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

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

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**ABSTRACT** **The effects of two mutations, which map at the *rplL* 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 $tRNA^{Leu}$ *in vitro*. The *rplL*-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 as a result of a mutation in the structural gene (*rpsL*) for protein S12 (7) or to neamine as a result of a changed protein S17 (8). The *rpsL* 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 GTPase-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 a 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.

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## 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 P1 transductions were performed as described by Miller (26). Introduction of the various *rplL* alleles into UD132, which is genetically identical to XAc, was done by cotransduction with $Arg^+$. The donor for the *rplL*-159 allele was UL314. The $rplL^+$ allele originates from UE123 and the *rplL*-265 allele originates from VT265. The resulting strains UY211, 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 *rplL* 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 *rplL* genotype. In all cases, the *rplL* 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*, *zij*::Tn*10-115* derivative of O17 by cotransduction with Tet-R. The *rplL*-159 and *rplL*-265 alleles were then introduced by P1 transduction by using UL314 and UY154 as donors and selecting for $Arg^+$ 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′ 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

Table 1. Strains of *E. coli* K-12 used in this study

| Designation | Sex; extrachromosomal markers | Chromosomal markers | Comment | Source or ref. |
|---|---|---|---|---|
| O17 | $F^-$ | *rplL*$^+$ | | 14 |
| UY128 | $F^-$ | *rpoB*, *argE*, *zij*::Tn*10-115* | O17 derivative | This paper |
| UY134 | $F^-$ | *rplL*-159 | O17 derivative | This paper |
| UY143 | $F^-$ | *rplL*-265 | O17 derivative | This paper |
| XAc | $F^-$ | Δ(*prolac*)Ara, *gyrA*, *rpoB*, *argE*(UAG) | | J. Miller |
| UY211 | F | Δ(*prolac*)Ara, *gyrA*, *rplL*$^+$ | XAc derivative | This paper |
| UY131 | $F^-$ | Δ(*prolac*)Ara, *gyrA*, *rplL*-159 | XAc derivative | This paper |
| UY154 | $F^-$ | Δ(*prolac*)Ara, *gyrA*, *rplL*-265 | XAc derivative | This paper |
| UD132 | $F^-$ | Δ(*prolac*)Ara, *gyrA*, *rpoB*, *argE*(UAG) | XAc derivative | 16 |
| KL37 | $F^-$ | Δ(*trpABtonB*)*argH*, *glyV55*, *glyS*$_H$ | | E. Murgola |
| UE123 | $F^-$ | Δ(*trpABtonB*)*glyS*$_H$, *glyV55* | KL37 derivative | E. Murgola and this laboratory |
| UE125 | $F^-$ | Δ(*trpABtonB*)*glyS*$_H$, *glyV55*, *glyT*Su$^+$56 | KL37 derivative | E. Murgola and this laboratory |
| UE135 | $F^-$ | Δ(*trpABtonB*)*glyS*$_H$, *glyV55*, *glyT*Su$^+$159, *rplL*-159 | KL37 derivative | E. Murgola and this laboratory |
| UL314 | $F^-$ | *rplL*-159, Δ(*trptonB*) | KL37 derivative | This laboratory |
| VT265 | Hfr | *rplL*-265, *rpsH*, *rpsL*, *metB*-1 | | E. Dabbs |
| CSH23 | F'*lac*$^+$*proA*$^+$,*B*$^+$ | Δ(*prolac*)*su*$^-$, *rpsE*, *thi* | | 26 |

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 Polymix 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 Polymix 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$^+$159) showed a resemblance to *rspD* (S4) strains in misreading of certain nonsense mutations (unpublished results). Su$^+$159 strains are known to be double mutants harboring a *glyT*-derived $tRNA_2^{Gly}$ 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 $tRNA_2^{Gly}$ 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

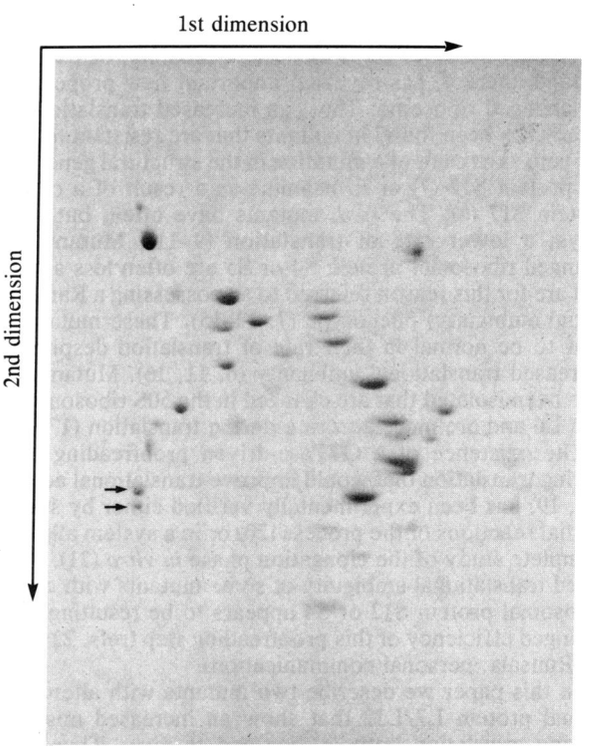


FIG. 1. 70S ribosomal proteins from UE135 and UE125 were mixed and analyzed in two-dimensional electrophoresis (33). The upper and lower small arrows indicate the position of protein L7/L12 from a *rplL*$^+$ and *rplL*-159 strain, respectively. Origin and direction of electrophoresis is indicated by large arrows.

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

| Strain | Relevant genotype | Position 84 | | Position 117 | | Position 181 | | Position 189 | | | Position 220 | | | Position 228 | | Position 280 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | UAG | UAA | UAG | UAA | UAG | UAA | UAG | UAA | UGA | UAG | UAA | UGA | UAA | UAG | UGA |
| UY211 | *rplL*$^+$ | 1.0 | 1.5 | 0.4 | 0.5 | 0.9 | 0.4 | 0.9 | 0.5 | 88 | 8.3 | 7.1 | 92 | 1.7 | 3.2 | 5.8 |
| UY131 | *rplL*-159 | 2.9 | 2.9 | 0.7 | 0.9 | 3.0 | 1.4 | 2.9 | 1.8 | 270 | 14 | 8.6 | 210 | 6.3 | 4.1 | 18 |
| UY154 | *rplL*-265 | 3.7 | 4.4 | 0.8 | 0.9 | 4.8 | 2.6 | 5.6 | 3.7 | 920 | 15 | 8.4 | 460 | 9.3 | 7.1 | 26 |

Position refers to the position of the nonsense codon in the *lacI* part of a fused *lacIlacZ* gene. Data are shown as β-galactosidase activity ($\times 10^4$) related to the value in a corresponding strain without nonsense mutation.

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 *rplL* derivatives of UD132 so obtained were then infected with a F′ factor containing a hybrid *lacIlacZ* 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 *lacIlacZ* 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 *lacIlacZ* 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 $tRNA_4^{Leu}$ and $tRNA^{Phe}$. 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.

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

| Relevant genotype | Error $\times 10^4$* | Error $\times 10^4$† | Proofreading factor‡ |
|---|---|---|---|
| *rplL*$^+$ | 5.1 | 5.9 | 93 |
| *rplL*-159 | 8.1 | 9.9 | 84 |
| *rplL*-265 | 13 | 23 | 36 |

Error determinations were done by using precharged $tRNA_4^{Leu}$ and $tRNA^{Phe}$ and purified factors together with ribosomes as indicated.

*With 70S ribosomes.

†With 70S 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 70S 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.

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.

All *in vitro* experiments were also done by using purified $tRNA_2^{Leu}$ instead of $tRNA_4^{Leu}$, together with $tRNA^{Phe}$ 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 quantitative 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 4. Rates of protein synthesis *in vivo* and *in vitro*

| Relevant genotype | Rate, amino acids per sec | |
|---|---|---|
| | *In vivo* | *In vitro* |
| *rplL*$^+$ | 12 | 8.9 |
| *rplL*-159 | 11 | 7.5 |
| *rplL*-265 | 12 | 5.0 |

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).

are compared *in vivo* and *in vitro* and with each other, a certain discrepancy might be found when the *in vivo* rates of translation are compared to these obtained *in vitro*.

## 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 missense 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 then 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* isogeneic 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 found in a strain that carries a *glyT*-derived AGA/G missense 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 $tRNA_2^{Gly}$ to read AGA/G instead of GGA/G, is normally lethal since $tRNA_2^{Gly}$ 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 $tRNA_{GGG}^{Gly}$, $tRNA_{GGU/C}^{Gly}$, or the $tRNA_{AGA/G}^{Gly}$ 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 amino-acyl-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 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 *rspD* (S4) mutants (not shown). Furthermore, we find that the *rplL*-associated misreading is seen in the case of both $tRNA_2^{Leu}$ and $tRNA_4^{Leu}$. 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 vitro* (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 mutant ribosomes could have 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–$tRNA^{Phe}$ 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-

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.

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