Escherichia coli Cells Bearing a Ribosomal Ambiguity Mutation in rpsD Have a Mutator Phenotype That Correlates with Increased Mistranslation

Sergey Balashov and M. Zafri Humayun*

University of Medicine and Dentistry of New Jersey—New Jersey Medical School, Department of Microbiology and Molecular Genetics, International Center for Public Health, Newark, New Jersey 07101-1709

Received 18 March 2003/Accepted 23 May 2003

In Escherichia coli mutA cells, a mutation in a gene specifying a glycine tRNA (glyV) leads to a significant mutator phenotype (14, 25). The mutA allele differs from the wild-type (glyV⁺) tRNA gene by a single base substitution that alters the anticodon in such a way as to promote Asp→Gly mistranslation in mutA cells. Subsequent to this finding, other work revealed that mutations in many other genes that specify tRNAs (10, 26) or tRNA-modifying enzymes (32) lead to a similar phenotype presumably mediated by mistranslation. Moreover, exposure to streptomycin, an antibiotic known to promote mistranslation, induces a very similar phenotype in cells bearing certain mutations in rpsL, the gene specifying the 30S ribosomal protein S12 (6). Thus, mistranslation appears to be causally required for provoking the mutator phenotype, termed TSM for translational stress-induced mutagenesis (12). Interestingly, the mutator phenotype induced by mutA is suppressed in cells defective for RecABC-mediated homologous recombination functions (20, 21). mutA cells have an error-prone DNA polymerase activity (2) that is most likely to be a modified form of DNA polymerase III (1). The mechanism by which mistranslation leads to the TSM phenotype is not known, but two hypotheses are considered elsewhere in this communication.

Certain mutations in genes specifying the 30S ribosomal protein S4 (4, 19, 33), ribosomal protein S5 (28), 16S RNA (17), or 23S RNA (16) confer a so-called ribosomal ambiguity (Ram) phenotype characterized by decreased growth rates, increased sensitivity to streptomycin, and increased translational errors. It has been hypothesized that reduced proofreading by defective ribosomes in Ram cells leads to a generalized increase in translational errors (5). The best-studied Ram mutants are those that affect rpsD, the gene encoding S4. S4 is an RNA-binding protein that plays an essential role in the assembly of the 30S ribosomal subunit by binding to the 5′-terminal 500-nucleotide region of 16S rRNA (15, 31). Interaction with 16S rRNA may place S4 close to the ribosomal decoding center, which would be consistent with the effect of S4 mutants on ribosomal accuracy. S4 is an autoregulator that translationally represses the mRNA transcribed from the alpha operon encompassing the genes for ribosomal proteins S13, S11, S4, and L17 (13, 27, 29). Recently, S4 was shown to act also as a transcription factor that tightly binds to RNA polymerase and is involved in antitermination of Rho-dependent termination of transcription (30).

Here we examined whether cells bearing rpsD14, a widely used allele known to confer a Ram phenotype, also confer a mutator phenotype in a way that correlates with increased mistranslation. The wild-type rpsD allele codes for a 206-amino-acid-long polypeptide, whereas the rpsD14 allele codes for a 182-amino-acid-long truncated protein because of a +1 frameshift mutation (insertion of a C after G535) (9). Whereas ribosomal ambiguity mutations (such as certain rpsD mutations) increase mistranslation, ribosomal restrictive mutations (such as those in rpsL, which encodes the 30S ribosomal protein S12) increase translational accuracy and are streptomycin resistant (Strₚ). We have previously isolated and characterized rpsL1408, which bears a K87E mutation in S12, with very interesting dual properties: in the absence of streptomycin, rpsL1408 cells show hyperaccurate translation (high restrictivity relative to rpsLₚ wild-type cells), whereas in the presence of 100 μg of streptomycin/ml, rpsL1408 cells display significantly elevated mistranslation levels (6).

To determine whether the rpsD14 allele conferred a TSM phenotype that correlated with mistranslation, we first sought to demonstrate that cells bearing the allele showed increased translational errors by using a nonsense suppression assay. Table 1 lists the strains used in this study, and Table 2 shows the relative mistranslation levels for strains harboring the rpsD14 and rpsL1408 alleles measured as translational (phenotypic) suppression of nonsense codons in plasmid-borne lacZ alleles (16, 18) (see Table 1 for a list of plasmids). Nonsense suppression is up to threefold higher in rpsD14 cells than in wild-type cells, and this increased suppression is eliminated in rpsD14 rpsL1408 double mutants in the absence of streptomycin, as predicted from the contrasting effects of these two mutations, a finding that is consistent with similar earlier ob-
Table 1. Bacterial strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Genotype and/or phenotype</th>
<th>Source, derivation, and/or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>E. coli</strong> strains</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AM110</td>
<td>recA938::Tn9-200 (Cm') in KH2</td>
<td>This laboratory (A. A. M. A1 Mamun and M. Z. Himayun, unpublished data; 24)</td>
</tr>
<tr>
<td>AM121</td>
<td>lacI (Ind') malF3089::Tn10 (Tet')</td>
<td>This laboratory (3)</td>
</tr>
<tr>
<td>BS1408</td>
<td>rpsL1408 (Str') ara Δ(pro-B-lac) thi F' (lacI lacZ proB')</td>
<td>This laboratory (6)</td>
</tr>
<tr>
<td>BS1480^a</td>
<td>F^- rpsL1408 (Str') ara argE Δ(pro-B-lac) nalA rif thi F'(lacI lacZ proB')</td>
<td>This work (P1 BS1408 × XAc to Str')</td>
</tr>
<tr>
<td>BS1481^b</td>
<td>F^- rpsL1408 rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (P1 BS1408 × UL478 to Str')</td>
</tr>
<tr>
<td>BS1482</td>
<td>ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (conjugation CC105 × XAc; Pro^- Rif^+ Nat^+)</td>
</tr>
<tr>
<td>BS1483</td>
<td>rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (conjugation CC105 × UL478; Pro^- Rif^+ Nat^+)</td>
</tr>
<tr>
<td>BS1484</td>
<td>rpsL1408 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (conjugation CC105 × BS1480; Pro^- Rif^+ Nat^+)</td>
</tr>
<tr>
<td>BS1485</td>
<td>rpsL1408 rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (conjugation CC105 × BS1483 to Cm')</td>
</tr>
<tr>
<td>BS1486^c</td>
<td>rpsL1408 rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (P1 AM110 × BS1483 to Cm')</td>
</tr>
<tr>
<td>BS1487^d</td>
<td>rpsL1408 rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>This work (P1 AM121 × BS1483 to Tet')</td>
</tr>
<tr>
<td>CC105</td>
<td>ara Δ(pro-B-lac) thi F' (lacI lacZ proB')</td>
<td>J. H. Miller (8)</td>
</tr>
<tr>
<td>UL478</td>
<td>F^- rpsD14 ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>E. Goldman (19)</td>
</tr>
<tr>
<td>XAc</td>
<td>F^- ara argE Δ(pro-B-lac) nalA rif thi F' (lacI lacZ proB')</td>
<td>E. Goldman (7, 19)</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pSG163</td>
<td>lacZ (UAG)</td>
<td>A. Dahlberg (18)</td>
</tr>
<tr>
<td>pSG853</td>
<td>lacZ (UAA)</td>
<td>A. Dahlberg (18)</td>
</tr>
<tr>
<td>pSG3/4</td>
<td>lacZ (UGA)</td>
<td>A. Dahlberg (18)</td>
</tr>
</tbody>
</table>

^a Other markers are thr-1 araC14 leuB6 Δ(gap-proA)62 lacY1 tss-33 gvrV44 (AS) gskK2 (Oc) λ-Rac-O hisG4 (Oc) rfb1 mdg-51 rpsL31 (Str') kdgK51 mtl-1 metB1 thi-1.
^b Transfer of the rpsL allele was confirmed by PCR amplification and DNA sequence analysis of the transductants.
^c Antibiotic-resistant transductants were tested for UV sensitivity by streaking on Luria-Bertani agar alongside wild-type cells and exposing the streaks to UV.
^d Plasmids were derived from pACYC184 (18).

Table 2. Relative mistranslation in rpsD14 and rpsL1408 cells

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Strain</th>
<th>Relevant genotype</th>
<th>β-Galactosidase activity (Miller units)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSG163 (UAG)</td>
<td>XAc</td>
<td>Wild type</td>
<td>38.6 ± 1.7</td>
</tr>
<tr>
<td>UL478</td>
<td>rpsD14</td>
<td></td>
<td>115.7 ± 12.2</td>
</tr>
<tr>
<td>BS1480</td>
<td>rpsL1408</td>
<td></td>
<td>18.6 ± 1.1</td>
</tr>
<tr>
<td>BS1481</td>
<td>rpsD14 rpsL1408</td>
<td></td>
<td>25.3 ± 2.4</td>
</tr>
<tr>
<td>pSG853 (UAA)</td>
<td>XAc</td>
<td>Wild type</td>
<td>19.6 ± 1.5</td>
</tr>
<tr>
<td>UL478</td>
<td>rpsD14</td>
<td></td>
<td>27.3 ± 3.8</td>
</tr>
<tr>
<td>BS1480</td>
<td>rpsL1408</td>
<td></td>
<td>9.7 ± 2.4</td>
</tr>
<tr>
<td>BS1481</td>
<td>rpsD14 rpsL1408</td>
<td></td>
<td>15.2 ± 3.4</td>
</tr>
<tr>
<td>pSG3/4 (UGA)</td>
<td>XAc</td>
<td>Wild type</td>
<td>106.5 ± 6.0</td>
</tr>
<tr>
<td>UL478</td>
<td>rpsD14</td>
<td></td>
<td>249.2 ± 23.8</td>
</tr>
<tr>
<td>BS1480</td>
<td>rpsL1408</td>
<td></td>
<td>17.2 ± 1.9</td>
</tr>
<tr>
<td>BS1481</td>
<td>rpsD14 rpsL1408</td>
<td></td>
<td>30.5 ± 6.6</td>
</tr>
</tbody>
</table>

* Values shown are averages ± standard deviations of results from three experiments carried out as described previously (6). – STR, without streptomycin; + STR, with streptomycin.
wild-type strain (BS1482; see Table 1 for strain derivation) displays a normal low level of papillation characterized by a small number of dark lacZ+ colonies growing on a colorless lawn of lacZ cells. In contrast, BS1483, a strain carrying an rpsD14 allele in an isogenic background, shows numerous papillae, a hallmark of a mutator strain. Introduction of the rpsL1408 allele, as demonstrated above, should increase translational accuracy in rpsD14 cells, which in turn should suppress the mutator phenotype of rpsD14 cells in the absence of streptomycin, as indeed was observed for BS1485 (rpsD14 rpsL1408) cells. When streptomycin is included in the agar (Fig. 1B), both BS1485 (rpsD14 rpsL1408) and BS1484 (rpsL1408) cells display a mutator phenotype (6), which is consistent with the reversal of the rpsL1408 effect by streptomycin and parallels similar reversals in growth effects as noted above. Table 4 summarizes spontaneous mutation rates in rpsD14 and rpsD14 rpsL1408 cells, confirming that rpsD14 confers a mutator phenotype that is eliminated by rpsL1408 in the absence of streptomycin. Therefore, the mutator phenotype displayed by rpsD14 cells is strongly correlated with mistranslation.

It is interesting that whereas the rpsD14 mutator effect is nullified by rpsL1408 in the absence of streptomycin, the mutator effect of streptomycin in rpsL1408 rpsD14 cells is unaffected, an expected result because in the presence of streptomycin rpsL1408 promotes rather than restricts such translational errors. In a sense, the rpsL1408 effect is dominant over that of rpsD14 in both the presence and absence of streptomycin.

We have previously shown that expression of the TSM mutator phenotype does not require SOS induction in mutA cells (20) or in rpsL1408 cells exposed to streptomycin (6). Figure 1C shows that the mutator phenotype of rpsD14 cells persists in lexA1 (Ind-) cells, showing that SOS induction is also not required in rpsD14 cells. However, in contrast to the situation in mutA cells (14, 20, 21), mutA cells (32), and streptomycin-exposed rpsL1408 cells (6), the rpsD14 mutator phenotype is not suppressed in recA cells. This last finding is similar to the lack of a recA requirement for the mutator phenotypes displayed by alaV<sup>Glu</sup> and gly<sup>Glu</sup> mutator tRNAs (10) and argues that there is no absolute requirement for RecBC-mediated recombination genes for the expression of the mutator phenotype.

Two hypotheses to explain how translational errors can lead to a mutator phenotype have been proposed. The epsilon subunit mistranslation hypothesis suggests that in mutA cells, low-level Asp→Gly mistranslation creates a small fraction of inactive epsilon protein (the proofreading subunit of DNA polymerase III holoenzyme), which when recruited into a func-

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Generation time&lt;sup&gt;a&lt;/sup&gt; (min)</th>
<th>Growth at 44°C&lt;sup&gt;b&lt;/sup&gt;</th>
<th>STR sensitivity&lt;sup&gt;c&lt;/sup&gt; (MIC: µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>− STR</td>
<td>+ STR</td>
<td>− STR</td>
</tr>
<tr>
<td>BS1482</td>
<td>Wild type</td>
<td>27</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>BS1483</td>
<td>rpsD14</td>
<td>49</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>BS1484</td>
<td>rpsL1408</td>
<td>42</td>
<td>44</td>
<td>+</td>
</tr>
<tr>
<td>BS1485</td>
<td>rpsD14 rpsL1408</td>
<td>35</td>
<td>41</td>
<td>+</td>
</tr>
</tbody>
</table>

<sup>a</sup> Generation times were determined from viable-cell counts of two culture aliquots taken at the beginning and the end of the logarithmic phase of growth in Luria-Bertani medium at 37°C (14, 20, 21), and streptomycin (STR). The following standard equation was used: \( G = t / [3.3 \log (b/b_0)] \), where \( G \) is the generation time, \( t \) is the time interval in minutes, \( b \) is the cell count at the beginning of the time interval, and \( b_0 \) is the cell count at the end of the time interval used.

<sup>b</sup> Cells (from fresh overnight colonies grown on Luria-Bertani agar at 37°C) were streaked on Luria-Bertani agar and incubated overnight at 44°C.

<sup>c</sup> MICs of streptomycin (STR) were determined by using the Etest (AB Biodisk, Solna, Sweden) in accordance with the procedures described by the manufacturer.
The induced pathway hypothesis generalizes the mechanism to many types of mistranslation beyond Asp-Gly misreading and postulates that increased translational errors generate a signal (increased protein turnover or creation of tRNAs that are error-prone). Thus, both hypotheses invoke a mechanism for the modiﬁcation of ribosomal functioning. Cold Spring Harbor Symp. Quant. Biol. 34:101–109.

At present, no experimental basis is available to interpret the apparent requirement for homologous recombination functions for the expression of the TSM phenotype under different mistranslation conditions. A speculation, and experimental evidence available to date cannot be unequivocally interpreted as supporting one mechanism over the other.

We thank E. Goldman for the XAc and UL478 strains and for helpful discussion.

This work was supported in part by NIH grant GM58253.

REFERENCES


