RNP complex between MBP-L5 protein and yeast 5S rRNA. Purified MBP-L5 fusion protein (5.5 μM) was incubated with purified yeast 5S rRNA (0.28 μM) as described in *Materials and methods*. Reaction mixtures were analyzed on an 8% non-denaturing polyacrylamide gel. After electrophoresis, the gel was stained with ethidium bromide (**A**) and silver (**B**). A similar sample was analyzed by Western analysis (**C**) using anti-HA antibodies. Positions of the MBP-L5 fusion protein-5S rRNA complex and free 5S rRNA are indicated on the left. In each panel: lane 1, free RNA only; lane 2, MBP-L5 fusion protein only; and lane 3, MBP-L5 fusion protein plus 5S rRNA.

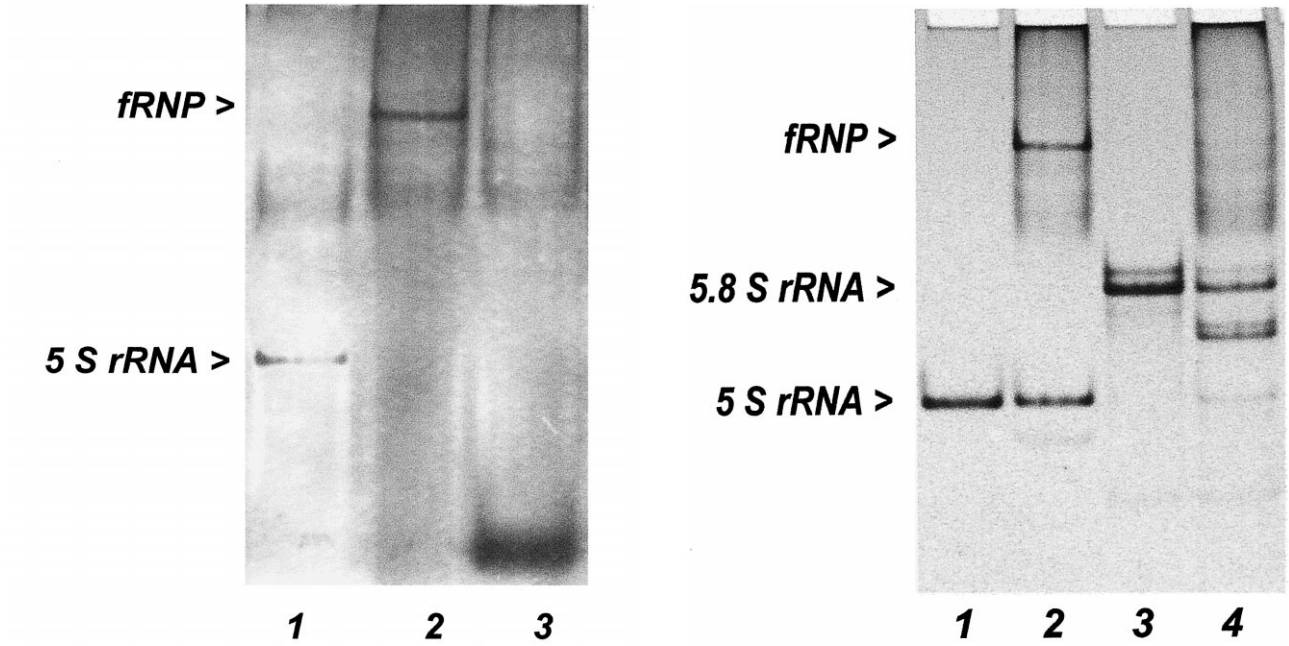

Figure 4. The MBP-L5-5S rRNA complex is sensitive to RNase T1 (**A**) and the MBP-L5 fusion protein does not bind yeast 5.8S rRNA (**B**). **A.** The MBP-L5 fusion protein-5S rRNA complex was assembled as described in *Materials and methods* and subjected to RNase T1 digestion. Reaction mixtures were analyzed on non-denaturing gels. RNPs were detected by silver staining. Lane 1, free 5S rRNA; lane 2, RNPs; lane 3, RNPs treated with RNase T1. **B.** Purified MBP-L5 fusion protein was incubated with purified yeast 5.8S rRNA or 5S rRNA. Reaction mixtures were analyzed on a non-denaturing gel and the gel was stained with silver. Locations of the fRNP complex, free 5.8S rRNA, and free 5S rRNA are indicated on the left. Lane 1, free 5S rRNA; lane 2, MBP-L5 fusion protein plus 5S rRNA; lane 3, free 5.8S rRNA; lane 4, MBP-L5 fusion protein plus 5.8S rRNA.

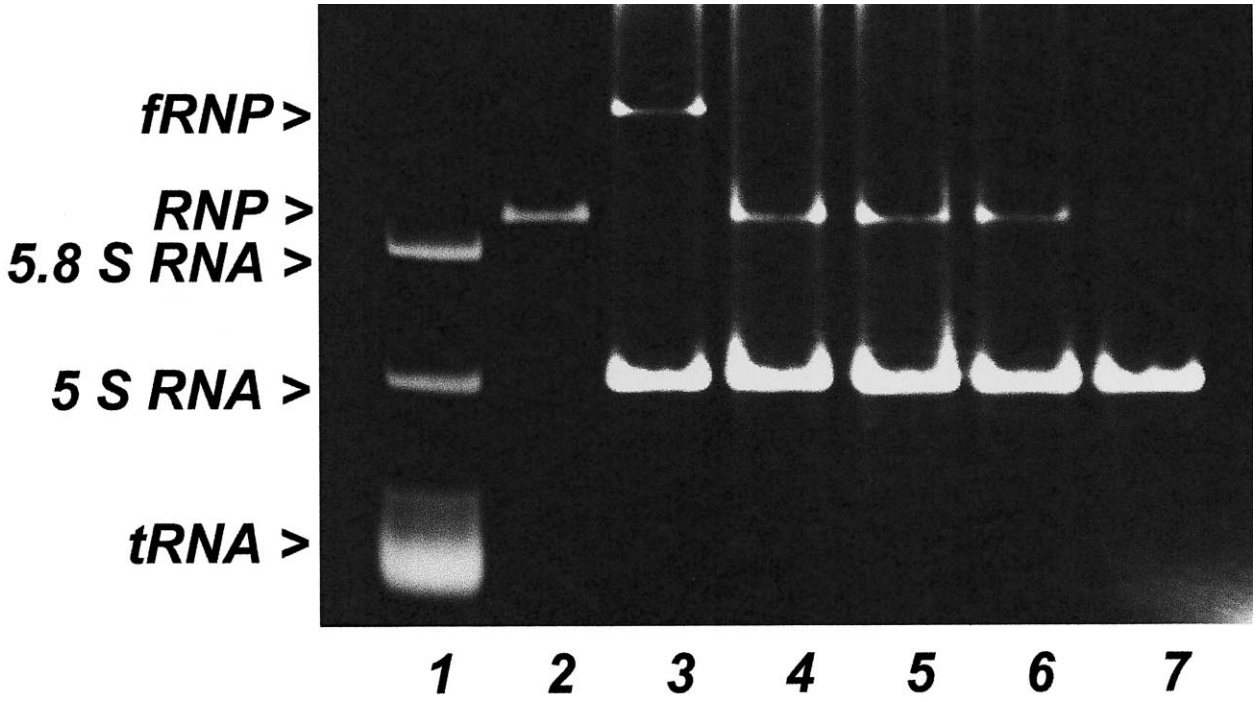

Figure 5. Effects of factor Xa digestion on the MBP-L5 fusion protein-5S rRNA complex. The MBP-L5 fusion protein-5S rRNA complex was treated with factor Xa for 30 min, 1 h, 3 h, and 19 h (lanes 4 through 7, respectively). Undigested, control fRNP complex is shown in lane 3. Lane 1 shows marker RNAs, 5.8S, 5S, and 4S tRNA. Lane 2 shows the RNP isolated from purified yeast 60S ribosome. Samples were analyzed on a non-denaturing polyacrylamide gel. RNPs were detected by ethidium bromide staining of the gel. Locations of the fusion protein-RNA complex (fRNP), the L5-RNA complex (RNP), 5.8S rRNA, 5S rRNA, and 4S tRNA are indicated on the left.

from purified ribosomes (*figure 5*, lane 2). Factor Xa cleavage of the fusion protein-RNA complex was completed after 30 min of treatment. Based on the intensity of the ethidium-stained RNA band, it appeared that the amount of 5S rRNA bound to the RNP complex was not detectably altered following removal of the MBP moiety. These results provided additional evidence that MBP does not contribute directly to 5S rRNA binding. Further digestion (> 3 h) of the fusion protein-RNA complex with factor Xa resulted in RNP degradation, presumably due to a low level of RNase contamination in the factor Xa sample used for the study.

A published report showed that the *Xenopus* L5 protein produced by factor Xa treatment of a fusion protein could bind the homologous 5S rRNA [15]. However, our preliminary studies indicated that the yeast L5 protein produced by factor Xa cleavage of the MBP-L5 fusion protein was not active in RNA binding (data not shown). The MBP-L5 fusion protein that was treated identically, except without factor Xa, retained about 70–80% of its RNA binding activity. A speculative explanation for the present observations is that the MBP moiety may serve as an intra-molecular chaperon that maintains the L5 moiety in a conformation that is capable to bind RNA. Removal of the MBP from the fusion protein in the absence of the RNA resulted in a L5 protein molecule that was incapable of RNA binding. However, once the L5 moiety of the fusion protein interacted with the RNA, the MBP moiety was no longer required to maintain an active L5 conformation for RNA binding. Consistent with the speculation is our previous observation that the RNP complex is formed only when 5S rRNA is added to the in vitro reaction at the beginning of the translation reaction. Full-length L5 protein fails to bind 5S rRNA [12].

Binding of the fusion protein with 5S rRNA strongly affected its interaction with factor Xa. Complete cleavage of the MBP moiety from the fusion protein took at least 19 h *(figure 6)*, while near complete cleavage of the MBP protein from the RNP complex occurred in 30 min *(figure 5)*. This difference in the kinetics of digestion suggests that binding of the 5S rRNA has altered the protein conformation significantly, making the MBP cleavage site more exposed to factor Xa. In support are previous findings on the biochemical/biophysical properties of the RNP complex and the individual RNA and protein com-

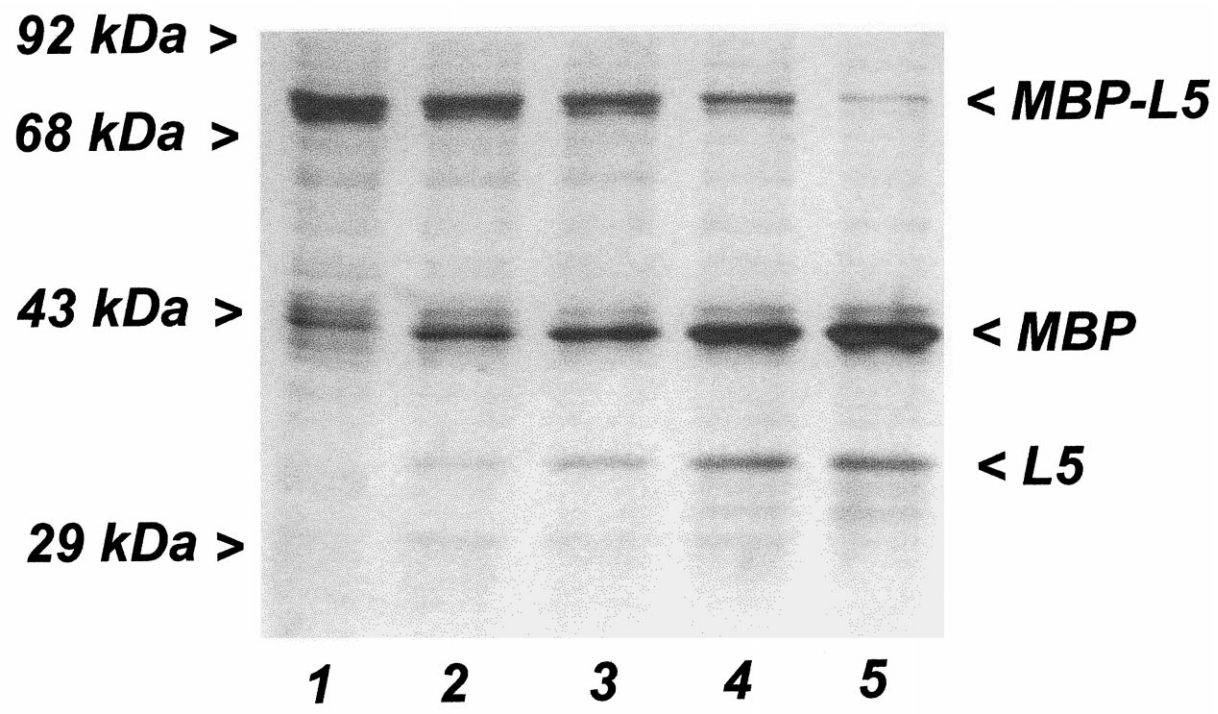

Figure 6. Digestion of MBP-L5 fusion protein by factor Xa as a function of time. Purified fusion protein was digested with factor Xa as described in *Materials and methods*. Lane 1, undigested fusion protein. The MBP-L5 fusion protein was treated with factor Xa for 30 min, 1 h, 3 h, and 19 h (lanes 2 through 5, respectively). Samples were analyzed on a SDS-containing polyacrylamide gel. Protein products were detected by silver staining.

ponents that both molecules undergo conformational changes upon dissociation of the complex [5-8].

Previously, we have shown that substitutions of two highly conserved arginine residues (R282 and R285) by methionine in L5 protein resulted in a mutant protein that has lost about 50% of its RNA binding capability, as determined by the in vitro coupled transcription-translation system [13]. In the present study, we examined the property of this mutant protein as a fusion protein. Accordingly, the L5 mutant gene was subcloned into pMAL-c2 and expressed in *E. coli* as a MBP-L5(R282, 285M) fusion protein. Expression and purification conditions for the mutant fusion protein were identical to those used for the wild-type protein.

Both the mutant and wild-type fusion proteins were tested for binding with 5S rRNA over a range of protein concentrations (1.3–5.5 μM) and at a constant 5S rRNA concentration (0.28 μM). RNP formation was analyzed by the mobility shift assay. As shown in *figure 7*, RNP formation was dependent on the protein concentration for both the wild-type and the mutant protein. The mutant fusion protein bound approximately 55–65% less 5S rRNA than the wild type at high protein concentrations approaching saturation. This finding is consistent with the earlier published data in which the RNP complex was assembled from 5S rRNA and L5 protein in the coupled transcription-translation reaction [13]. Furthermore, analysis of the initial slope of the binding curves revealed that the RNA binding property of the wild-type and the mutant MBP-L5 protein is 26.1 ± 1.6 and 9.9 ± 1.6 units of RNP/μM of protein, respectively. Using the present system, we further showed that the mutant protein could not bind yeast 5.8S rRNA *(figure 8A)*.

Figure 8B shows the electrophoretic patterns of the mutant and the wild-type protein-RNA complexes. The mutant complex migrated (with a $R_f = 0.33 \pm 0.007$) slightly and reproducibly slower than the wild-type complex (with a $R_f = 0.35 \pm 0.008$) in non-denaturing gels with a $P < 0.01$ ($n = 15$). Also shown in *figure 8B* are the electrophoretic patterns of the mutant and wild-type RNP complexes generated by factor Xa treatment of the fRNP complexes formed with the respective fusion proteins. The mutant RNP complex still migrated slower than the wild-type. Thus, the difference in the electrophoretic mobility of the RNP complexes is not likely due to the presence of the MBP protein in the fusion protein. We speculate that the mutations in L5 protein not only have lowered the RNA binding capability of the protein, but

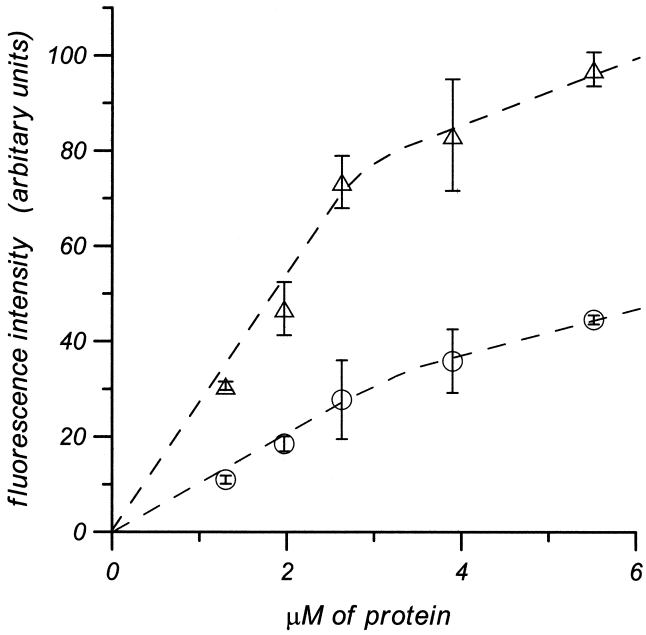

Figure 7. Formation of RNP complexes between the wild-type MBP-L5 fusion protein, the mutant fusion protein with L5 containing R282, 285M mutations, and yeast 5S rRNA. Varying concentrations (1.3 to 5.5 μM) of the wild-type or mutant fusion protein were incubated with a fixed concentration (0.28 μM) of purified 5S rRNA as described in *Materials and methods*. Samples were analyzed on non-denaturing polyacrylamide gels. After electrophoresis, gels were stained with ethidium bromide. The intensities of the RNP bands were determined using the AlphaImager™ System. Values represent the means ± SE of two independent determinations. Wild type fusion protein (Δ) and mutant fusion protein (O).

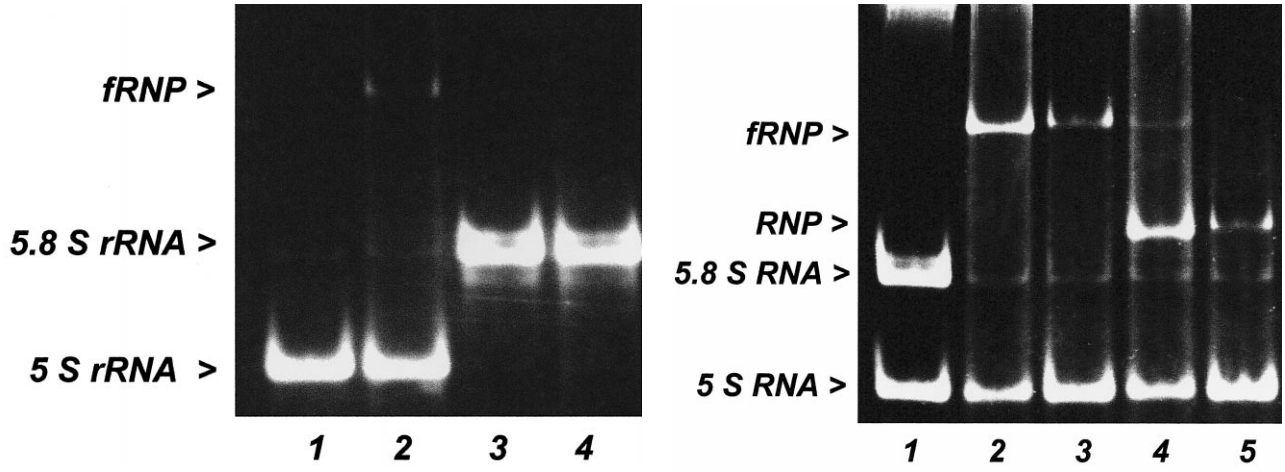

Figure 8. A. The mutant MBP-L5 fusion protein does not bind yeast 5.8S rRNA. **B.** The RNP complex consisting of the wild-type fusion protein and 5S rRNA migrates faster than that of the mutant fusion protein and 5S rRNA on non-denaturing gels. **A.** Purified mutant MBP-L5 fusion protein was incubated with purified yeast 5.8S rRNA or 5S rRNA. Reaction mixtures were analyzed on a non-denaturing gel which was stained with ethidium bromide after electrophoresis. Locations of the fRNP complex, free 5.8S rRNA, and free 5S rRNA are indicated on the left. Lane 1, free 5S rRNA; lane 2, mutant MBP-L5 fusion protein plus 5S rRNA; lane 3, free 5.8S rRNA; lane 4, mutant MBP-L5 fusion protein plus 5.8S rRNA. **B.** RNP complexes were formed with either the wild-type MBP-L5 fusion protein or the mutant MBP-L5 fusion protein and yeast 5S rRNA as described in *Materials and methods*. An aliquot of the RNP complexes was treated with factor Xa and analyzed on an 8% non-denaturing polyacrylamide gel. Complexes were detected by staining with ethidium bromide. The photo is a representative of several independent determinations. Lane 1, yeast 5.8S rRNA and 5S rRNA as markers; lane 2, the RNP complexes with wild-type fusion protein; lane 3, the RNP complexes with mutant fusion protein; lane 4, wild-type RNP complexes produced by factor Xa, digestion; and lane 5, mutant RNP complexes produced by factor Xa, digestion. Positions of the fusion protein-RNA complex (fRNP) and of factor Xa-treated RNP complex (RNP) are indicated on the left. Positions of the yeast 5.8S rRNa and 5S rRNA are also indicated.

also have changed the conformation of the resulting RNP complex. Consistent with these findings are our published results that the mutant RNP complexes are less thermal stable than the wild-type [13]. The present findings also provide a reasonable interpretation regarding the previously observed instability of 60S ribosomal subunit containing the mutant RNP complex

In summary, we demonstrated an experimental system for the in vitro assembly of yeast ribosomal protein L5 with 5S rRNA. The RNP formed appeared to be specific and faithful. The relative electrophoretic mobilities of the RNA-protein complex formed in vitro and isolated from mature yeast ribosomal subunits were similar on polyacrylamide gels. The system reported here should be useful in future studies to identify and characterize the RNP complex.

Acknowledgments

We acknowledge the Robert A. Welch Foundation (AQ 1360) for the support to J.C.L. We also thank Dr. V.A. Pekkel, Department of Pathology of this University for his help in the use of the AlphaImager system.

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