Differential Effects of Replacing *Escherichia coli* Ribosomal Protein L27 with Its Homologue from *Aquifex aeolicus*

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The *rpmA* gene, which encodes 50S ribosomal subunit protein L27, was cloned from the extreme thermophile *Aquifex aeolicus*, and the protein was overexpressed and purified. Comparison of the *A. aeolicus* protein with its homologue from *Escherichia coli* by circular dichroism analysis and proton nuclear magnetic resonance spectroscopy showed that it readily adopts some structure in solution that is very stable, whereas the *E. coli* protein is unstructured under the same conditions. A mutant of *E. coli* that lacks L27 was found earlier to be impaired in the assembly and function of the 50S subunit; both defects could be corrected by expression of *E. coli* L27 from an extrachromosomal copy of the *rpmA* gene. When *A. aeolicus* L27 was expressed in the same mutant, an increase in the growth rate occurred and the “foreign” L27 protein was incorporated into *E. coli* ribosomes. However, the presence of *A. aeolicus* L27 did not promote 50S subunit assembly. Thus, while the *A. aeolicus* protein can apparently replace its *E. coli* homologue functionally in completed ribosomes, it does not assist in the assembly of *E. coli* ribosomes that otherwise lack L27. Possible explanations for this paradoxical behavior are discussed.

Protein L27 is one of the smallest and most basic polypeptides in the *Escherichia coli* ribosome. Previous studies of a mutant of *E. coli* in which the *rpmA* gene, which encodes L27, was deleted showed that this protein is important for both the assembly and the function of the ribosome (46). In the absence of L27, the assembly of the large or 50S ribosomal subunit is severely perturbed, resulting in the accumulation of a 40S precursor particle that is deficient also in proteins L16, L20, and L21. Although completed subunits lacking only L27 are assembled in the deletion mutant, they are impaired in peptidyltransferase activity, most likely because of a defect in the binding of aminoacyl-tRNA to the A site (46). The approximate position of protein L27 in the *E. coli* 50S subunit has been localized by immunoelectron microscopy to the base of the central protuberance, in the vicinity of the peptidyltransferase center (24). The exact location of L27 is still unknown, however, despite the availability of a high-resolution crystallographic structure of the 50S subunit from the archaeon *Haloarcula marismortui*, as this particle does not contain an L27 homologue. In the recently published 5.5-Å crystallographic structure of the *Thermus thermophiles* 70S ribosome, electron density has been ascribed to protein L27, but the protein has not yet been fitted to this density (51).

While the effects of L27 deletion upon peptidyltransferase and tRNA binding could be due to long-range interactions or consequences of the influence of L27 on assembly, several additional lines of evidence suggest that the protein is present at the peptidyltransferase center. For example, L27 has been affinity labeled by inhibitors of peptidyltransferase such as chloramphenicol, carbomycin, tylosin, spiramycin, and puromycin (1, 4, 27, 35, 40). Puromycin is of particular interest, as it mimics the aminoacyl moiety of the A-site tRNA and serves as a substrate for peptidyltransferase (25). This mimicry was used to advantage in the design of a transition-state analogue for peptide transfer in which puromycin represents the A-site-bound aminoacyl-tRNA (44). X-ray crystallography of this analogue complexed with the *H. marismortui* 50S subunit has been used to determine the location of the peptidyltransferase center (2, 28).

Further evidence for the role of L27 comes from affinity-labeling studies with a tRNA\(^{\text{Phe}}\) derivative containing the photoreactive nucleoside 2-azidoadenosine at its 3′ terminus (39). When bound to ribosomal A or P sites, this probe cross-links predominantly to L27 (47, 49) and to nucleotides U2506 and U2585 of the 23S rRNA (48). The corresponding nucleotides in the crystallographic structure of the *H. marismortui* 50S subunit are located, as expected, in the vicinity of the transition-state analogue which marks the site of peptide transfer, but no proteins are seen within 20 Å of this site. These results conflict with the fact that L27 must be within 2 to 4 Å of the azido group at the 3′ terminus of the tRNA for cross-linking to occur. The presence of this protein at the peptidyltransferase center of the *E. coli* 50S subunit may therefore represent a significant difference between bacterial and archaeal ribosomes.

To further investigate the properties of this interesting protein, we set out to clone, overexpress, and purify protein L27 for additional structural and functional studies. Preliminary experiments with *E. coli* L27 indicated that it is largely unstructured in solution. We therefore decided to focus upon the homologous protein from the hyperthermophilic bacterium *Aquifex aeolicus* in the expectation that this protein would display greater conformational stability. This prediction was borne out by both circular dichroism (CD) and nuclear magnetic resonance (NMR) measurements. We also investigated

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Planck-Institut für Proteins, Germany. Eppendorf model 2510 electrophorator with cells plated electroporated according to the manufacturer’s instructions.

Electrophoresis of proteins. Gel electrophoresis of proteins was carried out with an SE250 Mighty Small II gel apparatus (Hoefer Scientific Instruments). Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) of proteins was performed as described previously (31). Extraction and electrophoresis of ribosomal proteins by two-dimensional (2D) PAGE were done by the procedure of Butler and Wild (11). For extraction of ribosomal proteins from whole cells, cell pellets were first resuspended in 1 mL of 10 mM Tris-HCl (pH 7.4)–15 mM magnesium acetate–60 mM KCl–7 mM β-mercaptoethanol. For extraction of proteins from ribonucleoprotein (RNP) particles obtained by sucrose density centrifugation, the RNPs were sedimented, resuspended in the centrifugation buffer but without sucrose, and resedimented. The final pellet was dissolved in 8 M urea containing 1% β-mercaptoethanol prior to extraction. Protein concentrations were determined using the Bio-Rad Laboratories Protein Assay Reagent with bovine serum albumin as a standard.

**Materials and methods**

**Materials.** Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs. Cell intestinal alkaline phosphatase and DNSS (grade II from bovine pancreas) were obtained from Boehringer Mannheim. Taq polymerase was obtained from Sigma-Aldrich, Inc. All enzymes were used in accordance with the manufacturer’s instructions. Primers for PCR were obtained from Sigma-Genosys; those used for sequencing were the reverse sequencing and the type III or IV forward sequencing primers provided by Qiagen, Inc., for pQE-series plasmids.

**Bacterial strains and plasmids.** Strains and plasmids are described in Table 1. Plasmid pSBETa was kindly provided by Hans-Henning Steinbüchel of the Max-Planck-Institut für Züchtungsforschung, Cologne, Germany. A. eccentricus chromosomal DNA was a gift from Robert Huber, Universität Regensburg, Germany. Molecular biology. Unless otherwise stated, bacteria were grown at 37°C in L broth with shaking or at 37°C on the same medium solidified with 2% (wt/vol) agar. Antibiotics were included in the media as appropriate at a concentration of 50 μg/ml (ampicillin or kanamycin) or 30 μg/ml (chloramphenicol). Plasmids for transformation or restriction analysis were prepared by the plasmid miniprep procedure (3). Those for sequencing were prepared by use of Wizard Miniprep (Promega) with modifications for automated sequencing as suggested by the manufacturer. DNA was sequenced at the University of Massachusetts DNA Sequencing Facility by automated fluorescent sequencing. Electrophoresis of DNA was performed with agarose gels in Tres-borate-EDTA buffer using standard procedures (30). PCR products and DNA fragments that had been purified on agarose gels were recovered using a Qiagen II gel extraction kit (Qiagen).

Plasmids were introduced into cells made competent with calcium chloride by standard procedures (30), except for the simultaneous transformation of strain IWX312 with both plasmids pAVM3 and pREP4; the latter was done using an Eppendorf model 2510 electrophorator with cells plated electroporated according to the manufacturer’s instructions.

**Cloning of the A. eccentricus rpmA gene.** The A. eccentricus rpmA gene was amplified by PCR using Taq polymerase and A. eccentricus chromosomal DNA (concentration, 50 nM) as a template. The forward and reverse primers were 5′-GGAGTGTTGAAACATGCGAAGTAAGAC-3′ and 5′-CGAGAAACAAGCAAGTGGTG ACTTTTCC, respectively, based in bold type differ from the A. eccentricus chromosomal sequence (13). NilH and HindIII restriction sites are underlined, and the initiation and stop codons are in italic type. A single product of about the expected length (321 bp) was obtained. The product was gel purified, digested with both NilH and HindIII, and ligated in expression vector pQE70 that had been cleaved with SplI and HindIII. SplI and NilHIII produce compatible termini. The use of an NilHIII site (rather than an SplI site) in the PCR product allowed the identity of the alanine codon in the second position of the gene to be preserved. The resulting plasmids were introduced into recA mutant strain XL1-Blue. Plasmids isolated from transformants, and the presence of the insert was confirmed by restriction digestion analysis. Both strands of one such isolate, designated pAAL27, were sequenced to confirm that the gene had been amplified and cloned without error. The A. eccentricus rpmA gene in pAAL27 is downstream of an isopropyl-β-D-galactopyranoside (IPTG)-inducible T5 promoter and a Shine-Dalgarno sequence. The T5 promoter is recognized by the E. coli host polymerase, and the lac operator sequences in pAAL27 allow repression of transcription by the lac repressor protein encoded on a separate plasmid, pREP4.

**Protein expression.** To test protein expression, strain XL1-Blue was transformed with both pAAL27 and pREP4, and the synthesis of A. eccentricus L27 was induced in early-log-phase cultures by the addition of IPTG. Although the presence of the inducer slowed growth, it did not result in significant production of the A. eccentricus protein, as judged by one-dimensional (1D) SDS-PAGE of whole-cell extracts. Inspection of the A. eccentricus rpmA coding sequence revealed that the poor expression of A. eccentricus L27 was most likely due to the presence of eight AGG or AGA arginine codons, which are rare in E. coli but common in A. eccentricus. Such codons are known to be particularly deleterious to protein expression in E. coli (7, 12, 18, 29, 37, 52). The simplest solution was to increase the copy number of the arg gene, which encodes a smaller Arg operon in A. eccentricus than is the corresponding operon from a plasmid, can decode both types of rare Arg codons (17, 36).

**Subcloning of the argU gene.** The argU gene, together with its promoter and terminator, was excised from pSBETa with BamHI and SalI to yield a 568-bp fragment, and overhangs were filled in with T4 DNA polymerase. Attempts to ligate this blunt-ended fragment into the XbaI site of pAAL27, also treated with DNA polymerase, were unsuccessful. Therefore, the fragment was cloned into the XbaI site of plasmid pCR-Blunt and then excised as a 650-bp SpeI-XbaI fragment. Since SpeI and XbaI produce compatible termini, this fragment could be ligated into XbaI-cleaved pAAL27. Introduction of the argU gene into pAAL27 resulted in copious expression of A. eccentricus L27 upon induction with IPTG. One clone, pAAL27, which contained the rpmA gene in a counter-clockwise orientation, was chosen for further study.

**Sucrose density gradient centrifugation.** Cultures were grown to an A599 of 0.5, chilled briefly on ice, and harvested by centrifugation. Cell pellets were washed in 0.5 volume of 20 mM HEPES-KOH (pH 7.8)–6 mM MgCl2–100 mM NaCl and resedimented. After resuspension in the same buffer containing 16% (wt/vol) sucrose, cells were lysed by the lysozyme freeze-thaw method as described by Bommer et al. (6), but with an extra freeze-thaw step. Lysates were clarified at 12,000 × g for 1 h at 4°C, and 1.5 to 3 A599 units were loaded onto 15 to 30% (wt/vol) sucrose density gradients made in 20 mM HEPES-KOH (pH 7.5)–10 mM MgCl2–150 mM NH4Cl–2 mM spermidine–0.05 mM spermine–4 mM β-mercaptoethanol. Centrifugation was done at 80,000 × g for 20 h in a Beckman SW28 rotor. For isolation of 70S ribosomes as analyzed by protein content, up to 100 A599 units were loaded per gradient and centrifugation was

**TABLE 1. Strains and plasmids used in this work**

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L90</td>
<td>Δ(lac-proAB)</td>
<td>16</td>
</tr>
<tr>
<td>IWX312</td>
<td>Δ(lac-proAB); kan</td>
<td>46</td>
</tr>
<tr>
<td>XL1-Blue</td>
<td>Δ(lac-proAB); recA endA hisAR17 (F’ proAB lacB lacZAM15 Ta10)</td>
<td>10</td>
</tr>
<tr>
<td>Top10+</td>
<td>Δ(mur-hsdR-mcrB-RecA) recA1 endA</td>
<td>Invitrogen Co.</td>
</tr>
<tr>
<td>BL21</td>
<td>Δ(lacUV5 promoter)</td>
<td>38</td>
</tr>
<tr>
<td>BL21(DE3)</td>
<td>Δ(lacUV5 promoter)</td>
<td>38</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pQE70</td>
<td>Amp’ (LacI-regulated T5 promoter upstream of MCS)</td>
<td>Qiagen</td>
</tr>
<tr>
<td>pREP4</td>
<td>Kana lacI</td>
<td>Qiagen</td>
</tr>
<tr>
<td>pAAL27</td>
<td>pQE70, A. eccentricus rpmA</td>
<td>This work</td>
</tr>
<tr>
<td>pAVM3</td>
<td>pAAL27 argU</td>
<td>This work</td>
</tr>
<tr>
<td>pET3a</td>
<td>Amp’ (T7 RNA polymerase promoter upstream of MCS)</td>
<td>38</td>
</tr>
<tr>
<td>pSBETa</td>
<td>pET3a argU</td>
<td>32</td>
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<td>pET3a E. coli rpmA</td>
<td>Worner, unpublished data</td>
</tr>
<tr>
<td>pLYS</td>
<td>Cam’, T7 gene 3.5 (encoding T7 lysozyme)</td>
<td>46</td>
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<td>pCR-Blunt</td>
<td>Kana ccdB</td>
<td>Invitrogen</td>
</tr>
<tr>
<td>pEE</td>
<td>Amp’, pBR322 containing an E. coli chromosome fragment that includes the rplU-rpmA operon</td>
<td></td>
</tr>
</tbody>
</table>

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*a* Only relevant markers are listed.

*b* MCS, multiple cloning site.
done at 95,000 × g for 19 h in a Beckman SW28 rotor. Gradients were pumped out of the tubes with a displacing solution of 40% (v/v) sucrose containing uracil (40 μg/ml) to help locate the bottom of the gradients. Absorbance profiles were monitored at 254 nm using an ISCO UA-5 absorbance monitor.

**TABLE 2. Effect of expression of the L27 protein on the rate of growth of strain IW312**

<table>
<thead>
<tr>
<th>Strain</th>
<th>IPTG (mM)</th>
<th>L27 protein expressed from the plasmid</th>
<th>Doubling time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IW312(pQE70)</td>
<td>0</td>
<td>0.005 A. aeolicus L27</td>
<td>105</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0</td>
<td>0.005 A. aeolicus L27</td>
<td>76</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0.005</td>
<td>0.005 A. aeolicus L27</td>
<td>72</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0.01</td>
<td>0.01 A. aeolicus L27</td>
<td>70</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0.02</td>
<td>0.02 A. aeolicus L27</td>
<td>70</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0.03</td>
<td>0.03 A. aeolicus L27</td>
<td>72</td>
</tr>
<tr>
<td>IW312(pAVM3)(pREP4)</td>
<td>0.04</td>
<td>0.04 A. aeolicus L27</td>
<td>78</td>
</tr>
<tr>
<td>IW312(pEE)</td>
<td>0</td>
<td>0.04 E. coli L27</td>
<td>48</td>
</tr>
<tr>
<td>LG90 (wild type)</td>
<td>0</td>
<td>0.04 E. coli L27</td>
<td>27</td>
</tr>
</tbody>
</table>

**RESULTS**

**Complementation.** Strain IW312 lacks *E. coli* L27 owing to the replacement of the gene (*rpmA*) that encodes it by a gene conferring kanamycin resistance (46). Plasmid pAVM3 directs the expression of *A. aeolicus* L27 when cells harboring the plasmid are induced with IPTG. Attempts to introduce pAVM3 into strain IW312 were unsuccessful, despite the fact that the parent plasmid, pQE70, could be easily introduced. Although the plates did not contain inducer, we suspected that leaky synthesis of *A. aeolicus* L27 was sufficiently high to be deleterious to growth of the mutant. In this case, the host did not contain the repressor plasmid, pREP4, since the selectable marker on pREP4 is Kan' and strain IW312 is already kanamycin resistant. However, simultaneous transformation of the mutant with both plasmids did result in viable transforms. Plasmids were isolated from three of these and subjected to restriction digestion analysis; all three were shown to contain pREP4 in addition to pAVM3.

One such isolate was grown on solid medium at a number of different IPTG concentrations (data not shown). A control strain, IW312(pQE70), grew equally well at all IPTG concentrations from 0 to 1 mM. The test strain that contained the *A. aeolicus* rpmA gene, IW312(pAVM3)(pREP4), grew faster than the control on plates without IPTG, suggesting that there was still some leaky expression of *A. aeolicus* L27. Growth increased further with 0.01 mM IPTG but progressively slowed in the range of 0.05 to 0.1 mM, with no growth at 0.5 to 1 mM, indicating again that excess expression of the *A. aeolicus* protein is detrimental to cell growth. Moderate expression of *A. aeolicus* L27, however, can compensate for the lack of the homologous *E. coli* protein.

These results were confirmed with liquid medium (Table 2). The strain containing the *A. aeolicus* rpmA gene again grew faster (doubling time, 76 min) than the control (doubling time, 105 min) in the absence of inducer. A further increase in growth rate was observed with 0.005 to 0.03 mM IPTG, while the highest concentration of IPTG tested (0.04 mM) decreased the growth rate. An optimal concentration of 0.01 mM IPTG was therefore adopted for subsequent experiments. Although *A. aeolicus* L27 was able to partially complement the missing *E. coli* L27, a greater improvement in growth was obtained when the *E. coli* protein was expressed from plasmid pEE (doubling time, 48 min). However, neither of the IW312 derivatives grew as fast as the wild-type strain, LG90 (doubling time, 27 min).

**Protein expression.** Protein expression was analyzed by 2D PAGE. Proteins extracted from the 70S ribosomes of wild-type strain LG90 (Fig. 1a) yielded the expected pattern of spots, including that of L27. When purified *A. aeolicus* L27 was mixed with this extract, a prominent additional spot corresponding to heterologous L27 was observed in the gel pattern (Fig. 1b). Figure 1c shows the pattern of ribosomal proteins in whole-cell extracts of strain IW312(pAVM3)(pREP4) grown in the presence of inducer. As anticipated, *E. coli* L27 is absent, while a spot corresponding to *A. aeolicus* L27 is clearly visible. When IW312 contains instead plasmid pEE, which expresses *E. coli* L27, the spot corresponding to *E. coli* L27 is restored (Fig. 1d). The contents of proteins in 70S ribosomes from strains IW312(pQE70) and IW312(pAVM3)(pREP4) grown in the presence of inducer are shown in Fig. 1e and f, respectively. As expected, the 70S ribosomes of both strains lack *E. coli* L27, but the latter now contain *A. aeolicus* L27. These results demonstrate that the heterologous protein can be assembled into *E. coli* ribosomes.

**Analysis of ribosome assembly in the presence of *A. aeolicus* L27.** Cell extracts from different strains were centrifuged...
through sucrose density gradients, and the resulting absorbance profiles were compared. The wild-type parent strain, LG90, shows a normal profile, with large amounts of 70S ribosomes and small peaks corresponding to 30S and 50S subunits (Fig. 2a). Owing to the absence of L27 in strain IW312, a 40S precursor to the 50S ribosomal subunits accumulates, so that the relative amount of completed 70S is reduced (46). This finding is shown for strain IW312 containing control plasmid pQE70 (Fig. 2b; profiles from a culture without the plasmid were identical). Resupply of the E. coli L27 protein from plasmid pEE largely removes this defect (Fig. 2c). However, when the A. aeolicus protein is supplied from plasmid pAVM3 (Fig. 2d), assembly seems even more perturbed than with no L27 at all, with a smaller peak of the 50S subunits relative to the 40S precursor. This finding could, in theory, reflect a greater propensity of 50S subunits to associate with 30S subunits to form 70S couples. The result would be a corresponding increase in the 70S material and a decrease in the 30S peak. However, such a redistribution is not apparent, suggesting that the shortage of free 50S subunits in strain IW312(pAVM3)(pREP4) is due to a decrease in their net synthesis rather than to increased sequestration into 70S couples.

Since this comparison is complicated by poor resolution of the 30S, 40S, and 50S peaks, a second set of gradients were centrifuged for longer times (data not shown). Analysis of the peak areas confirmed that the ratio of 50S material to 30S material in strain IW312(pQE70) was one-third lower than that in LG90 and that the ratio in IW312(pAVM3)(pREP4) was two-thirds lower. This result can be explained by the presence of a slowly sedimenting precursor to the 50S subunits in the 30S peak or by the breakdown of a precursor to the 50S subunits. Either way, expression of the A. aeolicus protein does not improve 50S subunit assembly in the mutant. Thus, the expression of A. aeolicus L27 presumably increases the rate of growth of the mutant by improving the function of the otherwise L27-deficient 50S subunits rather than by aiding their assembly.

Structural comparison of L27 from E. coli and A. aeolicus. E. coli and A. aeolicus L27 proteins were purified and renatured as detailed in Materials and Methods and subjected to CD and NMR analyses. The CD spectrum of E. coli L27 (Fig. 3a) indicates that the protein is unstructured. This inference was reinforced by 2D heteronuclear NMR analysis of a 15N-labeled sample of the E. coli protein, which demonstrated that there was no dispersion of the backbone amide or side-chain amine resonances (data not shown). The CD spectrum of A. aeolicus L27 is clearly different from that of E. coli L27 at 20°C but resembles that of the E. coli protein at 80°C. Together, these observations suggest that A. aeolicus L27 may be at least partially structured, a conclusion that was confirmed by 1D proton NMR analysis. Figure 4 shows that there are several striking differences between the NMR spectra of the E. coli and A. aeolicus proteins. In particular, the methyl groups of the isoleucine, valine, and leucine side chains, at 0 to 1.0 ppm, and the amide resonances, at 6.8 to 9.8 ppm, are much more dispersed in A. aeolicus L27 than in the E. coli protein. The NMR spectrum of the E. coli protein at 80°C differed very little from...
that at 20°C. In contrast, the dispersion of nonpolar side chain resonances in *A. aeolicus* L27 was reduced but nevertheless still showed evidence of structure.

**DISCUSSION**

The effects of heterologous protein expression on ribosome assembly in an *E. coli* ribosomal protein deletion mutant have not previously been reported. In two earlier studies, heterologous ribosomal proteins were expressed in the presence of a truncated (33) or undersynthesized (22) homologous ribosomal proteins were expressed in the presence of a bacterial sequence showed that only three of these are unique to *A. aeolicus*. These are Ala 5, Tyr 67, and Pro 83. While 9 and 12 different amino acids are seen at the latter two positions, respectively, Ala 5 is unique to *A. aeolicus*; all other bacterial sequences are neither identical nor similar to those in the *E. coli* homologue. It is possible that differences occur in portions of the polypeptide that are critical for assembly of this protein in *E. coli*. To assess the extent of phylogenetic variation in L27, sequences related to that of *E. coli* L27 were obtained from GenBank. Thirty-five bacterial and 22 eukaryotic sequences were obtained. No archaeal homologues were found, and the majority of the eukaryotic sequences are known to be from organelles. An alignment of the *E. coli* and *A. aeolicus* L27 sequences (Fig. 5) revealed that 16 amino acids in the *A. aeolicus* sequence are neither identical nor similar to those in the *E. coli* sequence. An alignment of all bacterial sequences showed that only three of these are unique to *A. aeolicus*. These are Ala 5, Tyr 67, and Pro 83. While 9 and 12 different amino acids are seen at the latter two positions, respectively, Ala 5 is unique to *A. aeolicus*; all other bacterial sequences have lysine, barring two arginines. All eukaryotic sequences also have lysine or arginine at this position, with the exceptions of one histidine and one alanine. The presence of a positively charged side group at this position might be required for assembly in *E. coli* (and other organisms). Alternatively, it is possible that the 15-amino-acid C-terminal extension of the *A. aeolicus* protein (relative to *E. coli* L27) impedes its assembly into *E. coli* ribosomes. A third possibility is that *A. aeolicus* L27 may be too structured in its unassembled state to be efficiently incorporated into the subunit structure. The *E. coli* protein is one of the least structured of the *E. coli* ribosomal proteins in solution (15), and it is possible that its assembly depends upon such flexibility. The *A. aeolicus* protein, on the other hand, readily adopts structure in solution.
that is very robust. As such, it may lack the plasticity necessary for assembly.

The L27 proteins from *E. coli* and *A. aeolicus* had very different CD spectra, and each one was essentially invariant, despite the use of several different renaturation protocols. For *E. coli* L27, this appears to be the case because the isolated protein is not structured at all, while *A. aeolicus* L27 seems to readily adopt some structure that is highly stable and independent of the renaturation procedure. For the *E. coli* protein, the lack of structure was confirmed by 2D heteronuclear NMR analysis. However, there remained a possibility that suitable renaturation conditions had not been found, since the protein was purified under denaturing conditions. Dijk and coworkers extracted *E. coli* L27 from ribosomes using mild “nondenaturing” conditions, so that the resulting protein should have been as close to its native folded state as possible. The CD spectrum that they obtained for their protein (15) was very similar to ours, although they predicted that it contained 50% β-sheet structure. Prediction of secondary structure from the CD spectrum was not attempted in the present study for two reasons. First, such predictions can be difficult to perform with confidence. Second, protein concentrations in the present study were estimated by the Bradford assay, while more accurate quantitation would be required to allow structural prediction. Nonetheless, CD spectroscopy is quite useful for comparing the degrees of structure in different preparations of related proteins, as was done here. The proton NMR spectra for the proteins measured by Littlechild and collaborators (23) and Morrison et al. (26) were also similar to ours, although they concluded that this technique could not entirely discount the presence of structure. However, given the similarity of the CD and 1D proton NMR spectra, we conclude that *E. coli* L27 is unstructured in solution regardless of the method of isolation.

The fact that the *A. aeolicus* protein gives a CD spectrum that is different from that of *E. coli* L27 suggests that it is structured in solution to at least some extent. This idea is reinforced by the observation that after thermal denaturation...
at 80°C, its spectrum resembles that of E. coli L27 at 20°C. Moreover, proton NMR analysis shows clear evidence of structure, some of which persists even at 80°C. As such, the A. aeolicus protein is a much better candidate for physical and structural studies, such as X-ray crystallography, than its E. coli counterpart. Efforts are also under way to identify the amino acids within L27 that are cross-linked from the acceptor terminus of tRNA. This information will define more precisely the proposed juxtaposition of the tRNA with protein L27 at the peptidyltransferase center.

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