The stalk protein L12 (generally referred to as L7/L12) is the only multi-copy protein in bacterial ribosomes (1, 2). It is involved in the interaction of the ribosome with the elongation factors EF-G\(^2\) and EF-Tu, activation of the GTPase activity of both factors, and associated reactions during translation (3–6). L12 is comprised of three regions: the N-terminal domain, which participates in the dimerization of L12 (for review see Ref. 7) and its binding to L10 (for review see Ref. 8), the globular C-terminal domain (CTD) (9, 10), which interacts with the elongation factors (11–14), and a hinge region, which connects the two domains (15, 16). It was generally accepted that, in the bacterial ribosomal large subunit, two L12 dimers bind to L10 to form a pentameric complex, L10•(L12)\(^2\)•(L12)\(^2\) (7, 17). Recent studies, however, have demonstrated that some bacterial and archaeal species contain ribosomes with three L12 dimers that form a heptameric complex L10•(L12)\(^2\)•(L12)\(^2\)•(L12)\(^2\) (6, 18, 19). This finding opened a new stage in the discussion as to the significance of multiple stalk dimers in the ribosome.

The L10-L12 stalk protein complex constitutes a highly flexible region in the ribosome (8, 20–25), and its detailed structure within the ribosome has not been resolved by x-ray crystallography (26–29). However, some structural features of isolated L12 have been determined. An earlier study of *Escherichia coli* L12 by x-ray crystallography revealed the structure of the CTD to be comprised of three \(\alpha\) helices (\(\alpha_4\), \(\alpha_5\), and \(\alpha_6\)) and a central three-stranded \(\beta\) sheet (10). This structure was confirmed by a recent cryo-crystallographic study of L12 from *Thermotoga maritima* (30) and also by NMR analyses of E. coli L12 (16). More recently, the crystal structure of the complex between the N-terminal domain (helices \(\alpha_1\) and \(\alpha_2\)) of L12 and L10 from *T. maritima* was elucidated using a reconstituted sample (6). In this complex, helices \(\alpha_1\) and \(\alpha_2\) of the L12 N-terminal domain participate in binding to the long C-terminal helix \(\alpha_8\) of L10, as well as in the formation of the homodimer. Three L12 dimers bind in the same manner, side by side, to the C-terminal helix of L10 (6). The hinge region (\(\alpha_3\)) of each L12 monomer seems to adopt a random coil structure, which is supported by the NMR data (20, 21, 24).

Although it has long been known that the CTD of L12 participates in EF-G-dependent GTP hydrolysis (6, 7), the detailed mechanism of the GTPase activation is not fully understood. Recent cryo-electron microscopy data showed that the L12 CTD directly interacts with the \(G^*\) domain of EF-G (13). Site-directed mutagenesis and kinetic studies suggested that helices...
sequences for *T. thermophilus* ribosomal proteins L10 (Tt-L10) and L12 (Tt-L12) were inserted into the expression vectors pGEX6P-1 (GE Healthcare) and pET11c (Novagen), respectively. A chimeric L10 protein (Ec-Tt-L10), which was composed of residues 1–132 of Ec-L10 fused with residues 132–172 of Tt-L10 (see Fig. 2A), was constructed using PCR (36) and cloned into pET28c (Novagen). Chimeric L12 proteins were also prepared: TrEc-L12, which comprises residues 1–36 of Tt-L12 fused with residues 37–120 of Ec-L12, and Ec-Tt-L12, which comprises residues 1–36 of *E. coli* L12 fused with residues 37–124 of Tt-L12 (see Fig. 2A), were cloned into pET28c and pET3a, respectively. The other chimeric L12 proteins that were constructed in this study were Ec-α3Tt-L12, Ec-α4Tt-L12, Ec-α5Tt-L12, and Ec-α6Tt-L12, which comprise Ec-L12 in which residues 37–50 (from α3), residues 63–77 (from α4), residues 80–94 (from α5), and residues 100–113 (from α6), respectively, were replaced with the equivalent regions from Tt-L12 (see Fig. 5). These chimeric L12 proteins were cloned into pET3a.

**Protein Expression and Purification**—Proteins were expressed in *E. coli* strain BL21 (DE3) pLysS (Novagen) by the addition of isopropyl β-D-thiogalactopyranoside. The cells were lysed by grinding with alumina and then resuspended in buffer A, which contained 20 mM Tris-HCl (pH 7.6), 10 mM MgCl₂, 20 mM NH₄Cl, and 5 mM 2-mercaptoethanol (17). All of the proteins, except Ec-Tt-L10 and Tt-L10, were obtained in the soluble (S100) fraction. The S100 fractions that contained the proteins were dialyzed against buffer B, which contained 20 mM NaOAc (pH 4.5), 7 mM urea, and 5 mM 2-mercaptoethanol and then applied to a column of CM-cellulose (Whatman) that had been equilibrated with the same buffer. Ec-L12, Ec-Tt-L12, TtEc-L12, Ec-α3Tt-L12, Ec-α4Tt-L12, Ec-α5Tt-L12, and Ec-α6Tt-L12 were all eluted in the flow-through fraction, whereas Ec-L10, Ec-L11, and Tt-L11 were eluted in a stepwise manner using buffer B containing 0.04–0.12 M LiCl. Individual proteins that had been eluted in the flow-through fraction were dialyzed against buffer C, which consisted of 20 mM Tris-HCl (pH 8.0), 6 mM urea, and 5 mM 2-mercaptoethanol and then purified using an AKTA fast protein liquid chromatography system with a Resource Q column (GE Healthcare) and a linear gradient of 0–200 mM LiCl in buffer C. The fractions that contained Ec-L10, Ec-L11, and Tt-L11 were individually dialyzed against buffer D, which consisted of 20 mM NaOAc (pH 5.0), 6 mM urea, and 5 mM 2-mercaptoethanol and purified using an AKTA fast protein liquid chromatography system with a Resource S column (GE Healthcare) and a linear gradient of 0–200 mM LiCl in buffer D. The S100 fraction that contained Tt-L12 was heated to 70 °C for 10 min to remove the *E. coli* proteins, and the Tt-L12 protein was then purified in a single step by DE-cellulose column chromatography (Whatman) in buffer C. The insoluble GST-Tt-L10 and Ec-Tt-L10 proteins were solubilized in 8 M urea and purified by the same column chromatography as Ec-L10, described above. The purity of the proteins was confirmed by SDS-polyacrylamide gel electrophoresis (see Fig. 2B).

**Formation of the L10+L12 Stalk Complex**—The purified L10 and L12 proteins were individually dialyzed against buffer E, which consisted of 20 mM Tris-HCl (pH 7.6), 300 mM KCl, and 5 mM 2-mercaptoethanol. An L10 protein (Ec-L10, GST-Tt-L10, and GST-L12) was expressed and purified as described above. To construct the L10+L12 stalk complex, excess GST-Tt-L10 was added to a solution containing Ec-L10 and GST-L12 at a ratio of 1:1. The solution was dialyzed against buffer E, and the complex was purified by gel filtration. The purity of the complex was confirmed by SDS-polyacrylamide gel electrophoresis.

**EXPERIMENTAL PROCEDURES**

**Plasmid Construction**—The coding regions for *E. coli* ribosomal proteins L10, L11, and L12 (designated hereafter as Ec-L10, Ec-L11, and Ec-L12, respectively) and for *T. thermophilus* L11 (Tt-L11) were inserted into the *E. coli* expression vector pET7a (Novagen) and cloned, as described previously (35). The coding
L10, or Ec-Tt-L10) was mixed with an L12 protein (Ec-L12, Tt-L12, or one of the chimeric L12 proteins) in buffer E at a molar ratio of 1:8. The complexes were formed by incubation at 37 °C for 5 min. In the case of the complex that contained GST-Tt-L10, the GST moiety was removed by digestion with PreScission Protease (GE Healthcare) according to the manufacturer’s instructions and subsequent heating at 70 °C for 30 min. The complexes that formed were analyzed by 6% polyacrylamide (acrylamide/bisacrylamide ratio 39:1) native gel electrophoresis at 6.5 V/cm with a buffer system that contained 50 mM Tris-HCl (pH 7.6), 10 mM MgCl2, 60 mM NH4Cl, and 5 mM 2-mercaptoethanol at 37 °C for 5 min. The resulting ribosome samples were assayed for GTPase activity in the presence of E. coli EF-G.

FIGURE 1. Higher EF-G-dependent GTPase activity is obtained with the reconstituted L10-L12 complex and L11 from T. thermophilus than with their counterparts from E. coli. 50 S core particles (2.5 pmol) that lacked L11, L10, and L12 were preincubated with L11 (10 pmol) and the L10-L12 complex (10 pmol) from T. thermophilus (Tt) or E. coli (Ec), together with 30 S subunits (7.5 pmol) at 37 °C for 5 min. The resulting ribosome samples were assayed for GTPase activity in the presence of E. coli EF-G.

Functional Assays with the Chimeric Ribosomal Stalk Complex

**Results**

High EF-G-dependent GTPase Turnover Activity with the Reconstituted T. thermophilus Stalk Protein Complex—Mixing of the purified wild-type Tt-L10 and Tt-L12 proteins resulted in the formation of a stable complex (33). This L10-L12 complex, together with Tt-L11, was assembled onto E. coli 50 S core particles that were deficient in the equivalent proteins (38). The functionality of the T. thermophilus proteins was assessed by measuring EF-G-dependent GTP hydrolysis. The activity was compared with that obtained with E. coli proteins that had been reconstituted under the same conditions (Fig. 1). The T. thermophilus L10-L12 complex, together with Tt-L11, yielded ribosomes with 2.5-fold higher activity than that of ribosomes that had been reconstituted with Ec-L10-L12 and Ec-L11. This higher activity seems to be mainly due to the T. thermophilus...
Functional Assays with the Chimeric Ribosomal Stalk Complex

L10-L12 complex, because in the presence of Ec-L11, this complex still gave more than 2-fold higher activity than that of *E. coli* reconstituted ribosomes, whereas the Ec-L10-Ec-L12 complex in the presence of Tt-L11 did not markedly stimulate the GTPase activity. It is therefore likely that the higher activity is due to a structural feature of the Tt-L10-Tt-L12 complex. This might be either the presence of three Tt-L12 dimers or other structural elements that are found in each L12 molecule.

Construction of Chimeric L10 and L12 Proteins from the *E. coli* and *T. thermophilus* Orthologs—Sequence comparisons show that the C-terminal α8 region of Tt-L10 contains an additional stretch of amino acids that is absent in Ec-L10. It has been suggested that this extra region is involved in the binding of the third Tt-L12 dimer (6, 18). We recently confirmed by biochemical approaches that the α8 region of Tt-L10 is involved in the binding of the three Tt-L12 dimers (33). To characterize the structure and function of the *T. thermophilus* heptameric complex Tt-L10(Tt-L12)2(Tt-L12)2(Tt-L12)2, we prepared constructs that allowed the expression in *E. coli* of Tt-L10, Ec-L10, and a chimeric L10 protein, in which the α8 region of Ec-L10 was replaced with that of Tt-L10 (Ec-Tt-L10) (Fig. 2A). We also prepared constructs for the expression of Tt-L12, Ec-L12, and two chimeric L12 proteins: Ec-Tt-L12, in which the region that corresponded to α1 and α2 (i.e. the binding site for L10) in Tt-L12 was replaced with that of Ec-L12, and Tt-Ec-L12, in which the α1-α2 region of Ec-L12 was replaced with that of Tt-L12 (Fig. 2A). The proteins were expressed in *E. coli* cells and purified (Fig. 2B), as described under “Experimental Procedures.” Only the Tt-L10 protein was expressed and isolated as a GST-fusion protein, Tt-Ec-L12, did not cause this L12-dependent supershift (Fig. 2B). When a chimeric L10 and L12 protein was overexpressed in *E. coli* and purified, as described under “Experimental Procedures.” A, L10 and L12 proteins used in this study. Ec-L10, full-length *E. coli* L10 (residues 1–164); Tt-L10, full-length *T. thermophilus* L10 (residues 1–172); Ec-Tt-L10, a chimeric protein composed of residues 1–312 of *E. coli* L10 fused with residues 132–172 of *T. thermophilus* L10; Ec-L12, full-length *E. coli* L12 (residues 1–120); Tt-L12, full-length *T. thermophilus* L12 (residues 1–124); Ec-Tt-L12, a chimeric protein composed of residues 1–36 of *E. coli* L12 fused with residues 37–124 of *T. thermophilus* L12; Tt-Ec-L12, a chimeric protein composed of residues 1–36 of *T. thermophilus* L12 fused with residues 37–120 of *E. coli* L12. The *E. coli* and *T. thermophilus* sequences are shown in white and gray, respectively. Each protein was overexpressed in *E. coli* and purified, as described under “Experimental Procedures.” B, SDS-PAGE of purified protein samples. Lane 1, Ec-L10; lane 2, GST-Tt-L10; lane 3, Ec-Tt-L10; lane 4, Ec-L12; lane 5, Tt-L12; lane 6, Ec-Tt-L12; lane 7, Tt-Ec-L12. C, schematic illustration of putative assembly modes of the purified chimeric L10 and L12 proteins.

FIGURE 2. Construction and expression of chimeric L10 and L12 proteins derived from the *E. coli* and *T. thermophilus* orthologs. A, L10 and L12 proteins used in this study. Ec-L10, full-length *E. coli* L10 (residues 1–164); Tt-L10, full-length *T. thermophilus* L10 (residues 1–172); Ec-Tt-L10, a chimeric protein composed of residues 1–312 of *E. coli* L10 fused with residues 132–172 of *T. thermophilus* L10; Ec-L12, full-length *E. coli* L12 (residues 1–120); Tt-L12, full-length *T. thermophilus* L12 (residues 1–124); Ec-Tt-L12, a chimeric protein composed of residues 1–36 of *E. coli* L12 fused with residues 37–124 of *T. thermophilus* L12; Tt-Ec-L12, a chimeric protein composed of residues 1–36 of *T. thermophilus* L12 fused with residues 37–120 of *E. coli* L12. The *E. coli* and *T. thermophilus* sequences are shown in white and gray, respectively. Each protein was overexpressed in *E. coli* and purified, as described under “Experimental Procedures.” B, SDS-PAGE of purified protein samples. Lane 1, Ec-L10; lane 2, GST-Tt-L10; lane 3, Ec-Tt-L10; lane 4, Ec-L12; lane 5, Tt-L12; lane 6, Ec-Tt-L12; lane 7, Tt-Ec-L12. C, schematic illustration of putative assembly modes of the purified chimeric L10 and L12 proteins.

Binding of the chimeric stalk protein complexes to the *E. coli* 50 S core was confirmed by acrylamide/agarose composite gel electrophoresis (Fig. 3), in which the band of 50 S core particles (lane 1) was clearly shifted upward by the addition of Ec-L10 with Ec-L11 (lane 3) and supershifted by the addition of Ec-L12 together with Ec-L10 and Ec-L11 (lane 4). When a chimeric L12, Ec-Tt-L12, was added instead of Ec-L12, a similar supershift was observed (lane 5). However, another chimeric L12 protein, Tt-Ec-L12, did not cause this L12-dependent supershift (lane 6). When the chimeric Ec-Tt-L10 was used, with Ec-L11, the band shift of the 50 S core was observed (lane 7). Furthermore, the L12-dependent supershift was also observed when either Tt-L12 (lane 9) or Tt-Ec-L12 (lane 11) was added further.

FIGURE 2. Formation of Chimeric Stalk Protein Complexes—To investigate which structural feature of the *T. thermophilus* stalk complex Tt-L10-Tt-L12 is responsible for the higher GTPase activity, we used the chimeric L10 and L12 proteins, described above (Fig. 2A). By using Ec-Tt-L10 and Tt-Ec-L12, we would expect to form a heptameric complex (Fig. 2C), in which the functional regions for rRNA binding (the N-terminal domain of L10) and elongation factor binding (the C-terminal domain of L12) are composed of *E. coli* sequences, respectively. In contrast, by using Ec-L10 and Ec-Tt-L12, we would expect to form the pentameric complex, in which the factor binding site is composed of *T. thermophilus* L12 sequences (Fig. 2C).

Binding of the chimeric stalk protein complexes to the *E. coli* 50 S core was confirmed by acrylamide/agarose composite gel electrophoresis (Fig. 3), in which the band of 50 S core particles (lane 1) was clearly shifted upward by the addition of Ec-L10 with Ec-L11 (lane 3) and supershifted by the addition of Ec-L12 together with Ec-L10 and Ec-L11 (lane 4). When a chimeric L12, Ec-Tt-L12, was added instead of Ec-L12, a similar supershift was observed (lane 5). However, another chimeric L12 protein, Tt-Ec-L12, did not cause this L12-dependent supershift (lane 6). When the chimeric Ec-Tt-L10 was used, with Ec-L11, the band shift of the 50 S core was observed (lane 7). Furthermore, the L12-dependent supershift was also observed when either Tt-L12 (lane 9) or Tt-Ec-L12 (lane 11) was added further.
together with Ec-Tt-L10 and Ec-L11. However, this supershift was not observed upon the addition of Ec-L12 (lane 8) or Ec-Tt-L12 (lane 10). These data suggest that the chimeric complexes Ec-L10-Ec-Tt-L12, Ec-Tt-L10-Tt-L12, and Ec-Tt-L10-Tt-Ec-L12 were formed on the 50 S core particles, as expected.

Because the exact number of copies of the L12 variants within the reconstituted stalk complexes was not clear from the gel analysis (Fig. 3), we performed a quantitative analysis using L12 samples that had been radiolabeled with [14C]iodoacetamide at a single cysteine, as described under “Experimental Procedures.” 50 S ribosomal particles that had been reconstituted with L10 proteins contained 4.18 copies of Ec-L12 or 4.12 copies of Tt-Ec-L12, which implied that there were two

A single Cys was introduced by the A63C mutation in Ec-L12 or the A68C mutation in Tt-L12. Each mutant L12 was labeled with [14C]iodoacetamide, as described under “Experimental Procedures.” 50 S ribosomal particles that had been reconstituted with L10 proteins contained 4.18 copies of Ec-L12 or 4.12 copies of Tt-Ec-L12, which implied that there were two

| Reconstituted particles | Specific activity of L12 | Radioactivity bound/A350 | Copies of L12/particle |
|-------------------------|------------------------|--------------------------|-----------------------|
| 50 S core + Ec-L10 + Ec-L12 | 95 | 15306 ± 186 | 4.18 ± 0.05 |
| 50 S core + Ec-L10 + Tt-L12 | 111 | 14935 ± 1472 | 3.94 ± 0.35 |
| 50 S core + Ec-L10 + Ec-Tt-L12 | 124 | 19731 ± 681 | 4.12 ± 0.14 |
| 50 S core + Ec-L10 + Ec-Tt-Ec-L12 | 125 | 62152 ± 1235 | 5.26 ± 0.26 |
| 50 S core + Ec-Tt-L10 + Ec-L12 | 95 | 14448 ± 444 | 3.94 ± 0.12 |
| 50 S core + Ec-Tt-L10 + Tt-L12 | 111 | 26775 ± 1040 | 6.25 ± 0.24 |
| 50 S core + Ec-Tt-L10 + Ec-Tt-L12 | 124 | 24842 ± 1014 | 5.19 ± 0.21 |
| 50 S core + Ec-Tt-L10 + Ec-Tt-Ec-L12 | 125 | 30275 ± 783 | 6.27 ± 0.16 |
| 50 S core + Ec-L12 | 95 | 1137 ± 357 | 0.31 ± 0.10 |
| 50 S core + Tt-L12 | 111 | 807 ± 268 | 0.21 ± 0.06 |
| 50 S core + Ec-Tt-L12 | 124 | 1269 ± 389 | 0.27 ± 0.08 |
| 50 S core + Tt-Ec-L12 | 125 | 745 ± 221 | 0.25 ± 0.05 |

The amino acid sequence of the C-terminal moiety, which was responsible for the high GTPase activity, was compared between the T. thermophilus and E. coli proteins (Fig. 5A). There are four regions in which the amino acid sequence differs markedly between them. These divergent areas lie within four helical regions: α3, α4, α5, and α6, according to crystal structure data from isolated T. maritima L12 (30). It should be noted that α3 forms the hinge region. To identify the main region that is responsible for the high GTPase activity, the divergent areas in Ec-L12, which correspond to amino acids 43–47, 60–75, 87–94, and 101–113, were replaced with the corresponding amino acids, 43–52, 65–80, 92–98, and 105–117, respectively, from Tt-L12. These chimeric proteins were designated Ec-α3Tt-L12, Ec-α4Tt-L12, Ec-α5Tt-L12, and Ec-α6Tt-L12, respectively (Fig. 5B). These proteins were overexpressed and purified (Fig. 5C), as described under “Experimental Procedures,” and used for further GTPase assays. Only the ribosomes that had been reconstituted with Ec-α6Tt-L12, not the other chimeric proteins, showed 2-fold higher GTPase activity than that of the ribosomes that contained the wild-type Ec-L12 (Fig. 6A).

Characterization of the Interaction between the Ec-Tt-L12-containing Ribosome and EF-G—As shown in Fig. 4, ribosomes that contained the chimeric pentameric complex Ec-L10-Ec-Tt-L12 showed 2.5-fold higher GTPase activity than that of the wild-type E. coli ribosome. We further characterized the mode of interaction of the Ec-Tt-L12-containing ribosome with EF-G. The GTPase turnover of EF-G with the Ec-Tt-L12-containing ribosome was inhibited by the antibiotic fusidic acid in a manner similar to that with the wild-type E. coli ribosome.
Natural text:

**Functional Assays with the Chimeric Ribosomal Stalk Complex**

![Diagram](Image)

**DISCUSSION**

Rather than the pentameric stalk complex that is found in *E. coli*, Ec-L10(Ec-L12)\(^2\)(Ec-L12)\(^2\), *T. thermophilus* ribosomes contain a heptameric complex that is composed of six copies of the Tt-L12 protein with a single copy of Tt-L10, Tt-L10(Tt-L12)\(^2\)(Tt-L12)\(^2\) (18). Here we have characterized this complex by constructing chimeric L10 and L12 proteins. The chimeric Ec-Tt-L10 protein, in which the \(\alpha8\) region of Ec-L10 was replaced with the equivalent \(\alpha8\) region from Tt-L10, could bind six copies of Tt-L12. Moreover, Ec-Tt-L10 also bound six copies of Tt-Ec-L12, in which the \(\alpha1\) and \(\alpha2\) regions of Ec-L12 were replaced with the corresponding region from Tt-L12 (Table 1). The results clearly indicated that the C-terminal \(\alpha8\) region of Tt-L10 was responsible for the binding of all six copies of Tt-L12 and that the \(\alpha1\) and \(\alpha2\) regions of Tt-L12 participated in the binding to the \(\alpha8\) region of Tt-L10, as well as in the formation of the homodimer. Our biochemical data were consistent with previous crystal structure data for the L10-L12 complex of *T. maritima* from Diaconu *et al.* (6). Because the \(\alpha8\) region of Tt-L10 has an additional sequence that is absent in Ec-L10, it is likely that this extra sequence is the binding site for the third Tt-L12 dimer.

Understanding the relationship between the number of copies of L12/ribosome and ribosome function is of interest. Because the *T. thermophilus* heptameric complex, which contains six copies of L12, resulted in 2.5-fold higher EF-G-dependent GTPase activity than the *E. coli* pentameric complex (Fig. 1), we examined whether this higher activity was due to the presence of six copies of Tt-L12 or to elements within each Tt-L12 molecule. We showed that the activity obtained with a chimeric heptameric complex, Ec-Tt-L10(Tt-Ec-L12)\(^2\)(Tt-Ec-L12)\(^2\)(Tt-Ec-L12)\(^2\), was at a level comparable with that obtained with the *E. coli* pentameric complex, whereas the activity obtained with another chimeric pentameric complex, Ec-L10\(^2\)(Ec-Tt-L12)\(^2\)(Ec-Ec-L12)\(^2\), was comparable with that obtained with the *T. thermophilus* heptameric complex. Our results imply that the higher EF-G-dependent GTPase activity that is observed with the *T. thermophilus* stalk complex is not principally because of the presence of six copies of Tt-L12, but rather a structural element within the CTD of Tt-L12 is responsible for the higher activity. It is therefore likely that the contribution of the third stalk dimer to the activity is only small, at least at 37 °C. The results are consistent with our previous functional study on the heptameric stalk complex from *Pyrococcus horikoshii*, i.e., loss of the third stalk dimer from the heptameric complex has only a slight effect on ribosome function (19). Therefore, the question of the functional difference between the pentameric and heptameric stalk complexes in the ribo-
some remains unanswered. The third stalk dimer may play its role only at very high temperatures. It is also conceivable that the complex with three stalk dimers is more stable than the complex with two stalk dimers, and this may make the assembly of the complex at extremely high temperatures more efficient. However, it should be mentioned that the heptameric stalk complex might not be found in ribosomes from all thermophilic species (45).

It has been reported that the EF-G-GTP complex has the ability to bind to ribosomes in the absence of L12, although the binding is further enhanced by the presence of L12 (5, 46, 47). This implies that L12 is not essential for the binding of EF-G to the ribosome but enhances its binding. However, L12 may be essential for the correct recruitment of EF-G into the GTPase-activating site of the ribosome or alternatively for the GTPase activation itself. The present study, using the chimeric proteins, indicated that the CTD of Tt-L12 contained structural features that are required for the higher GTPase activity. Although the L12 orthologs are well conserved between E. coli and T. thermophilus, there are four divergent areas within the α3, α4, α5, and α6 regions of the CTD (Fig. 5A). The present study, using chimeric L12 proteins, also demonstrated that the unique amino acid sequence in the α6 region of Tt-L12 was required for the higher GTPase activity (Fig. 6A). Although the α4 and α5 regions are known to provide the binding sites for the elongation factors (12), the Tt-L12 sequences from the α4 and α5 regions gave only a slight stimulation of the GTPase activity (Fig. 6A). Therefore, it seems to be unlikely that the enhancement of GTPase activity by Tt-L12 is due to an increased binding of EF-G. The detailed role of α6 in EF-G-dependent GTPase turnover is not known at present. It is interesting that the amino acid residues in the α6 region of Tt-L12 differ from the corresponding ones in Ec-L12 (Gln105, Glu106, Glu109, Ile110, Lys113, Ala116, and Val117; Fig. 5A) are found on the same side of the helix (Fig. 6B) and that this side faces toward α4, to which the translation factors presumably bind (12). We infer that α6 can interact with α4 within L12, at least during certain stages of factor binding, and this interaction between α4 and α6 may be relevant for the enhancement of GTPase turnover by Tt-L12. The interaction may be involved in the rearrangement of the ribosome-EF-G-GDP complex that causes dissociation of EF-G from the ribosome. Furthermore, we should not rule out the possibility of a direct or indirect role for α6 in the activation of
EF-G-dependent GTPase activity at the functional center of the ribosome.

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