Involvement of ribosomal protein L7/L12 in control of translational accuracy

[rppl mutants/misreading in vivo, in vitro/reconstituted ribosomes/poly(U)/proofreading]

LEIF A. KIRSEBOM AND LEIF A. ISAKSSON

Department of Molecular Biology, Biomedical Center, University of Uppsala, Box 590, 751 24 Uppsala, Sweden

Communicated by Diter von Wettstein, October 1, 1984

ABSTRACT The effects of two mutations, which map at the rppl locus and both give a changed 50S ribosomal protein L7/L12, were studied. Both mutations are associated with an increased misreading of all three nonsense codons in vivo and ribosomes from the mutants give an increased misreading of the phenylalanine codon UUU by tRNA<sup>Lys</sup> in vitro. The rppl-associated misreading in vitro is not limited to a particular type of mRNA or tRNA. Results from a translational proofreading assay, using mutant ribosomes, suggest that protein L7/L12 is involved in the control of translational accuracy by contributing to the efficiency of a translational proofreading step(s).

Mutants with alterations in most ribosomal proteins have now been isolated in Escherichia coli by various approaches and the corresponding genes have been mapped (1, 2). Many of these mutants are not associated with any obvious phenotypic property and the mutation is only manifested as an electrophoretic shift of the mutated protein (3, 4). Surprisingly enough, a number of viable mutants has been found that even lack a protein in the ribosome (5). Others have been selected as being resistant towards some of the many antibiotics that normally interact with the ribosome and disturb the translational process (for a review, see ref. 6).

A closer study of some ribosomal mutants, or pseudorevertants thereof, has revealed important new properties of the changed ribosome. Thus, an increased translational accuracy has been found in mutants that are resistant to streptomycin and to steptomycin as a result of a mutation in the structural gene (rppl) for protein S12 (7) or to teicoplanin as a result of a changed protein S17 (8). The rppl mutants have often, but not always, a lower rate of translation (9–11). Mutants with changed ribosomal protein S4 or S5 are often less accurate and are for this reason referred to as possessing a Ram (ribosomal ambiguity) phenotype (7, 12–15). These mutants appear to be normal in their rate of translation despite their increased translational ambiguity (6, 11, 16). Mutants have also been isolated that are changed in the 50S ribosomal protein L6 and are more accurate during translation (17).

The existence of a G1Pase-driven proofreading step(s) during translation that would improve translational accuracy (18, 19) has been experimentally verified either by studying partial reactions of the process (20) or in system allowing a complete study of the elongation phase in vitro (21). The altered translational ambiguity of some mutants with changed ribosomal protein S12 or S4 appears to be resulting from a changed efficiency of this proofreading step (refs. 22 and 23; T. Ruusala, personal communication).

In this paper we describe two mutants with altered ribosomal protein L7/L12 that show an increased misreading during translation both in vivo and in vitro. Thus, an increased level of mistranslation can also arise as a result of a mutational change in the 50S ribosomal subunit even though the codon–anticodon interaction presumably takes place at the 30S subunit (24, 25). Our finding that protein L7/L12 is involved in control of proofreading efficiency gives additional support to the concept that translational accuracy is affected by the kinetic properties of the process besides the simple codon–anticodon interaction.

MATERIALS AND METHODS

Bacterial Strains and Genetic Procedures. The genotypes and derivations of the E. coli K-12 strains used in this study are listed in Table 1. Generalized PI transductions were performed as described by Miller (26). Introduction of the various rppl alleles into U132, which is genetically identical to XAc, was done by cotransduction with Arg<sup>+</sup>. The donor for the rppl-159 allele was UL314. The rppl<sup>+</sup> allele originates from UE123 and the rppl-265 allele originates from VT265. The resulting strains UY121, UY131, and UY154, respectively, were used for the in vivo measurements of read-through of nonsense codons in vivo. To facilitate the genetic constructions involving rppl alleles, which do not give an easily scoreable phenotype, a rifampicin resistance mutation in the nearby and thus closely linked gene, rpoB, was used for a tentative classification of transductants with respect to their rppl genotype. In all cases, the rppl allele was finally verified by gel electrophoresis.

Strain O17 was used as reference for the in vitro assays since it is already well characterized (16, 23, 27). Therefore, we constructed UY128, an argE<sup>+</sup> zij::Tn10-115 derivative of O17 by cotransduction with Tet-R. The rppl-159 and rppl-265 alleles were then introduced by PI transduction by using UL314 and UY154 as donors and selecting for Arg<sup>+</sup> followed by a screening for Tet-S and Rif-S. The resulting strains, UY134 and UY143, were used for the preparation of ribosomes to be tested in the poly(U)-directed in vitro system.

Media and Growth Conditions. Strains to be used for ribosome preparations and subsequent assay in the poly(U)-directed in vitro system were grown at 37°C with good aeration to late-exponential phase in a modified LB medium supplemented with 0.2% glucose (28). After cooling on ice, the cells were harvested by centrifugation and stored at −80°C until use. Cells to be used for preparation of purified L7/L12 protein from UY134, UY143, and UE123 were grown in the TY2 medium (29) in a LKB fermentor to late-exponential phase. They were then cooled, harvested by centrifugation and washed with 0.9% NaCl, and stored at −80°C.

To test the read-through of nonsense codons in vivo, strains UY211, UY131, and UY154, with the different F<sup>+</sup> factors (16, 44), were grown in an M9 medium supplemented with 0.2% glucose and the recommended concentrations of amino acids (30) but lacking proline and arginine. The cells were grown to mid-exponential phase, cooled on ice, and kept on ice until tested for β-galactosidase activity. Measurements of translational read-through by β-galactosidase

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
activity were performed as described by Andersson et al. (16). Step-time determinations were made as described elsewhere (16, 31).

Preparation Procedures and in Vitro Translation Systems. Ribosomes were prepared as described by Jelenc (32) but stored in Polyimix buffer at −80°C. Ribosomal proteins from 70S ribosomes were analyzed in a two-dimensional gel electrophoresis system (33).

Poly(U)-directed in vitro translation (34) and error measurements were performed as described elsewhere (16, 21, 23, 27). The L7/L12-depleted 70S ribosomal core particles were prepared as described by Hamel et al. (35) and stored in Polyimix buffer (34) at −80°C. Reconstituted 70S ribosomes were made by adding purified L7/L12 protein in excess to 70S core particles lacking this protein. The amount of L7/L12 protein to be added for optimal rate of protein synthesis was always determined by titration. The proofreading factor was determined for 70S ribosomes from O17, UY134, and UY143 according to the method described by Ruusala et al. (21). Rate of protein synthesis in vitro was determined and corrected for the fraction of ribosomes that are not active (36).

RESULTS

Mutation Affecting Ribosomal Protein L7/L12. Preliminary observations had indicated that a strain with a missense suppressor (Su^159) showed a resemblance to rpsD (54) strains in misreading of certain nonsense mutations (unpublished results). Su^159 strains are known to be double mutants harboring a glyT-derived tRNA^gly^ missense suppressor (Su^AGA/G) together with another closely linked but unknown mutation (37, 38). Since several 50S ribosomal protein genes, coding for proteins L1, L10, L11, and L12, are located close to glyT (3), we compared the electrophoretic properties of the ribosomal proteins from a Su^159 strain (UE135) and another strain (UE125), which does not show such misreading of nonsense mutations. The UE125 control strain also carries an independently selected glyT-derived tRNA^gly^ missense suppressor [Su^56, (39)]. The primary sequences of the suppressor tRNA from a Su^56 and a Su^159 strain are identical (N. E. Prather and E. Murgola, personal communication). The results of the electrophoretic analysis showed that the spot containing protein L12 (together with its acetylated form L7) from UE135 migrated faster in the second dimension of an electrophoretic system (33) than the corresponding spot from UE125. When ribosomal proteins from the two strains were mixed, the electrophoretic mobility difference between L7/L12 from UE125 and UE135 was large enough to give rise to a double L7/L12 spot with the one migrating fastest in the second dimension originating from UE135 (Fig. 1). Taken together, the data indicate that the Su^159 composite phenotype of UE135 originates from one mutation giving the glyT-derived missense Su^AGA/G suppressor together with a second closely linked mutation in the structural gene for protein L12 (rplL-159) (unpublished data). Analysis of purified L7/L12 in other electrophoretic systems supports this interpretation (not shown).

Effect of rplL. Mutations on Read-Through of Nonsense Codons in Vivo. To examine misreading properties in vivo, the rplL-159 mutation as well as another allele (rplL-265 in strain
VT265) were transduced into strain UD132. This recipient strain does not carry any nonsense codon suppressor gene and contains a deletion for the entire lac operon. The rpl derivatives of UD132 so obtained were then infected with a F' factor containing a hybrid lacI+ lacZ gene coding for a fused protein with β-galactosidase activity. By introducing nonsense codons (UGA, UAG, and UAA) at different locations in the lacI part of the hybrid gene it is possible to get an estimate of the translational read-through of these nonsense codons at different codon contexts (16). As can be seen in Table 2, a mutation in the ribosomal protein L7/L12 might be associated with an increased read-through of all three nonsense codons in vivo. The magnitude of this read-through is, however, dependent on the location of the nonsense codon in the lacI part of the fused lacI+ lacZ gene. Furthermore, it can be seen that the read-through level in the rplL-265 strain is consistently higher than in a rplL-159 derivative.

Thus, it appears that mutations in the structural gene for ribosomal protein L12 might give an increased translational misreading of nonsense codons in vivo. In this respect they are similar to rpsD mutants, which are known to give an increased read-through capability of nonsense codons in the same fused lacI+ lacZ system (16). The phenotypic similarities are even more pronounced since the increased read-through of nonsense codons in rplL strains can be counteracted by the introduction of a rpsL (S12) mutation in analogy with what is already known to be the case for rpsD mutations (not shown).

**Determination of Translational Misreading in a Poly(U)-Directed In Vitro System.** Since mutations in ribosomal protein L7/L12 are associated with an increased read-through of nonsense codons in vivo, which presumably takes place at the translational level, we wanted to measure the missense error frequency in vitro in a poly(U)-directed system (16, 21, 23). Ribosomes from control and mutant strains were examined for some of their properties during translation using purified translation factors and the tRNA isoacceptors tRNA4eU and tRNA55P. As can be seen (Table 3), the misincorporation of leucine at the phenylalanine codon UUU is increased when ribosomes from the rplL mutants were used. This is particularly true in the case of rplL-265.

To verify that the increased translational error actually results from the mutationally altered L7/L12 protein, the mutant protein was purified and added to wild-type ribosomal particles that had been depleted of their L7/L12 protein. The reconstituted ribosomes so obtained were next assayed for translational misreading in the poly(U)-directed in vitro system (Table 3). When L7/L12 protein from a rplL-265 strain was added to core particles, an increase by a factor of 3.9 in the error was observed. A consistent increase in translational misreading was also seen when L7/L12 protein from a rplL-159 strain was used. These results strongly suggest that the increased translational error seen in vitro is indeed resulting from the mutation affecting ribosomal protein L7/L12.

In vitro experiments were also done by using purified tRNA4eu instead of tRNA46U, together with tRNA55P with qualitatively the same results (not shown). Furthermore, when an alternating poly(U-G)-directed in vitro system was used together with mutant ribosomes we obtained a similar increase in the error level, measured by methionine over valine incorporation (27), as in the poly(U)-directed system (not shown). Therefore, it appears that the increase in translational error in vitro that results from a rplL mutation is not limited to a particular type of mRNA or tRNA.

**Decreased Translational Proofreading Efficiency in rplL Strains in Vitro.** As described above, the rplL mutants show an increased translational misreading in vivo and in vitro in a similar fashion as rpsD mutants with their well-studied Ram phenotype (16). In the case of rpsD strains the increased translational error in vitro is correlated with a decreased efficiency of the proofreading step(s) during translation (23). As a comparison, ribosomes from the two rplL mutant strains were therefore tested in a proofreading assay (21, 23). A slightly decreased proofreading efficiency was obtained in the case of rplL-159, and a more profound effect was seen in the case of rplL-265. The magnitude of the decreased proofreading that was obtained with the rplL-265 ribosomes is in qualitative agreement with the observed increase in the error level found in a normal misreading assay (Table 3). This correlation would suggest that the increased translational error in the rplL-265 mutant strain mainly results from a decreased efficiency at the proofreading step(s). The results of the proofreading experiments are also in line with the observation that the rplL-159 mutation imposes a milder effect in general on translational misreading than does the rplL-265 mutation.

**Estimation of the Rate of Protein Synthesis in Vivo and in Vitro.** The rpsD Ram mutants do not show any alteration in the rate of protein synthesis in vivo or in vitro (16). The rplL Ram mutants described here do, however, show significantly lowered rates of protein synthesis in the poly(U)-directed in vitro system (Table 4). Such a result is not obtained when rates of protein synthesis in vivo are determined by step-time estimates using β-galactosidase induction (16, 31). In this case no significant difference in step-time is seen when the wild-type and the rplL mutants are compared. Therefore, even though the level of misreading that characterizes rplL+, rplL-159, and rplL-265 strains is rather consistent when they

---

**Table 2. Read-through in vivo of nonsense codons**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Position 84</th>
<th>Position 117</th>
<th>Position 181</th>
<th>Position 189</th>
<th>Position 220</th>
<th>Position 280</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>UAG</td>
<td>UAA</td>
<td>UAG</td>
<td>UAA</td>
<td>UAG</td>
<td>UAA</td>
</tr>
<tr>
<td>UY211</td>
<td>rplL+</td>
<td>1.0</td>
<td>1.5</td>
<td>0.4</td>
<td>0.5</td>
<td>0.9</td>
<td>0.4</td>
</tr>
<tr>
<td>UY131</td>
<td>rplL-159</td>
<td>2.9</td>
<td>2.9</td>
<td>0.7</td>
<td>0.9</td>
<td>3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>UY154</td>
<td>rplL-265</td>
<td>3.7</td>
<td>4.4</td>
<td>0.8</td>
<td>0.9</td>
<td>4.8</td>
<td>2.6</td>
</tr>
</tbody>
</table>

Position refers to the position of the nonsense codon in the lacI part of a fused lacI+ lacZ gene. Data are shown as β-galactosidase activity ($\times 10^5$) related to the value in a corresponding strain without nonsense mutation.

---

**Table 3. Leucine misincorporation in a poly(U)-directed in vitro system**

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Error $\times 10^4$</th>
<th>Error $\times 10^4$</th>
<th>Proofreading factor†</th>
</tr>
</thead>
<tbody>
<tr>
<td>rplL+</td>
<td>5.1</td>
<td>5.9</td>
<td>93</td>
</tr>
<tr>
<td>rplL-159</td>
<td>8.1</td>
<td>9.4</td>
<td>84</td>
</tr>
<tr>
<td>rplL-265</td>
<td>13</td>
<td>23</td>
<td>36</td>
</tr>
</tbody>
</table>

†Error determinations were done by using precharged tRNA4eu and tRNA55P and purified factors together with ribosomes as indicated.

‡With 705 ribosomes.

§With 705 ribosomes, reconstituted from purified L7/L12 from rplL+ or rplL strains and L7/L12-deficient ribosomal core particles from an rplL+ strain.

The proofreading factor, determined with 705 ribosomes, is defined as the ratio between the number of elongation factor Tu (EF-Tu)-GTP consumed per molecule of incorporated leucine divided by the corresponding value per incorporated phenylalanine.
found in a strain that carries a glyT-derived AGA/G mis-sense suppressor together with a closely linked unspecified mutation. This second mutation partly compensates for the deleterious effects caused by the suppressor mutation (37, 38). A mutation in the glyT gene, that in this case converts tRNA\(^{\text{Gly}}\) to read AGA/G instead of GGA/G, is normally lethal since tRNA\(^{\text{Gly}}\) is the only GGA reader in the cell (42). Thus, the rplL-159 mutation might well be the compensatory mutation already indicated (37, 38) since rplL maps very close to glyT (2). It is quite possible that the weak Ram phenotype of the rplL-159 mutation could compensate for the loss of GGA reading by allowing tRNA\(^{\text{AGA}}\), tRNA\(^{\text{AGC}}\), or the tRNA\(^{\text{Gly}}\) suppressor itself to read GGA by misreading. Another explanation for the selective advantage of the rplL-159 mutation could be compensation for the mutational loss of native GGA reader by a similar mechanism as has already been suggested to explain pseudo-revertants of certain aminoacyl-tRNA ligase mutants. Some of these compensatory mutations are known to be located in genes for ribosomal proteins and are proposed to act by slowing down translation, which would give a sparing effect of limiting amounts of aminoacyl-tRNA (6). Our findings that rplL mutants and their control strain are indistinguishable in vivo in rates of growth and translation would, however, tend to make this second explanation less likely.

The rplL-265 mutation was isolated as a spontaneous pseudo-revertant of rplL-159. This mutation was from dependence on streptomycin for growth, which is the result of mutation in the 30S ribosomal protein S12 (E. Dabbs, personal communication). Such a selection is well known to give Ram mutants with changed ribosomal protein S4 or S5. A functional interplay between mutations affecting the two ribosomal subunits is further indicated by our findings that the rplL-159- and rplL-265-dependent Ram phenotype in vivo is counteracted by the very same mutations in rpsL (S12), which also counteract the Ram phenotype of several rpsD (S4) mutants (not shown). Furthermore, we find that the rplL-associated misreading is seen in the case of both tRNA\(^{\text{AGA}}\) and tRNA\(^{\text{AGC}}\). Misreading by these two tRNAs should be at the first and the third bases of the codon, respectively. A similar observation has been made on rpsD-dependent misreading by these tRNAs in vivo (23). Thus, the increased error is not sensitive to the nature of the codon–anticodon mismatch irrespective of whether the ribosomal mutation affects the 30S subunit, as in the case of rpsD, or the 50S subunit as discussed here.

The reason for the decreased proofreading efficiency of the rplL mutants can only be speculated. Protein L7/L12 is present in four copies in the ribosome unlike other ribosomal proteins, which are present in at most unit amounts. It is part of, or is close to, the binding site for translation factors EF-Tu, EF-G, and initiation factor 2 (IF2) and is also of importance for their GTPase activities (25). One possibility therefore would be that the GTPase activity of some of the factor(s) is affected as a result of the L7/L12 alteration. The forward flows of both cognate and non-cognate aminoacyl-tRNA, as well as the proofreading efficiency, are controlled by the combined effects of GTP hydrolysis together with displacements from equilibrium that counteract the back reaction(s) (18, 19). It is possible that even a small disturbance of the process could have a profound effect on the translational accuracy. Alternatively, the mutation may give an altered affinity for some translation factor(s). So far, our preliminary experiments indicate, however, that the apparent \(K_m\) values for EF-Tu–GTP–Phe–tRNA\(^{\text{Phe}}\) or EF-G–GTP are not altered as a result of the rplL mutations (not shown).

The three-dimensional structure of the COOH-terminal part of the wild-type L7/L12 is known (43) and more structural information about this important protein will soon be available. Clearly, a detailed knowledge about the functional impact of the L7/L12 alterations together with an elucida-

---

**Table 4. Rates of protein synthesis in vivo and in vitro**

<table>
<thead>
<tr>
<th>Relevant genotype</th>
<th>Rate, amino acids per sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>rplL*</td>
<td>12</td>
</tr>
<tr>
<td>rplL-159</td>
<td>11</td>
</tr>
<tr>
<td>rplL-265</td>
<td>12</td>
</tr>
</tbody>
</table>

**Note:** Translational step-time in vivo was calculated from times required for the synthesis of β-galactosidase following enzyme induction (16, 31). Rates in vitro were measured by \([^{14}C]\)phenylalanine incorporation in a poly(U)-dependent translation system using 70S ribosomes from strains as indicated (36).

**DISCUSSION**

Mutations that affect ribosomal protein L12, or its N-acetylated form L7, have been described previously but they have not been demonstrated to give any apparent phenotypic effect (3, 40). Here we have shown that a particular strain with a glyT-derived tRNA mis-sense suppressor (38) also harbors a mutation in rplL that gives an electrophoretically altered L12 protein. When this rplL-159 mutation was separated from the glyT mutation (unpublished data) it was found that the ribosomal mutation is associated with a decreased translational accuracy both in vivo and in vitro. The general importance of protein L7/L12 for the control of translational accuracy is indicated by the finding that another uncharacterized mutation (rplL-265), which was isolated by E. Dabbs, was shown to be even more severely affected in this respect.

Ribosomal ambiguity mutants with a changed protein S4 have been extensively studied earlier. Such mutants have been found to show an essentially normal rate of translation despite their increased translational misreading. On the other hand, a coupling between decreased rate and increased accuracy might be seen in the case of some mutants with changed protein S12 (9). The lack of a simple relation between translational rate and accuracy is probably fully explained by the finding that S4 and S12 mutants mainly appear to be associated with a changed efficiency of the translational proofreading step(s) (ref. 23; T. Ruusala, personal communication). The rplL mutants investigated here appear to have the same rate of translation in vivo as the control strain. These estimates for translational step time (11 or 12 amino acids per sec) are significantly lower than other published estimates [17 or 18 amino acids per sec (16)]. This discrepancy probably has a technical explanation since previous published estimates are based on a too low amino acid content of the β-galactosidase enzyme. If the previous estimates are corrected for the proper value of this content (41) they become around 14 amino acids per sec, which is at or very close to the normal range of variation from one set of experiments to another. Nevertheless, within our set of data it appears that the rplL mutants are very similar to the control strain in translational step-time in vivo. Our determinations of translation rate in the poly(U)-dependent in vitro system do, however, consistently indicate that the active ribosomes from the rplL mutants are slower than normal ribosomes. However, since growth rates are identical within the limit of detection when rplL* and rplL isogenic mutant strains are compared in an amino acid-supplemented glucose minimal medium (not shown), we believe that the discrepancy between in vitro and in vivo rate determinations demands a more careful examination of the behavior of the mutant ribosomes in the in vitro systems.

The rplL-159 mutation described here was fortuitously

---

tion of the nature of the mutational defect could be directly incorporated into the structural models of protein L7/L12. This would help to clarify its interactions with some of the translation factors and other parts of the ribosome.

We are deeply indebted to Dr. Eric Dabbs and Prof. Emanuel Murgola for their generosity in making unpublished strains available to us and for valuable comments on their history. We are also much obliged to Prof. Charles G. Kurland for constructive criticism, comments on the manuscript, and availability of purified components for the in vitro systems. Thanks are due to Tarmo Ruusala, Siv Andersson, and Dan Andersson for guidance in using the in vitro systems and to Katarina Ringström for her technical assistance. The critical reading of the manuscript by several people in this department is gratefully acknowledged. This work was supported by grants from the Swedish National Science Research Council to L.A.I. (S-FO 3703-115 and B-BU 3703-114) and from the Swedish Cancer Society (520-B85-15X-A) and the Swedish National Science Research Council (B-BU 3218-111) to C. G. Kurland.