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The C-Terminus of Ribosomal Protein uS4 Contributes to Small Ribosomal Subunit Biogenesis and the Fidelity of Translation

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Abstract

Ribosomal protein uS4 is an essential ribosomal component involved in multiple functions, including mRNA decoding. Structural analyses indicate that during decoding, the interface between the C-terminus of uS4 and protein uS5 is disrupted and in agreement with this, C-terminal uS4 truncation mutants are readily isolated on the basis of their increased miscoding phenotypes. The same mutants can also display defects in small subunit assembly and 16S rRNA processing and some are temperature sensitive for growth. Starting with one such temperature sensitive *Escherichia coli* uS4 mutant, we have isolated temperature insensitive derivatives carrying additional, intragenic mutations that restore the C-terminus and ameliorate the ribosomal defects. At least one of these suppressors has no detectable ribosome biogenesis phenotype, yet still miscodes, suggesting that the C-terminal requirements for ribosome assembly are less rigid than for mRNA decoding. In contrast to the uS4 C-terminal mutants that increase miscoding, two *Salmonella enterica* uS4 mutants with altered C-termini have been reported as being error-restrictive. Here, reconstruction experiments demonstrate that contrary to the previous reports, these mutants have a distinct error-prone, increased misreading phenotype, consistent with the behavior of the equivalent *E. coli* mutants and their likely structural effects on uS4-uS5 interactions.

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1. Introduction

Early investigations into the decoding process identified ribosomal proteins uS4, uS5 and uS12 as key players involved in the ribosome's discrimination between cognate and near-cognate tRNAs [1]. The advent of high resolution structures of ribosomes has provided structural interpretations of decoding and the roles of these proteins. Sensing of correct codon-anticodon pairings in the decoding center is proposed to trigger large scale rearrangements of the 30S subunit, resulting in the transition from an 'open' to a 'closed' conformation and the eventual activation of GTP hydrolysis of aminoacyl-tRNA-EF-Tu•GTP [2]. These structural rearrangements on the 30S subunit include the disruption of interactions between ribosomal proteins uS4 and uS5 and the establishment of 16S rRNA-uS12 contacts. In support of this model, at least some of the mutations that influence decoding affect residues on the interface between proteins uS4 and uS5 [3]. In uS4, these alterations include amino acid substitutions as well as truncations that remove C-terminal residues, all of which are predicted to disrupt the uS4-uS5 interface (Fig.1). In addition to its role in decoding, ribosomal protein uS4 helps initiate the assembly of the small subunit by binding directly to 16S rRNA and at least some of the uS4 alterations that promote miscoding also display assembly defects [4]. uS4 is also a translational repressor [5] and is involved in transcription antitermination activities [6]. Consequently, mutations in uS4 can potentially have multiple effects on ribosome regulation, assembly and functionality.

A majority of the C-terminal uS4 mutants display increased miscoding [7]; one interpretation of this phenotype is that the altered ribosomes are predisposed to assume the closed conformation, even with near-cognate tRNAs, since the uS4-uS5 interface is already disrupted in the mutant ribosomes. However, at least some interface-disrupting uS4 mutants have an error-restrictive phenotype, which is difficult to reconcile with the open-closed model [8]. These uS4 mutations, isolated in *Salmonella enterica* carry a deletion of Val200 (V200) or a truncation at Glu201 (E201*) [9]. Puzzlingly, the same E201* mutation was recently generated in *E. coli* in our laboratory and was shown to have an error-prone, ribosomal ambiguity (*ram*) phenotype [7]. In an effort to reconcile these discrepancies and to investigate the roles of the C-terminal region of uS4 in multiple ribosomal functions, we have isolated and characterized intragenic suppressors of an *E. coli* uS4 C-terminal deletion mutant and have reconstructed the V200 and E201* mutations *de novo* both in *E. coli* and in *S. enterica*. We find that 16S rRNA processing, 30S biogenesis and translational fidelity are all affected by C-terminal truncations and that in contrast to earlier reports, the V200 and E201* alterations in uS4 have a distinct error-prone phenotype in both bacteria. Moreover, in the course of our investigations, we found that two of the well-characterized uS4 C-terminal truncation mutants also carry unanticipated mutations affecting ribosomal protein uS17.

2. Materials and methods

2.1. Bacterial strains and plasmids

The *E. coli* strains D12, D14, and D16 [10] carrying previously characterized temperature sensitive *rpsD* mutations were obtained from Dr. Monica Ryden-Aulin, Stockholm University, Sweden. Strain FG2 (F⁻ (*lac-pro*) *thi leu-48* (UGA) *prfB* (*ts*) *rpsL* (strep^R) *rpsD*

(+T536)) carries an *rpsD* mutation that was isolated in this laboratory as a faster-growing derivative of a slow-growing *prfB* mutant, carrying a temperature sensitive RF2 release factor. All *E. coli* uS4 mutants were reconstructed by P1 transduction in strain MC361 (F⁻ *ara* (*gpt-lac*)5 *thi prfB* (from *E. coli* B)) that carries the fully active A246 RF2 [11]. A *aroE721::kan* derivative of MC361 was transduced to Aro⁺ with P1 on each *rpsD* mutant and transductants were initially screened for temperature sensitivity, a phenotype of the *rpsD* mutations under investigation. The presence of *rpsD* mutations was confirmed by sequencing an amplified *rpsD* fragment.

The E201* and V200 mutations in *E. coli rpsD* were constructed by recombineering as previously described [7]. Briefly, using chromosomal DNA from a strain with a kanamycin resistance (KanR) cassette inserted immediately downstream of the *rpsD* coding region as a template, an *rpsD*-KanR fragment was amplified with forward primers carrying the desired *rpsD* mutation and a reverse primer flanking the 3' side of the KanR cassette. The amplified fragments were electroporated into strain MC323 (F⁻ *lacZ521* [*Tn10* linked] *rph-I*) expressing λ Red recombinase from pKD46 and kanamycin resistant transformants were selected. Increased misreading in this strain gives rise to dark blue colonies on X-gal containing medium. The *rpsD* gene was sequenced from blue colonies which were found to carry the E201* or V200 alterations. These mutations were then transferred into MC361 by electroporation of the purified *rpsD*-KanR fragment. Kanamycin resistance cassettes were removed by transient expression of the Flp recombinase from plasmid pCP20.

During the course of this work, it was found that strains D16, D12, and some of their derivatives carried an unanticipated *rpsQ* mutation that generates an E62K substitution in ribosomal protein uS17. Since the *rpsD* and *rpsQ* genes are very tightly linked, selected *rpsD* mutants lacking the *rpsQ* substitution were reconstructed in MC361 by recombineering, using the mutant oligonucleotide-directed approach described above. The *rpsD* and *rpsQ* genes were sequenced in all reconstructed strains to confirm absence of any undesired mutations.

The E201* and V200 mutations in *S. enterica rpsD* were constructed in a similar fashion. A KanR cassette was first inserted immediately after the *rpsD* stop codon by standard recombineering procedures and genomic DNA from the resulting strain used as a template to generate the E201* and V200 mutations. The amplified fragments were first introduced into MS72 (LT2 *pro::Tn10F' lacZ* (UGA)) expressing λ Red recombinase and darker blue colonies were identified and the *rpsD* gene was sequenced. Wild type and mutated *rpsD* genes along with the linked KanR cassettes were transferred to a wild type LT2 strain by KB1-mediated transduction. The KanR cassette was removed by expression of Flp recombinase. The *S. enterica* strains carrying the *rpsD* mutations described in Bjorkman *et al.* [9] were obtained from Dr. Diarmaid Hughes, Uppsala University, Sweden.

Plasmids p34-11 UGA and p12-6 UAG, carrying a UGA or UAG stop codon in the 5' coding region of the *lacZ* coding sequence respectively, were used to monitor stop codon readthrough. Plasmids pKD46, pKD4 and pCP20 were used for recombineering [12].

2.2 Growth and antibiotic sensitivity determinations, enzyme assays, and rRNA processing and ribosome analyses

Growth on solid media was analyzed by plating 10-fold serial dilutions of overnight cultures on LB plates and incubating them at 20°C, 37°C, and 42°C for 24 to 48 h. Minimal inhibitory concentrations (MIC) for streptomycin were determined using 2-fold serial dilutions of antibiotic in 96-well microtiter dishes.

Misreading levels were determined using two ampicillin resistant *lacZ* reporter constructs containing either a premature UGA (p34-11) or UAG (p12-6) stop codon. Logarithmically growing cells at 37°C or 42°C were permeabilized and *O*-nitrophenyl- β -D-galactoside was used as a substrate in assays of β -galactosidase activity. At least 3 independent cultures of each strain were tested and the activity was expressed in Miller units [13].

Isolation and analysis of total RNA from logarithmically growing cells at 37°C and 42 °C was carried out on 0.7% agarose/0.9% Synergel (Diversified Biotech, Dedham MA) gels, as described by Wachi *et al.* [14]. The agarose-Synergel system can separate 23S rRNA from 16S rRNA and its precursors. In addition, RNAs were also isolated from wild-type strains treated with chloramphenicol (final concentration of 60 mg/ liter) for 30 min, which inhibits rRNA processing and leads to accumulation of 17S precursors of 16S rRNA[15]. RNA lysates from at least 3 independent cultures of each strain were analyzed.

Detergent lysates of cells grown logarithmically at 37°C and 42°C were prepared as previously described [16], in a buffer containing 25 mM Tris HCl pH 8.0/60 mM KCl/10 mM MgCl₂. The lysates were loaded onto a linear 10 to 40% sucrose gradient (in 25mM TrisHCl, pH 7.8/50mM KCl/ 10mM MgCl₂/ 3mM DTT) and subjected to centrifugation in a Beckman SW28 for 18h at 38,191 $\times$ g. The ribosomal subunits and 70S ribosomes were eluted by displacement with 60% glycerol and analyzed on an ISCO gradient fractionator connected to an ISCO UV6 detector. At least two different lysates from each mutant or wild type strain were analyzed on sucrose gradients

3. Results and Discussion

3.1. Intragenic suppressors of uS4 C-terminal truncations

The wild type uS4 protein is 205 amino acids long (numbering throughout omits the N-terminal methionine, which is removed [17]). The well-studied uS4 mutants D12, D14, and D16 [18–22] as well as the recently isolated FG2 mutant all have C-terminal truncations in uS4 as shown in Tables 1 and S1. D12 has a 5 base-pair deletion (C528 – A532; hereafter designated ‘ 5’) in the *rpsD* gene that causes premature termination and generates a 179 amino acid protein. Strains D14 and FG2 have single base insertions of cytosine and thymine, respectively, after G535 (designated +C536 and +T536, respectively). These base insertions lead to premature termination and generate 181 amino acid long proteins. The last four amino acids in these truncated proteins are also altered. In D16, a G to A base change at G509 results in the conversion of TGG (Trp) at codon 169 to a TAG stop codon and generates a 168 amino acid long uS4 protein [21]. All the four C-terminal truncated mutants were reconstructed in MC361 and all displayed the prominent temperature sensitive phenotype previously reported for these mutants.

In an effort to determine the contribution of the C-terminus of uS4 to distinct ribosomal functions, we have isolated and characterized temperature-insensitive derivatives of Δ 5, a profoundly temperature sensitive uS4 truncation mutant that does not revert back to a wild type *rpsD* sequence. Streaking of overnight cultures of this truncation mutant (reconstructed in MC361) on LB plates at 42°C yielded multiple temperature-insensitive mutants. Of the 13 colonies analyzed, 9 carried intragenic mutations in the *rpsD* gene. Among these 9 mutants, 6 unique intragenic suppressors were uncovered (Tables 1 and S1), while 3 of the suppressor mutations were encountered more than once. The 4 remaining strains (Δ 5rev6, Δ 5rev9, Δ 5rev11 and Δ 5rev12) retained the original 5 bp deletion present in the starting Δ 5 strain, but had no additional changes in *rpsD*. They thus carry extragenic suppressor mutations that render them temperature insensitive. Sequencing of *rpsE*, *rpsL*, *prfA* and *prfB* candidate genes encoding proteins uS5, uS12, RF1 and RF2, respectively, did not reveal any alterations and the identity of the extragenic suppressor mutations remains unknown.

The relevant DNA and amino acid sequences of the 6 temperature insensitive mutants carrying intragenic changes are shown in Tables S1 and 1. In the original Δ 5 mutant, the 5 bp deletion shifts the reading frame, leading to the synthesis of a shortened protein. In the intragenic suppressors, additional deletions of 1, 4 or 7 base pairs have the effect of restoring the original, zero reading frame and permit the synthesis of near-full-length proteins that are 2–4 residues shorter than wild type. In addition, in all the intragenic suppressor mutants, the regions of *rpsD* spanning the original 5 bp deletion and the sites of the suppressor mutations encode amino acids that differ from wild type uS4 (Table 1). The greatest number of changes is seen in Δ 5rev4, which differs at 10 positions, followed by Δ 5rev2, which differs at 8 positions. These amino acid substitutions and adjacent deletions all involve a small solvent-exposed loop (Fig.1). The intragenic suppressor mutants show varying degrees of growth recovery at 42°C (Fig. 2). Interestingly, Δ 5rev1 and Δ 5rev4 which show less robust growth at 42°C than the other suppressor mutants also grow poorly at 20°C.

3.2. An altered uS17 protein is present in some uS4 mutant strains

Ribosomal protein uS17 has been associated with neamine resistance and the restriction of miscoding in *E. coli* [23, 24]. In our efforts to identify the extragenic suppressors present in strains Δ 5rev6, Δ 5rev9, Δ 5rev11 and Δ 5rev12, the *rpsQ* gene was sequenced and all of these strains were found to carry a mutated *rpsQ* gene encoding a uS17 protein with an E62K alteration. Surprisingly, the same *rpsQ* mutation was present in the starting MC361 Δ 5 strain used to isolate all the temperature insensitive suppressors. Moreover, even though we had reconstructed each of the intragenic suppressor mutants in MC361 using P1 transduction, 5 of these (Δ 5rev1, Δ 5rev2, Δ 5rev3, Δ 5rev4, and Δ 5rev13) also carried the altered *rpsQ* gene, as did MC361 carrying *rpsD* (G509A). Only one reconstructed, temperature insensitive strain (Δ 5rev5) as well as MC361 *rpsD* (+C536) and MC361 *rpsD* (+T536) had wild type *rpsQ* sequences. The *rpsD* and *rpsQ* genes are separated by approximately 7.5 kb and are thus co-transduced at high frequency by P1 phage. The origin of the *rpsQ* (E62K) mutation is unclear. The *rpsD* mutants Δ 5, +C536 and G509A were isolated in the 1970s in separate experiments and in three independent strains, as suppressors of streptomycin dependence and were transferred by P1 transduction into a wild type strain, 017, at that time [10]. DNA sequencing showed that our isolates of strains D12 and D16 carry the same *rpsQ* (E62K)

mutation, while the 017 and D14 strains do not. Our multiple attempts to generate the *rpsQ* (E62K) alteration on its own, using two different strategies were unsuccessful, possibly suggesting that it may be inviable without uS4 alterations. The independent generation of the same *rpsQ* mutation in D12 and D16 strains is likely significant; proteins uS4 and uS17 bind to distinct regions of the 5' domain of 16S rRNA during the same early stage of 30S assembly [25] and conceivably, the uS17 E62K alteration may influence the assembly kinetics of uS4 mutant ribosomes at this early stage. That said, our limited experiments comparing the growth and other characteristics of isogenic wild type and *rpsQ* mutant strains indicate that some *rpsD rpsQ* double mutants are even more acutely temperature sensitive and their ribosome profiles show greater defects than *rpsD* single mutants (compare for instance, growth of Δ rev4 in Figs 2 and S1). It is thus unclear what, if any interaction occurs between the altered uS17 and uS4 proteins. However, the presence of an altered uS17 in D12 strains may confound interpretation of the several genetic and biochemical studies that have used this strain [19, 20, 22, 26].

All subsequent analyses described below used reconstructed strains with wild type *rpsQ* genes. In addition to the Δ rev5, +C536 and +T536 strains described above lacking the *rpsQ* mutation, the original temperature sensitive Δ 5 mutant as well as the temperature insensitive intragenic suppressor Δ rev4 were reconstructed by recombineering methods in MC361 and the *rpsQ* genes were re-sequenced to ensure that only wild type uS17 proteins were present in these strains. The growth on solid medium of the reconstructed Δ 5 mutant and Δ rev5 and Δ rev4 strains at various temperatures is depicted in Fig S1.

3.3. Miscoding and Ribosomal Properties of uS4 Mutant Strains

The availability of wild type, uS4 truncation mutants and intragenic suppressors with restored C-termini allow us to ask what activities are affected by loss of the uS4 C-terminus and which of these activities are restored in the suppressors. All 3 uS4 truncation mutants (Δ 5, +C536 and +T536) supported increased readthrough of UAG and UGA stop codons at both 37°C and 42°C and readthrough levels were consistently higher at 42°C (Fig. 3). In the Δ 5 mutant, UAG and UGA readthrough was each 5.7-fold higher than the wild type strain. In two derivatives of the Δ 5 truncation mutant carrying intragenic suppressors and a restored uS4 Cterminus (Δ rev4 and Δ rev5) stop codon readthrough was reduced relative to the original Δ 5 truncation, although it was still higher (average ca. 2-fold) than wild type. An earlier report describing some unsequenced temperature insensitive derivatives of a Δ 5 strain indicated that the *ram* phenotype (assayed by suppression of an amber mutation in phage T4) was lost in these strains [10]. The discrepancy between these earlier results and ours likely derives from the relative insensitivity of the phage growth assay used to monitor miscoding events.

Analysis of lysates on sucrose gradients showed that when grown at 37°C, none of the mutants had ribosome profiles that differed from wild type (not shown). In contrast, when grown at 42°C, the 3 C-terminal truncation mutants had grossly abnormal profiles, with little or no free 30S subunits and greatly increased levels of free 50S subunits (Fig 4). Impaired 30S assembly likely underlies both of these changes. When grown at 42°C, the 2 intragenic suppressor mutants showed varying levels of recovery; Δ rev5 showed a ribosome profile

indistinguishable from wild type, while the profile of Δ rev4 showed an intermediate level of recovery, with a modestly increased 50S peak.

rRNA processing in each of the mutants was also examined by analysis of lysates from cells grown either at 37°C or 42°C on agarose-Synergel gels. None of the cells grown at 37°C showed any processing defects (not shown). When grown at 42°C, all truncation mutants showed substantial accumulation of 16S rRNA precursors, while processing was ameliorated in the C-terminal restored mutants (Fig. 5).

Overall these analyses show that rRNA processing, ribosome profiles and the accuracy of translation are all impacted by loss of the uS4 C-terminus, particularly at higher temperatures. Restoration of C-terminal region alleviates temperature sensitive growth and reduces ribosomal profile abnormalities in both the Δ rev4 and Δ rev5 mutants. In the

Δ rev5 mutant, restoration is complete and the ribosomal profile is indistinguishable from wild type. The restored C-terminus also reduces, but does not eliminate, stop codon readthrough, which remains substantially above wild type in both the Δ rev4 and Δ rev5 mutants. This is consistent with our previous studies on miscoding, where only a minority of the uS4, uS5 or uS12 mutants display processing or assembly defects [7, 27, 28]. Thus, while the C-terminus has a major effect on both miscoding and ribosome profiles, ribosome biogenesis appears to be less sensitive than decoding accuracy to the identity of the residues in the C-terminal region of uS4.

3.4. A re-examination of the Δ V200 and E201* alterations in uS4 in *E. coli* and *S. enterica*

S. enterica and *E. coli* are closely related organisms and can even engage in intergeneric mating to form hybrids [29]. The finding by Björkman *et al.* [9] that the E201* truncation of *Salmonella* uS4 has an error-restrictive phenotype, in contrast to the error-prone phenotype of the same *E. coli* mutant is, therefore, surprising, given this relatedness and the high degree of conservation of the translation system in general. The amino acid sequences of uS5 proteins are identical in both organisms and the uS4 proteins differ at a single position (Y181 vs. F181). From a structural perspective, the error-prone phenotypes of the E201* and Δ V200 *S. enterica* uS4 mutants are also unexpected, since this region of the C-terminus of uS4 makes extensive contacts with uS5 and these are likely to be disrupted by the mutations, particularly the E201* truncation. To explore this discrepancy, we have regenerated the E210* and Δ V200 alterations in uS4 in both organisms using recombineering techniques.

The Δ V200 and E201* alterations do not affect rRNA processing or ribosome profiles in either *E. coli* or *S. enterica* and with the exception of the *S. enterica* E201* mutant which grows 1.5-fold slower than wild type, do not affect growth (data not shown). In *lacZ* readthrough assays, the Δ V200 and E201* alterations promote substantial levels of readthrough of UGA and UAG stop codons in both organisms, indicating that they have a distinct *ram* phenotype (Fig. 6). In addition, the *E. coli* wild type strain and both uS4 mutants all displayed identical responses to streptomycin (MIC = 25 mg/l). The wild type *S. enterica* LT2 strain had an MIC of 50 mg/l while the Δ V200 and E201* uS4 mutants had essentially identical MICs of 25 mg/l (since the MIC method involves two fold dilutions, two-fold differences in MIC are not considered significant). The latter result is in contrast to

the results of Björkman *et al.* [9] who noted that their three error-restrictive *S. enterica* uS4 mutants (Q53L, V200 and E201*) all had substantially increased streptomycin MICs.

Given the discrepancy between our results and those of Björkman *et al.* [9], we asked if the original error-restrictive *S. enterica* strains possibly carried additional mutations affecting ribosomal protein uS12. Sequencing of *rpsL* genes revealed that strains TH5459 (uS4 Q53L), TH5661 (uS4 V200) and TH5665 (uS4 E210*) all had alterations in uS12, while other uS4 mutant strains from the same experiments (TH5602 (uS4 Q53P), TH5660 (uS4 I199N), TH5662 (uS4 K205N) TH6556 (uS4 K205T)), as well as the reconstructed E210* and delV200 strains generated by us during this work had wild type *rpsL* genes. The Q53L,

V200 and E201* uS4 mutants were originally isolated as suppressors of streptomycin-dependence and tellingly, the *rpsL* alterations in TH5459, TH5661 and TH5665 corresponded to the uS12 alteration used to select these uS4 mutants originally. Hence TH5459 and TH5661 had a G91D alteration in uS12 and TH5665 had a P90L change. It thus appears that the previously-reported error-restrictive and weak streptomycin resistance phenotypes associated with Q53L, V200 and E201* in uS4 are the result of uS4-uS12 double-mutant combinations and not due to single uS4 alterations.

By establishing that the V200 and E201* uS4 alterations in both *E. coli* and *S. enterica* have a *ram* phenotype, the results presented here help resolve a puzzling inconsistency in the literature [8]. With the exception of V200 and E201 and Q53L in *S. enterica* all other uS4 mutants isolated as suppressors of the streptomycin dependence, or the slower growth phenotypes associated with uS12-alterations have proved to be error-prone. The unique error-restrictive and S12-suppressing phenotypes of these three *S. enterica* mutants were unexpected and difficult to interpret. In addition, the results presented here are also consistent with a yeast two-hybrid analysis of uS4-uS5 interactions [30]. In the two-hybrid context, interaction of uS4 and uS5 is completely disrupted by the C-terminal truncations generated by the 5, +C536 and G509A uS4 mutants, consistent with loss of the uS4-uS5 interface interaction. However, in the yeast two-hybrid experiments, the V200 and E201* alterations also reduced interactions in at least some of the assays used, consistent with the ribosomal *ram* phenotypes and likely structural effects of these mutations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- C-terminal truncations of ribosomal protein uS4 cause misreading of the genetic code and confer temperature sensitive growth
- Temperature insensitive revertants carry intragenic suppressor mutations that restore the C-terminus
- The C-terminus-restored mutants show recovery of ribosome assembly but still miscode
- Mutations that delete Val200 or truncate uS4 at Glu201 in the C-terminus cause miscoding in both *E. coli* and *Salmonella enterica*.

Figure 1.

Location of ribosomal proteins uS4 and uS5 on the 70S ribosome and positions of mutations affecting uS4. The left panel depicts a surface rendering of the *E. coli* 70S ribosome based on the 3.5 Å crystal structure (pdb entry 2AVY; [31]). The 30S subunit is shown as light grey (16S rRNA) and dark grey (ribosomal proteins). Ribosomal proteins uS5 and uS4 are colored skyblue and palegreen, respectively. (Right panel) Cartoon rendering of ribosomal protein uS4 (palegreen) and its contact with ribosomal protein uS5 (skyblue). The region of uS4 affected by mutations (residues 166:205) is colored red and the α -carbons of every tenth residue from 160 to 200 are shown as spheres and numbered.

Figure 2.

Growth of *uS4* mutants on solid medium. Overnight cultures of the indicated strains were serially-diluted and 2.5 µl aliquots were spotted onto LB plates that were incubated at 20 °C (left panel), 37 °C (middle panel), or 42 °C (right panel) for 24–72 hrs. WT = wild type. A dagger (†) denotes the presence of the E62K *rpsQ* mutation affecting ribosomal protein S17 in the indicated strains.

Figure 3.

Effects of alterations in ribosomal protein uS4 on UGA (right panel) and UAG (left panel) readthrough. Wild type and mutant cells were transformed with ampicillin resistant, *lacZ* reporter plasmids carrying either in-frame UGA (p34-11) or UAG (p12-6) stop codons and assayed for β-galactosidase activity. Cells were grown at either 37 °C (solid black bars), or 42 °C (stippled grey bars) and β-galactosidase activities are expressed in Miller units. Each bar represents the average activity of at least 3 independent samples ($\pm$ SE).

Figure 4. Ribosome profiles of wild type and uS4 mutant strains grown at 42°C. Elution of free subunits, 70S ribosomes and polysomes was detected by monitoring the A₂₆₀ (y-axis) with an ISCO UV6 detector. The positions of 30S and 50S subunits and 70S ribosomes are indicated on selected gradients.

Figure 5.

Processing of rRNA in wild type and uS4 mutant cells grown at 42°C. Total RNAs extracted from logarithmically growing cells were electrophoresed on agarose-Synergel gels and stained with ethidium bromide. The second lane from the left contains RNAs from wild type cells treated with chloramphenicol, which inhibits 16S rRNA processing.

Figure 6.

Stop codon readthrough in wild type *E. coli* and *S. enterica* strains expressing wild type or mutant uS4 proteins. Bars represent the β-galactosidase activities (expressed in Miller units) of *E. coli* (EC) or *S. enterica* (Sal) strains expressing the indicated uS4 proteins and transformed with the UAG *lacZ* reporter plasmid p12-6 (left panel) or the UGA reporter, p34-11 (right panel). Each bar reflects the average activity of at least 3 independent samples ($\pm$ SE).

Table 1

Amino acid sequences of wild type and mutant uS4 proteins.

uS4 Mutant	C-terminal sequences of wild type and mutant uS4 proteins (from E162)
Wild type	EQREKPTWLEVDAGKMEGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 170180190200205 </div>
Δ5	EQREKPTWLEVDAGGRYV* <div style="display: flex; justify-content: space-around; width: 100%;"> 175179 </div>
Δ5rev1	EQREKPTWLEV---EGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 172178 </div>
Δ5rev2	EQREKPPGWKLMLV---EGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 167178 </div>
Δ5rev3	EQREKPTWLEVDV---EGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 174178 </div>
Δ5rev4	EQRESQPGWKLMLV---EGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 165178 </div>
Δ5rev5	EQREKPTWLEVEV---EGTFKRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 172178 </div>
Δ5rev13	EQREKPTWLEVDAGGRYV---KRKPERSDLSADINEHLIVELYSK* <div style="display: flex; justify-content: space-around; width: 100%;"> 175182 </div>
+C536	EQREKPTWLEVDAGKMARYV* <div style="display: flex; justify-content: space-around; width: 100%;"> 177181 </div>
+T536	EQREKPTWLEVDAGKMVRVYV* <div style="display: flex; justify-content: space-around; width: 100%;"> 177181 </div>
G509A	EQREKPT* <div style="display: flex; justify-content: space-around; width: 100%;"> 168 </div>

Stop codons are indicated with an asterisk (*) and a dash (-) denotes a residue deleted in an intragenic suppressor mutant. Grey shading indicates residues that differ from the wild type uS4 protein.