Interaction among silkworm ribosomal proteins P1, P2 and P0 required for functional protein binding to the GTPase-associated domain of 28S rRNA

Tomomi Shimizu, Masao Nakagaki¹, Yoshinori Nishi², Yuji Kobayashi², Akira Hachimori and Toshio Uchiumi*

Institute of High Polymer Research and ¹Department of Applied Biological Science, Faculty of Textile Science and Technology, Shinshu University, Ueda 386-8567, Japan and ²Graduate School of Pharmaceutical Sciences, Osaka University, Osaka 565-0871, Japan

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ABSTRACT

Acidic ribosomal phosphoproteins P0, P1 and P2 were isolated in soluble form from silkworm ribosomes and tested for their interactions with each other and with RNA fragments corresponding to the GTPase-associated domain of residues 1030–1127 (Escherichia coli numbering) in silkworm 28S rRNA in vitro. Mixing of P1 and P2 formed the P1–P2 heterodimer, as demonstrated by gel mobility shift and chemical crosslinking. This heterodimer, but neither P1 or P2 alone, tightly bound to P0 and formed a pentameric complex, presumably as P0(P1–P2)2, assumed from its molecular weight derived from sedimentation analysis. Complex formation strongly stimulated binding of P0 to the GTPase-associated RNA domain. The protein complex and eL12 (E.coli L11-type), which cross-bound to the E.coli equivalent RNA domain, were tested for their function by replacing with the E.coli counterparts L10.L7/L12 complex and L11 on the rRNA domain within the 50S subunits. Both P1 and P2, together with P0 and eL12, were required to activate ribosomes in polyphenylalanine synthesis dependent on eucaryotic elongation factors as well as eEF-2-dependent GTPase activity. The results suggest that formation of the P1–P2 heterodimer is required for subsequent formation of the P0(P1–P2)2 complex and its functional rRNA binding in silkworm ribosomes.

INTRODUCTION

It is generally accepted that ribosomal proteins modulate the structure and function of rRNAs (1). The acidic stalk protein complex, L10.L7/L12 in procaryotes, binds to a region around residues 1030–1127 in domain II of 23S rRNA, termed the GTPase-associated domain (2–4), and constitutes a part of the functional center, termed the GTPase center (5) or factor-binding center (6). Acidic L7/L12 protein (L7 differs from L12 by an acetylated N-terminus) is an important component of this functional center (7), although there is no evidence for its direct binding to RNA. This protein has characteristic properties: there are four copies, two homodimers, of L7/L12 per ribosome (8); they are flexible in the ribosome (9–11). The four copies of L7/L12 bind to L10 and form the pentameric complex (12); the L10 moiety of the complex appears to bind directly to the rRNA domain (13). Although it is well known that L7/L12 within the GTPase center participates in interaction of the ribosome with translation factors (G-proteins) (7,14), the functional significance of the four copies has been poorly understood (15–17).

The structure and function of the acidic phosphoproteins in eucaryotic ribosomes have been investigated mainly in rat (18–20), human (21), Artemia salina (22) and yeast (23,24). Animal ribosomes contain two kinds of the acidic proteins, P1 and P2 (reviewed in 25), whereas yeast ribosomes contain two P1-type proteins, P1α and P1β, and two P2-type proteins, P2α and P2β (24). These proteins bind to P0 protein, the equivalent of Escherichia coli L10, and presumably form the pentameric complex in the ribosome (22,26), designated here P0.P1/P2. The P0.P1/P2 complex binds to the GTPase-associated domain of 28S rRNA (27) and plays a crucial role in kingdom-specific interaction between 80S ribosomes with eucaryotic elongation factor 1α (eEF-1α) and elongation factor 2 (eEF-2) (28).

It is interesting that the eucaryotic ribosomes have more than two kinds of acidic proteins, unlike procaryotic ribosomes. There are two major interpretations for the states of P1 and P2 in the ribosome: (i) P1 and P2 form homodimers, as suggested by crosslinking (22,29); or (ii) they form heterodimers, suggested by yeast two-hybrid system (26,29,30) and the other genetic and biochemical studies (26,31–33). Isolated P1 and P2 proteins have a tendency to form oligomeric complexes (29), which may occur because of non-specific interactions. The instability of free P1 and P2 proteins in solution seems to bring ambiguity to the biochemical data. Moreover, the insolubility of isolated P0 protein makes in vitro binding experiments difficult.

We here used the ribosomal proteins from the silkworm Bombyx mori; proteins P1, P2 and P0 could be isolated in soluble form and were used for a study on their interaction in vitro. We show that formation of a P1–P2 heterodimer is significant for the subsequent assembly of a pentameric complex, probably in the form P0(P1–P2)2, and then its rRNA...
binding. To evaluate the in vitro binding data on the basis of ribosome function, we used a hybrid ribosome system developed recently (28), in which E. coli L10.L7/L12 complex and L11 on the 50S subunit were replaced with the eucaryotic counterparts P0.P1/P2 complex and eL12, respectively. The functions of the hybrid ribosomes carrying the silkworm ribosomal proteins were tested with eucaryotic elongation factors. The results provide evidence that formation of P1–P2 heterodimers is crucial for their binding to P0 and the subsequent interaction with the GTPase-associated domain of rRNA, from which derives factor-dependent ribosome function. We discuss an additional functional role of the P1–P2 heterodimer as a modulator of the RNA-binding protein P0.

MATERIALS AND METHODS

Silkworm ribosomes and ribosomal proteins

The high KCl/purumycin-treated 80S ribosomes were isolated from posterior silk glands of last instar larvae of B. mori (strain C132), according to the method previously described (34). Total proteins were extracted from the ribosome in 66% acetic acid, 33 mM MgCl2 and recovered by precipitation with 7 vol of cold acetone. The proteins were dialyzed against buffer A containing 20 mM sodium acetate, pH 4.5, 7.5 M urea and 5 mM 2-mercaptoethanol and then applied to a column of CM-cellulose (Whatman) equilibrated with the same buffer. Proteins were successively eluted with buffer A containing increasing concentrations of LiCl; P1, P2 and P0 were eluted in solutions containing 0, 0.05 and 0.08 M LiCl, respectively. The protein fractions were concentrated with Centricon YM-10 (Amicon). P1 and P2 fractions were dialyzed against buffer B containing 20 mM sodium phosphate, pH 6.5, 6 M urea, 100 mM LiCl, 5 mM 2-mercaptoethanol and further purified by ion exchange high performance liquid chromatography (HPLC) with DEAE-5PW (Tosoh) in a linear gradient of 100–300 mM LiCl. The P0 fraction was dialyzed against buffer C (buffer B except that the concentration of LiCl was 50 mM) and purified by HPLC with CM-5PW (Tosoh) in a linear gradient of 50–250 mM LiCl. The identities of the P1, P2 and P0 proteins were confirmed by reactivity with anti-P monoclonal antibody (35). P1 and P2 were also tested for partial amino acid sequencing using a protein sequencer (model PPSQ-21; Shimadzu).

Complex formation of P0, P1 and P2

Isolated proteins in the presence of 6 M urea were mixed together at a molar ratio of P0:P1:P2 of 1:3:3 and dialyzed against 0.3 M KCl, 20 mM Tris–HCl, pH 7.6, at 0°C. The same dialysis was also performed in the absence of P1 or P2. The P1–P2 complex was formed by mixing various ratios of P1 and P2 in 0.3 M KCl, 20 mM Tris–HCl, pH 7.6, at 0°C. Complex formation was confirmed by 6% native PAGE (acylamide/bisacylamide ratio 40:1) at 6.5 V/cm with a buffer system containing 5 mM MgCl2, 50 mM KCl and 50 mM Tris–HCl, pH 8.0. Samples were electrophoresed for 10 h at constant voltage and 4°C with buffer recirculation. The gel was stained with Coomasie Brilliant Blue. Bands of the complexes were cut out of the gel and the constituents were separated by SDS–PAGE as described by Laemmli (36), except that the gel contained 22% polyacrylamide and 0.44% bisacrylamide to improve separation of P1 and P2. The P0, P1 or P2 proteins on the gel were identified by immunoblotting using an anti-P monoclonal antibody that cross-reacts with the silkworm proteins. In some experiments, the P0.P1/P2 complex was further purified by gel filtration with G-3000SWXL (Tosoh) in a solution consisting of 100 mM KCl, 0.2 mM dithiothreitol, 20 mM Tris–HCl, pH 7.6.

Gel retardation assays

RNA fragments containing residues 1030–1127 of E. coli 23S rRNA and the equivalent region of B. mori 28S rRNA were synthesized with SP-6 RNA polymerase using cDNA as template (34). The transcripts were purified by gel filtration on a Sephadex G-50 column (Amersham Pharmacia). A solution (10 µl) containing 5 pmol [32P]RNA fragments, 20 mM MgCl2, 0.3 M KCl and 20 mM Tris–HCl, pH 7.6, was preincubated at 65°C for 5 min and then cooled to 30°C over 10 min. After the addition of a protein sample as indicated in the legend to Figure 5, the mixture was further incubated at 30°C for 10 min. RNA–protein binding was examined by 6% native PAGE under the same conditions as described above.

Functional assay using the hybrid system

Escherichia coli ribosomes deficient in L11 were obtained from strain AM68 (37), as described previously (28). The 50S subunits were incubated in a solution containing 50% ethanol and 0.5 M NH4Cl to remove specifically the L10.L7/L12 complex, as described in a previous report (28). The 50S core subunits deficient in L10.L7/L12 and L11 thus obtained were used to study function of the P0.P1/P2 complex. The 50S core subunits were incubated with the silkworm ribosomal proteins P0.P1/P2 (or samples without P1 and/or P2) and eL12, as described in the legend to Figure 7. The resultant E.coli–silkworm hybrid 50S subunit (0.13 µM) was tested for eucaryotic eEF-2-dependent GTPase activity in a solution (20 µl) containing 0.38 µM 30S subunit, 0.25 µM eEF-2, 150 µM [γ-32P]GTP, 10 µM MgCl2, 50 mM NH4Cl, 20 mM Tris–HCl, pH 7.6, and 0.2 mM dithiothreitol. The reaction was performed at 37°C for 10 min. Polyphenylalanine synthetase activity was assayed in a solution (100 µl) containing 0.1 µM hybrid 50S subunit, 0.5 µM 30S subunit, 10 µg poly(U), 0.4 µM E.coli [14C]Phe-tRNA (with 80 µg deacylated total tRNA), 200 µM GTP, 10 mM MgCl2, 75 mM NH4Cl, 50 mM Tris–HCl, pH 7.6, 0.2 mM dithiothreitol and 800 µg silk gland cytosol fraction as a source of elongation factors that was obtained by ammonium sulfate precipitation (40–60%) of the S200 fraction. The reaction was performed at 37°C for 10 min.

Chemical crosslinking

A mixture of P1 and P2 (9.5 nmol each) in 220 µl of 100 mM KCl, 20 mM triethanolamine HCl, pH 7.6, was incubated at 30°C for 10 min. After cooling to 0°C, the proteins were crosslinked with 18 mM 2-iminothiolane as described by Kenny et al. (38). The crosslinked sample was dialyzed against a solution containing 20 mM sodium phosphate, pH 6.5, 7.5 M urea, 100 mM LiCl, 5 mM iodoacetamide and loaded onto a column of DEAE-5PW (Tosoh) equilibrated with the same solution without iodoacetamide. Proteins were separated with a linear gradient of 100–300 mM LiCl (see Fig. 2A). Each fraction was concentrated with Centricon YM-10 (Amicon) and analyzed by SDS–PAGE under non-reducing and reducing conditions.
Analytical ultracentrifugation

Sedimentation equilibrium studies were performed with a Beckman Optima XL-I analytical ultracentrifuge using a double-sector centerpiece and sapphire windows, at three rotor speeds (12 000, 15 000 and 18 000 r.p.m.) and at 20°C. The sample was prepared as mentioned above. Absorbance scans at 280 nm were measured in the radial step mode at 0.001 cm intervals and data were collected taking the average of 16 measurements at each radial distance. Approach to equilibrium was considered to be complete when replicate scans separated by ≥6 h were indistinguishable. The partial specific volume of the protein was assumed to be 0.73 ml/g and the density of the solvent was assumed to be 1.00 g/ml. Analysis of the data was carried out utilizing the program Origin 4.1.

RESULTS

In vitro interaction between isolated silkworm P1 and P2

Animal ribosomes contain two kinds of the L7/L12-like acidic proteins, P1 and P2, unlike procaryotic ribosomes. We purified these proteins from ribosomes of the silkworm B.mori and, in addition, P0, corresponding to procaryotic L10. The identities of these proteins were confirmed by reactivity with monoclonal anti-P antibody (data not shown) and partial amino acid sequencing. The N-terminal sequence of P2 was MRYVAAYLLAVLGGKTTPAA, which is 70% identical to rat P2 (19) and 85% identical to Drosophila melanogaster (39). For P1, we used a peptide produced by V8 protease digestion (40), since the N-terminus of P1 was blocked, as in yeast (41). The sequence was LACVYSALIL, which is 90% identical to residues 7–16 of rat P1 (19) and 70% identical to the same residues of D.melanogaster (42). Isolated P0, P1 and P2 were soluble in a solution containing 0.3 M KCl in the absence of urea.

Isolated P1 and P2 showed characteristic mobilities in native PAGE (Fig. 1A, lanes 1 and 2). A smearing of the P1 sample (lane 1) may be due to instability of this protein under these electrophoresis conditions. By mixing P1 with P2, a new protein band appeared (lane 3) not detected in isolated P1 and P2. Unexpectedly, the gel mobility of the new band was faster than that of isolated P1 and P2 and its mobility did not change regardless of different molar ratios of added P1 and P2 (Fig. 1B). The new band was cut out of the gel and protein constituents were analyzed by SDS–PAGE, followed by immunoblotting using an anti-P monoclonal antibody that reacts with all of P0, P1 and P2 (Fig. 1C). This band contained P1 and P2 (lane 3), suggesting the formation of a complex composed of P1 and P2. Relative intensity between immunostained P1 and P2 components of the complex formed in vitro was apparently comparable with that of the proteins in intact ribosomes (lane 4). To confirm formation of a P1–P2 heterodimer, chemical crosslinking was performed. The P1/P2 mixture crosslinked with 2-iminothiolane was fractionated by ion exchange HPLC (Fig. 2A). Each fraction was analyzed by SDS–PAGE under non-reducing conditions (Fig. 2B). A crosslinked protein complex suggesting a dimer (28 kDa) was detected only in fraction H (lane 8). Crosslinked oligomers suggesting a trimer or tetramer were not formed. On reducing the 28 kDa complex (Fig. 2C), it separated into two protein components (lane 3) corresponding to P1 (lane 2) and P2 (lane 1), detected by immunoblotting analysis. No formation of either P1 or P2 homodimer was detected in this experiment.

Binding of P1–P2 heterodimer to P0

We then investigated complex formation with isolated silkworm P0, P1 and P2 using native PAGE (Fig. 3A). Although isolated P0 (lane 1) did not run in the gel under the conditions used, complex formation was deducible from the appearance of a new complex band or disappearance of the bands for free P1 (lane 2), detected by immunoblotting analysis. No formation of either P1 or P2 homodimer was detected in this experiment.
Although the complex composed of P0, P1 and P2 has been reconstituted in vitro with proteins from human (21) and rat (27,32), the stoichiometry of the constituents has not yet been established.

From sedimentation equilibrium experiments, a weight-average molecular weight is estimated by the following equation (43).

\[ M_{\text{app}} = \frac{2RT}{(1 - \bar{\rho})\omega^2} \left( \frac{d \ln c}{d r^2} \right)^{-1} \]

where \( r \) is the radius, \( c \) is the concentration of the sample, \( \bar{\rho} \) is the partial specific volume of the sample, \( \rho \) is the density of the solvent, \( \omega \) is the angular velocity of the rotor (in radians/s), \( R \) is the universal gas constant, \( T \) is the absolute temperature and \( M_{\text{app}} \) is the apparent molecular weight.

Thus, the absorbance at a specified wavelength and position in the solution column should be given by

\[ A(r) = A(r_0) \exp \left[ M_{\text{app}} H \left( r^2 - r_0^2 \right) \right] \]

where \( A(r) \) represents the absorbance at radius \( r \) and \( A(r_0) \) is the absorbance at \( r_0 \), the radius at the meniscus, and

\[ H = \frac{[(1 - \bar{\rho})\omega^2]/2RT} \]

Figure 4 shows the results of the sedimentation equilibrium experiments on the P0.P1/P2 complex at 12 000 r.p.m. Considering the facts that the P0.P1/P2 complex was prepared as a fraction with a single symmetrical peak on the gel filtration (data not shown) and that the complex gave a single band in native PAGE, we assume the P0.P1/P2 complex to be a single species. Figure 4 shows the non-linear least squares fitting with this assumption. The symmetrical residuals and the small range of 95% confidence intervals as shown in Figure 4 support this assumption. The non-linear least squares fitting with equation 2 gave the apparent molecular weight as 7.80 ± 0.39 × 10^4. All the results with various rotor speeds (12 000, 15 000 and 18 000 r.p.m.) gave similar values within the range of experimental error, showing that the further association of solute is not detectable in this range of protein concentrations. The apparent molecular weight is very close to the expected value (80 126) of a complex of P0:P1:P2 (1:2:2) considering the molecular weights of P0 (34 148), P1 (11 451) and P2 (11 538). The results from these sedimentation experiments, together with data from gel analyses (Figs 1–3), strongly suggest that the reconstituted P0.P1/P2 complex is composed of two P1–P2 heterodimers and a monomeric P0, i.e. P0(P1–P2)_2.
**Figure 5.** Binding of P0, P1 and P2 protein mixtures to GTPase-associated RNA domain. The 32P-labeled RNA fragment (5 pmol) containing residues 1030–1127 (E.coli numbering) of *B.mori* wild-type 28S rRNA (A) and its U1094/A1098 variant (B) were incubated in 10 µl of a solution without protein (lane 1) or with a mixture of P1 and P2 (87 pmol each, lane 2), P0 (29 pmol, lane 3) and the mixtures P0 (29 pmol) + P1 (87 pmol) (lane 4), P0 (29 pmol) + P2 (87 pmol) (lane 5) and P0 (29 pmol) + P1 (87 pmol) + P2 (87 pmol) (lane 6). The samples were analyzed by gel retardation, as described in Materials and Methods. (C) The *B.mori* U1094/A1098 variant (5 pmol) was incubated in 10 µl of a solution with increasing amounts of isolated P0: 0 (lane 1), 29 (lane 2), 58 (lane 3) and 120 pmol (lane 4). The samples were analyzed by the same gel retardation assay as in (B).

**RNA binding of P0.P1/P2 complex**

It has been shown that the P0.P1/P2 complex binds to the GTPase-associated domain of 28S rRNA, corresponding to residues 1030–1127 of *E.coli* 23S rRNA (27), presumably through the P0 moiety. To examine the importance of P1 and P2 in RNA binding, the experiment was performed in the absence of either of these proteins using the silkworm RNA fragment (Fig. 5A). Both P1 and P2, as well as P0, were required for binding to the RNA (lane 6). No RNA binding was detected with P1–P2 heterodimer (lane 2), P0 alone (lane 3), the P0–P1 pair (lane 4) or the P0–P2 pair (lane 5). This experiment was repeated using the U1094/A1098 RNA variant, instead of the wild-type C1094/G1098 RNA, as RNA probe, because the structure of the silkworm wild-type RNA is labile and its protein-binding ability is low compared with the U1094/A1098 RNA variant (34). The same results were obtained (Fig. 5B), except that only very weak binding was detected for the P0–P2 pair (lane 5) using the RNA variant. To confirm that P0, not P1/P2, binds directly to the RNA, a large excess of isolated P0 was added to the U1094/A1098 RNA. A faint complex band appeared when 24-fold P0 was added to the RNA (Fig. 5C), although a P1/P2 mixture of the same amounts showed no binding (data not shown). A stable RNA–protein complex was observed only in the presence of all of P0, P1 and P2, suggesting that the P1–P2 heterodimers greatly increased the binding affinity of P0 to the RNA.

**Functional properties of P0.P1/P2 complex**

It is important to evaluate the present *in vitro* binding data in the aspect of ribosome function. To test the function of P0, P1 and P2 proteins, we recently developed a useful hybrid system in which *E.coli* L10.L7/L12 and L11 bound to the GTPase-associated domain of 23S rRNA were replaced with rat P0.P1/P2 complex and el12 on the 50S subunit. Accessibility of the hybrid ribosome to eucaryotic elongation factors eEF-1α and eEF-2 has been established (28). We attempted to use this hybrid system to study the function of the silkworm ribosomal proteins. For this purpose, the prepared silkworm samples must cross-bind to the *E.coli* GTPase-associated RNA domain. As shown in Figure 6, silkworm P0.P1/P2 (lane 2) and L11-like protein el12 (lane 2) as well as both the proteins (lane 4) bound strongly to the *E.coli* RNA.

The silkworm protein samples were added to the core *E.coli* ribosomes deficient in L10.L7/L12 and L11 (28) and tested for function (Fig. 7). The hybrid ribosomes containing silkworm P0.P1/P2 and el12 showed eucaryotic eEF-2-dependent GTPase activity (Fig. 7A) and polyphenylalanine synthesis (Fig. 7B). These activities were comparable with those of the previous hybrid sample containing rat ribosomal proteins (28). Removing either P1 or P2 markedly reduced both the activities. In the absence of P1, addition of P0/P2 in 5-fold excess to the *E.coli* 50S subunit core gave no effect on the GTPase activity (Fig. 7A). Likewise, addition of excess amounts of P0/P1 did not recover the activity in the absence of P2. In the absence of P0, addition of P1/P2 gave no stimulation of the activity (data not shown).

To test whether the activity of the hybrid ribosome is due to binding of the silkworm proteins to the GTPase-associated RNA domain within the *E.coli* ribosome, a competition study was performed using the *E.coli* RNA fragment used in the binding assay (Fig. 6) as a competitor. On addition of the RNA competitor, polyphenylalanine synthetic activity of the ribosome sample was reduced to ∼35% of the original activity (Fig. 7C). Addition of RNA itself showed no effect on polyphenylalanine synthesis by the intact silkworm ribosomes. The results indicate that the activity of the hybrid ribosomes was caused by interaction between the silkworm ribosomal proteins and the *E.coli* GTPase-associated RNA domain.

**DISCUSSION**

The structure of the ribosomal GTPase center within the large subunit is not resolved well by the current X-ray crystallography (44,45), probably because of its flexible nature. Many lines of biochemical evidence, however, indicate that the pentameric acidic protein complex L10(L7/L12)2(L7/L12)x binds to the GTPase-associated domain of 23S rRNA and constitutes a major part of the functional center in procaryotic systems.
The eucaryotic proteins P0 and P1/P2 are the counterparts of procaryotic L10 and L7/L12, respectively. We here investigated interactions among silkworm P0, P1 and P2 by using the isolated proteins and showed that a P0:P1:P2 (molar ratio 1:2:2) pentameric complex was reconstituted in vitro, which appears to be a functional unit activating ribosomes by its binding to the GTPase-associated RNA domain. A major difference between the eucaryotic and procaryotic acidic proteins is that there are two types, P1 and P2 (three types in some plants; 46), of the proteins in eucaryotes that are expressed from different genes (19,21,24).

All of the present results lead to the conclusion that formation of a P1–P2 heterodimer in vitro (Figs 1 and 2). The P1–P2 heterodimer seems to be compact and stable, because the band of the dimer is distinct on native electrophoretic gels and its mobility is higher than isolated P1 and P2 (Fig. 1A). Formation of the heterodimer is consistent with previous data from different approaches (26,29,30,31,33). All of the present results lead to the conclusion that formation of the P1–P2 heterodimer is a key step in assembly of the P0(P1–P)2 pentameric complex and its rRNA binding to constitute the silkworm ribosomal GTPase center.

Binding of P0 to the GTPase-associated RNA domain appears to be the most crucial step linked directly to ribosome function. It is likely that the RNA-binding site lies in the N-terminal half of P0 protein (47,48) and the P1/P2-binding site in the C-terminal region (24) (Fig. 8). However, it has been very hard to investigate the molecular details of the RNA-binding mechanism of P0, because of its instability in the isolated state. Although rat P0 protein has been prepared from ribosomes (27) and by overexpression in E.coli cells (32), the isolated protein samples are insoluble without P1 and P2. In the present studies, we could isolate P0 protein in a soluble state from silkworm ribosomes. The amino acid sequence of silkworm P0 shows 69% identity with rat P0 (Fig. 8). One or some of the divergent parts of the molecule seem to contribute to the solubility of silkworm P0. From the fact that the very low RNA-binding ability of isolated P0 is significantly increased by addition of both P1 and P2 (Fig. 5), it is conceivable that the conformation of the RNA-binding site of P0 apparently changes on binding of P1–P2 heterodimers to the C-terminal side of the molecule. This allosteric conformational change induced by P1–P2 heterodimers may be important for RNA binding and ribosome function. We propose here that the P1–P2 heterodimer, but neither P1 nor P2 alone, plays a role in modulating the structure and function of P0 protein at least in the case of silkworm proteins.

There is a major difference between the present study and that of yeast mutants. The yeast mutant deficient in P1 and P2 was made by disruption of genes for both proteins (for P1α, P1β, P2α and P2β) (24). This P1/P2-deficient yeast ribosome retained P0 protein and showed a reduced level of polyphenylalanine synthetic activity. Growth of this strain was 3-fold slower compared with the wild-type (24). In contrast, our results indicate that isolated silkworm P0 hardly binds to the GTPase-associated RNA domain without P1 and P2 and does not activate the ribosome. One explanation for this discrepancy may be the difference in experimental conditions between in vitro and in vivo studies. In yeast cells, there may be components compensating for the P1–P2 modulation of P0. A certain ribosomal protein other than P1/P2 may play such a role. A second possible explanation is species differences. Unlike animal P0, yeast P0...
may bind to the RNA without the help of other proteins, although this has not yet been tested. Modes of interaction between P0 and P1/P2 and their functional significance may have diverged during evolution. In fact, identity of amino acid sequence around the P1/P2-binding region (28.6% identity) on P0 is lower than that of full-length P0 (44.2%) as well as the sequence around the P1/P2-binding region (28.6% identity) on E.coli (positions 44–67), which is most likely to participate in RNA binding (47), and the P1/P2-binding region (positions 182–293) (24) are indicated.

Some possible models of protein topography of P0, P1 and P2 on animal ribosomes have been presented (32): (i) P1–P1 and P2–P2 homodimers bind to P0 so that the two dimers come close to each other; (ii) as (ii), but the two dimers do not come close to each other. Our present results strengthen the idea of heterodimer formation and support model (ii) or (iii). The two E.coli L7/L12 homodimers are known to bind very close to each other on the C-terminus of L10 (17); if the same is true in animal ribosomes, then model (ii) may be the actual molecular arrangement. Previous P1–P1 and P2–P2 crosslinking with 2-iminothiolane (22) may be explained by model (ii).


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REFERENCES