**Ivan Mitroshin,* Azat
Gabdulkhakov and Maria Garber**Institute of Protein Research, Russian Academy
of Sciences, Institutskaya 4, Pushchino, Moscow
Region 142290, Russian FederationCorrespondence e-mail:
ivan-mitroshin@vega.protres.ru

Received 4 September 2013

Accepted 28 September 2013

The base of the ribosomal P stalk from *Methanococcus jannaschii*: crystallization and preliminary X-ray studies

The lateral P stalk in archaeal/eukaryotic ribosomes and the L12 stalk in bacterial ribosomes play a pivotal role in specific binding to the ribosome and recruiting translational factors during protein biosynthesis. The P stalk consists of the ribosomal proteins L11, P0 and P1. The proteins P0 and P1 form the complex that binds 23S rRNA through the N-terminal domain of the P0 protein. Ribosomal protein L11 binds to the same region of 23S rRNA and together with the protein P0 forms the base of the stalk. The structure of the ribosomal protein L11 from archaea has been solved, but with several missing segments. Here, the preparation and crystallization of a ternary complex consisting of the ribosomal protein L11, the two-domain N-terminal fragment of the ribosomal protein P0 and a specific fragment of 23S rRNA from the archaeon *Methanococcus jannaschii* are reported. The crystals belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 72.4$, $b = 88.5$, $c = 95.2$ Å, $\beta = 102.2^\circ$. A complete diffraction data set has been collected to a resolution of 2.9 Å using an in-house rotating-anode X-ray generator.

1. Introduction

In all organisms studied, ribosomes contain a functional site, referred to as the GTPase-associated centre, which is responsible for interactions between the ribosome and translation factors (Uchiyama *et al.*, 1999). The P stalk (the L12 stalk in bacteria) plays a very important role in the formation and function of this centre. This flexible lateral ribosomal protuberance is composed of ribosomal proteins L11, P0 (L10 in bacteria) and P1 (L12 in bacteria), and helices 42–44 of the large ribosomal RNA. It has been shown that in solution the isolated ribosomal proteins L10 and L12 form a stable complex which binds ribosomal RNA through the N-terminal domain of L10 (Pettersson & Liljas, 1979). Archaeal/eukaryotic P0 and P1 demonstrate the same properties (Casiano *et al.*, 1990). In bacteria, the binding regions for the L11 and L10 proteins are located on 23S rRNA near to each other, and this has also been shown for the archaeal L11 and P0 proteins (Kavran & Steitz, 2007). It was shown that the binding of the isolated bacterial L11 to 23S rRNA enhances the affinity of L10 to rRNA by 100-fold (Iben & Draper, 2008).

The size and primary structures of the archaeal P0 and P1 stalk proteins demonstrate high similarity to their eukaryotic counterparts. Archaeal P1 and eukaryotic P1/P2 proteins are much shorter than their bacterial counterpart L12, whereas the archaeal/eukaryotic P0 is much longer than the bacterial L10 and contains a specific insertion within the N-terminal domain and a C-terminal extension. The structure of the N-terminal RNA-binding domain of P0 and L10 is universally conserved in eukarya, archaea and bacteria. It has been demonstrated that the archaeal and eukaryotic P stalk proteins are able to replace corresponding L12 stalk proteins in the *Escherichia coli* ribosome *in vitro*. During translation such hybrid ribosomes recruited archaeal and eukaryotic elongation factors but were inactive towards bacterial factors (Uchiyama *et al.*, 1999; Nomura *et al.*, 2006).

In the last two decades, progress has been made in structural studies of the isolated components of the L12 stalk of bacterial ribosomes and the P stalk of archaeal ribosomes. High-resolution structures have been determined for the bacterial complex between L11 and a 58-nucleotide 23S rRNA fragment, for the bacterial

© 2013 International Union of Crystallography
All rights reserved

complex of L10 with six copies of the N-terminal domain of L12 and for a similar complex from archaea (Wimberly *et al.*, 1999; Diaconu *et al.*, 2005; Naganuma *et al.*, 2010). In 2010, the structure of the two-domain N-terminal fragment of the ribosomal protein P0 from the hyperthermophilic archaeon *Methanococcus jannaschii* was determined (Kravchenko *et al.*, 2010). This structure contains the conserved RNA-binding domain and a second domain which corresponds to a specific insertion in the sequences of archaeal and eukaryotic P0 proteins. Very recently, a supplemented model of the large ribosomal subunit from the archaeon *Haloarcula marismortui* has been published (Gabdulkhakov *et al.*, 2013). In this work two-thirds of the P0 protein, one dimer of the N-terminal domain of the P1 protein and a large part of L11 (with two missing segments) were visualized in the *H. marismortui* 50S ribosomal subunit. The aim of our study is to obtain the complete structure of the archaeal L11 protein and to analyse the influence of L11 on the affinity of P0 for 23S rRNA.

In the present work, expression of the gene and purification of the ribosomal protein L11 from *M. jannaschii* (MjaL11) and preparation and crystallization of the ternary complex between the specific fragment of 23S rRNA, the ribosomal protein MjaL11 and the N-terminal fragment of MjaP0 (MjaP0NTF) were performed. Preliminary crystallographic characterization of the crystals is also reported.

2. Materials and methods

2.1. Protein expression and purification

The pET11c-MjaL11 plasmid containing the gene of the ribosomal protein L11 from *M. jannaschii* (MjaL11) was transformed into competent *E. coli* BL21 (DE3, pUBS520) cells. The transformed cells were cultured in Luria–Bertani (LB) medium supplemented with 50 µg ml⁻¹ kanamycin and 100 µg ml⁻¹ ampicillin. The bacterial cells were grown at 310 K. When the OD₆₀₀ reached 0.6, the culture was induced with 0.4 mM isopropyl β-D-1-thiogalactopyranoside (IPTG). After 6 h of induction, the cells were harvested by centrifugation at 5000g for 20 min at 277 K. The cell pellets were resuspended in lysis buffer [100 mM Tris–HCl pH 7.0, 1 M NaCl, 1 mM dithiothreitol (DTT), 100 mM MgCl₂, 0.1 mM phenylmethylsulfonyl fluoride (PMSF)] and disrupted using sonication. Cell debris and ribosomes were removed by two consecutive centrifugation steps (10 000g for 30 min and 90 000g for 50 min). Further purification of MjaL11 was performed by heat treatment of the post-ribosomal fraction (20 min

at 348 K to remove the main contaminating *E. coli* proteins). The denatured proteins were removed by centrifugation (10 000g for 30 min at 277 K). Ammonium sulfate was then added to the supernatant to a final concentration of 1.7 M.

MjaL11 was initially purified by hydrophobic chromatography on a Butyl Sepharose column and was eluted with a linear reverse gradient of 1.7–0 M ammonium sulfate and 1–0.05 M NaCl. Peak fractions were dialysed against a buffer consisting of 100 mM Tris–HCl pH 7.0, 50 mM NaCl, 1 mM DTT and loaded onto a Heparin Sepharose column. The protein was eluted with a linear gradient of 100–600 mM NaCl. The peak fractions were dialysed against a buffer consisting of 100 mM sodium acetate pH 5.2, 50 mM NaCl, 1 mM DTT and loaded onto a Q Sepharose column. The MjaL11 protein did not bind to the Q Sepharose and eluted in the flowthrough fraction, whereas remaining *E. coli* proteins bound to the column. The purified MjaL11 sample was pooled, exchanged into a buffer consisting of 10 mM Tris–HCl pH 7.5, 50 mM NaCl, 1 mM DTT and concentrated to 10 mg ml⁻¹.

pET11c-MjaP0NTF plasmid containing the gene for the two-domain N-terminal fragment of the ribosomal protein P0 from *M. jannaschii* (MjaP0NTF) was transformed into competent *E. coli* BL21 (DE3, pUBS520) cells (as described in Kravchenko *et al.*, 2010). The purified MjaP0NTF sample was pooled, exchanged into a buffer consisting of 10 mM Tris–HCl pH 7.5, 50 mM NaCl, 1 mM DTT and concentrated to 10 mg ml⁻¹.

2.2. RNA-fragment synthesis and purification

The 74-nucleotide specific 23S ribosomal RNA fragment from *M. jannaschii* (Mja23SrRNA-74) was obtained by *in vitro* transcription with T7 RNA polymerase from linearized plasmid DNA. The reaction mixture was precipitated using ethanol and the RNA fragment was purified by gel electrophoresis in the presence of 8 M urea (see Kravchenko *et al.*, 2011).

2.3. RNA–protein complex preparation and crystallization

Formation of the RNA–protein complex was performed by mixing the individual isolated components. To obtain a 1:1:1 stoichiometric ratio of the P0NTF–L11–rRNA complex, a 1.2-fold molar excess of MjaP0NTF and MjaL11 was added to Mja23SrRNA-74 and incubated for 30 min at 277 K to form the complex. The rRNA fragment was preliminarily heated at 338 K for 10 min and cooled to room temperature. The mixture of components was injected onto a Superdex 75 10/30 column equilibrated in a buffer consisting of 100 mM Tris–HCl pH 7.0, 100 mM NaCl, 5 mM MgCl₂, 1 mM DTT and the ternary complex was separated from excess MjaP0NTF and MjaL11. Fractions containing the complex were pooled and concentrated to 2.5 mg ml⁻¹. The homogeneity of the complex was confirmed by nondenaturing gel electrophoresis [10% polyacrylamide gel (19:1) with a buffer consisting of 90 mM Tris–acetate pH 7.8, 5 mM MgCl₂]. The gel was run at 100 V for 2–4 h at 277 K.

Screening for initial crystallization conditions was performed by the hanging-drop vapour-diffusion method using commercially available crystal screening kits from Jena Bioscience (JBScreen Nuc-Pro 1, JBScreen Nuc-Pro 2, JBScreen Nuc-Pro 3 and JBScreen Nuc-Pro 4) and Qiagen (Nucleix) at 295 K. Droplets consisting of 1 µl P0NTF–L11–rRNA complex solution and 1 µl reservoir solution were equilibrated against 500 µl reservoir solution in 24-well plates. After 8–10 d, crystals (Fig. 1) appeared in condition No. 23 of JBScreen Nuc-Pro 1 (15% PEG 4000, 50 mM Tris–HCl pH 7.5, 150 mM KCl, 20 mM MgCl₂). The approximate dimensions of the crystals were 180 × 100 × 50 µm.

Figure 1
Crystal of the P0NTF–L11–rRNA complex from *M. jannaschii*.

Table 1

Statistics of data collection and processing.

Values in parentheses are for the outermost resolution shell.

Space group	$P2_1$ (No. 4)
Unit-cell parameters ($\text{\AA}$, $^\circ$)	$a = 72.4$, $b = 88.5$, $c = 95.2$, $\beta = 102.19$
Radiation source	PROTEUM X8
Wavelength ($\text{\AA}$)	1.5418
Temperature (K)	110
Detector	PLATINUM 135
Oscillation range ($^\circ$)	0.5
Number of frames	480
Resolution range ($\text{\AA}$)	50.0–2.9 (3.00–2.90)
Mosaicity ($^\circ$)	0.42
Total reflections	111108 (10700)
Total independent reflections	25542 (2432)
$R_{\text{merge}}^\dagger$	0.142 (0.506)
Average $I/\sigma(I)$	7.0 (2.1)
Average multiplicity	4.4 (4.4)
Completeness (%)	97.4 (96.9)

$^\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the i th measurement of reflection hkl and $\langle I(hkl) \rangle$ is the average intensity of reflection hkl .

2.4. X-ray diffraction analysis

X-ray diffraction data were collected from one crystal using the laboratory X-ray apparatus, which consisted of a PROTEUM X8 rotating-anode generator equipped with a Montel 200 focusing system (Bruker) and a PLATINUM 135 CCD detector (Bruker). The voltage of the generator was 45 kV, the generator current was 40 mA, the beam diameter was 0.2 mm, the crystal-to-detector distance was 60 mm and the angle of oscillation was 0.5° . A single crystal was flash-cooled at 110 K in a nitrogen-gas stream. Prior to flash-cooling, the crystals were soaked for a short time in cryosolution (15% PEG 4000, 15% glycerol, 50 mM Tris–HCl pH 7.5, 150 mM KCl, 20 mM MgCl_2). The X-ray data were processed using the PROTEUM2 program package (Bruker). The X-ray data-collection statistics are given in Table 1.

3. Results and discussion

MjaP0NTF (molecular mass 23 kDa) and MjaL11 (molecular mass 18 kDa) were purified using the protocols described above. The P0NTF–L11–rRNA complex was purified by Superdex 75 size-exclusion chromatography and the elution profile showed a peak corresponding to the complex (65 kDa). Nondenaturing gel electrophoresis was used to confirm the homogeneity and the presence of the complex (Fig. 2). P0NTF–L11–rRNA crystals were obtained using the hanging-drop vapour-diffusion method at 295 K with reservoir solution consisting of 15% PEG 4000, 50 mM Tris–HCl pH 7.5, 150 mM KCl, 20 mM MgCl_2 . A complete X-ray data set to 2.9 $\text{\AA}$ resolution was collected in-house using a PROTEUM X8 rotating-anode X-ray generator. The crystals belonged to space group $P2_1$, with unit-cell parameters $a = 72.4$, $b = 88.5$, $c = 95.2$ $\text{\AA}$, $\beta = 102.2^\circ$. Two molecules of the complex per asymmetric unit correspond to a specific volume (V_M) of 2.28 $\text{\AA}^3 \text{Da}^{-1}$ and a solvent content of 52.0%

Figure 2

The formation of a complex between MjaP0NTF, MjaL11 and Mja23SrRNA-74nt was analysed by nondenaturing gel electrophoresis [10% polyacrylamide gel (19:1), 90 mM Tris–acetate pH 7.8, 5 mM MgCl_2]. Lane 1, 23SrRNA-74; lane 2, P0NTF–23SrRNA-74; lane 3, L11–23SrRNA-74; lane 4, P0NTF–L11–rRNA complex formed from the isolated components in a 1:1:1 ratio under analytical conditions; lane 5, peak fraction corresponding to preparative purification of the P0NTF–L11–rRNA complex.

(Matthews, 1968). Structure determination and model building are under way.

This work was supported by the Russian Academy of Sciences, the Russian Foundation for Basic Research (grant No. 12-04-31349 mol_a) and the Program of RAS on Molecular and Cellular Biology.

References

- Casiano, C., Matheson, A. T. & Traut, R. R. (1990). *J. Biol. Chem.* **265**, 18757–18761.
- Diaconu, M., Kothe, U., Schlünzen, F., Fischer, N., Harms, J. M., Tonevitsky, A. G., Stark, H., Rodnina, M. V. & Wahl, M. C. (2005). *Cell*, **121**, 991–1004.
- Gabdulkhakov, A., Nikonov, S. & Garber, M. (2013). *Acta Cryst.* **D69**, 997–1004.
- Iben, J. R. & Draper, D. E. (2008). *Biochemistry*, **47**, 2721–2731.
- Kavran, J. M. & Steitz, T. A. (2007). *J. Mol. Biol.* **371**, 1047–1059.
- Kravchenko, O. V., Mitroshin, I. V., Gabdulkhakov, A. G., Nikonov, S. V. & Garber, M. B. (2011). *Crystallogr. Rep.* **56**, 751–753.
- Kravchenko, O., Mitroshin, I., Nikonov, S., Piendl, W. & Garber, M. (2010). *J. Mol. Biol.* **399**, 214–220.
- Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
- Naganuma, T., Nomura, N., Yao, M., Mochizuki, M., Uchiumi, T. & Tanaka, I. (2010). *J. Biol. Chem.* **285**, 4747–4756.
- Nomura, T., Nakano, K., Maki, Y., Naganuma, T., Nakashima, T., Tanaka, I., Kimura, M., Hachimori, A. & Uchiumi, T. (2006). *Biochem. J.* **396**, 565–571.
- Pettersson, I. & Liljas, A. (1979). *FEBS Lett.* **98**, 139–144.
- Uchiumi, T., Hori, K., Nomura, T. & Hachimori, A. (1999). *J. Biol. Chem.* **274**, 27578–27582.
- Wimberly, B. T., Guymon, R., McCutcheon, J. P., White, S. W. & Ramakrishnan, V. (1999). *Cell*, **97**, 491–502.