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KARL JÜRGENSTEIN

A context-dependent interplay of translational fidelity and genome stability in *Pseudomonas* species

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A context-dependent interplay of translational fidelity and genome stability in *Pseudomonas* species

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Institute of Molecular and Cell Biology, University of Tartu, Estonia

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Ref II – participated in planning the experiments, preformed experiments, analyzed data, provided the illustrations and statistical analysis, and wrote and edited the manuscript

Ref III – participated in planning the experiments, preformed experiments, analyzed data, provided the illustrations and statistical analysis, and wrote and edited the manuscript

ABBREVIATIONS

aaRS	aminoacyl-tRNA synthetase
ASL	anticodon stem loop
EF-Tu	elongation factor thermo unstable (translation GTPase)
MIC	minimum inhibitory concentration
mRNA	messenger RNA
Ram	ribosomal ambiguity (mutant with increased error rates)
RifR	rifampicin-resistant
ROS	reactive oxygen species
rRNA	ribosomal RNA
tRNA	transfer RNA
tmRNA	transfer-messenger RNA
TSM	translational stress-induced mutagenesis
NQO	4-Nitroquinoline 1-oxide
WGS	whole-genome sequencing
wt	wild type
Ψ	pseudouridine

1. INTRODUCTION

Every living cell depends on the faithful transmission of genetic information. The instructions stored in DNA are transcribed into RNA and then translated into protein, and at each of these steps the cell must balance the value of accuracy against the time and energy that accuracy demands. Of the three, protein synthesis is by far the least precise. Roughly one codon in every few thousand is read incorrectly, an error rate many orders of magnitude higher than that of DNA replication (Kramer and Farabaugh, 2007). For a long time this was regarded as a tolerable imperfection, since most mistranslated proteins are short-lived and degraded away before they can cause lasting harm. It has become increasingly clear, however, that translation errors are not always so fleeting, and that the accuracy of protein synthesis can echo back to influence the stability of the genome itself.

The notion that faulty proteins might raise the rate of DNA mutation is compelling in its simplicity. DNA is copied and repaired by proteins, and if those proteins are themselves assembled with errors, the machinery that safeguards the genome may become less dependable. Most of what is known about this connection comes from *Escherichia coli*, where mutations that elevate the translational error rate also increase the mutation rate (Balashov and Humayun, 2003). Whether the same holds true across the bacterial world is far less certain, since *E. coli* is in many respects an exceptional organism, and conclusions drawn from it alone do not always extend to other organisms.

The genus *Pseudomonas* offers a natural point of comparison. It is a large and metabolically versatile group of bacteria whose members inhabit environments ranging from soil and water to plant and animal hosts (Silby et al., 2011). *Pseudomonas putida* is a common soil bacterium valued in biotechnology for its tolerance of toxic compounds, whereas *Pseudomonas aeruginosa* is an opportunistic human pathogen known for acquiring antibiotic resistance with remarkable speed (Lister et al., 2009, Kivisaar, 2020). Both differ from *E. coli* in lifestyle niches, genome structure and content, and the architecture of their translational machinery, making them well suited to testing whether observations from enterobacteria reflect general biological principles or specific peculiarities.

Translational accuracy can be perturbed at several points along the protein synthesis pathway; this thesis approaches the question through two of them. The first is the chemical modification of transfer RNA. tRNAs are among the most heavily modified molecules in the cell, and the modifications clustered around the anticodon are especially important for reading

the genetic code faithfully. Among these are the pseudouridines placed in the anticodon stem-loop by the enzymes TruA and RluA, which had not previously been connected to mutagenesis. The second is the ribosome itself, and in particular the small-subunit protein uS5, which lies close to the site where codons and anticodons are matched and helps determine how strictly the ribosome distinguishes correct from incorrect pairings.

The central aim of this thesis was to establish how perturbing translational fidelity at these two levels affects spontaneous mutagenesis in *Pseudomonas*, and whether this relationship resembles that described in *E. coli*. The work rests on three studies. The first showed that the loss of anticodon stem-loop pseudouridines elevates mutation frequency in *P. putida*, and to a lesser extent in *P. aeruginosa* (Ref. I). The second compared translational error rates across *P. putida*, *P. aeruginosa*, and *E. coli*, and examined how the loss of these same modifications alters decoding accuracy (Ref. II). The third characterized a panel of mutations in ribosomal protein uS5 and their effects on both decoding and mutation rate (Ref. III). Together, these studies show that a process seemingly far removed from DNA can nevertheless shape the stability of the genome, and that the strength of this connection depends on the biological context in which it is measured.

2. REVIEW OF LITERATURE

2.1. Overview of translational fidelity

Life is a story written, read, and interpreted, then rewritten, re-read, and re-interpreted ad nauseam at three intimately connected levels: replication, transcription, and translation. Each stage, while markedly different in details, ultimately conveys the same task. That is to deliver genetic information from one molecular language to the next, as accurately as possible. However, accuracy does not come cheap. Every checkpoint, proofreading step, and corrective measure costs energy and time. Thanks to high-fidelity polymerases and repair pathways, DNA replication is executed with extraordinary accuracy, making only about one error per billion to ten billion nucleotides copied (Fijalkowska et al., 2012, Lee et al., 2012). Transcription is less accurate with RNA polymerase proofreading activity, allowing for an error rate of about 10^{-5} to 10^{-6} mistakes per nucleotide (Imashimizu et al., 2013). Translation, the final act where the genetic script becomes a working protein, is surprisingly tolerant in comparison. A wrong amino acid is incorporated into the polypeptide chain in every 10^3 to 10^4 codons translated (Kramer and Farabaugh, 2007, Mordret et al., 2019). This relative drop in fidelity is not a flaw, but a trade-off between accuracy, speed and the metabolic cost. Whereas replication mistakes are permanent and heritable, most translation errors vanish with the protein's turnover. And yet, consequences of mistranslation can ripple through the organism in unexpected ways, influencing fitness, adaptability, and, paradoxically, even genome stability. Mistranslation can trigger proteotoxic stress, alter stress responses, and even modulate antibiotic tolerance (Samhita et al., 2020).

Accuracy within the central dogma of molecular biology is shaped by the balance between risk and reward. For DNA replication, the stakes are highest: errors made here are fixed into the genetic record and passed to progeny. Therefore, the high energetic burden of proofreading by DNA polymerases, mismatch and other repair systems is justified (Schaaper and Mathews, 2013, Fijalkowska et al., 2012). Transcriptional fidelity is lower, reflecting the transient nature of RNA molecules (Imashimizu et al., 2013). RNA polymerases employ basic proofreading, but the cost of higher accuracy is not justified for short-lived transcripts that can just be remade. Indeed, organisms sometimes exploit transcriptional infidelity to create transient phenotypic diversity without genetic change (Traverse and Ochman, 2016). Translation is inherently more error-prone because the decoding process must handle a massive throughput: millions of codons

read per minute in a growing bacterial culture. Excessive proofreading would slow growth fatally (Drummond and Wilke, 2009). Therefore, translation must operate near the optimal boundary between accuracy and efficiency (Rodnina, 2016, Wohlgemuth et al., 2010).

The principles of translational fidelity are conserved across domains of life, but error rates and biases vary. Bacterial ribosomes often prioritize speed, while eukaryotic protein synthesis tends toward higher accuracy (Kramer and Farabaugh, 2007). Some of these differences reflect adaptation to ecological niches: slow-growing bacteria like *Mycobacterium tuberculosis* exhibit unusually high mistranslation rates, which may aid stress survival (Javid et al., 2014). Fast-growing enterobacteria maintain a more balanced error profile. Mitochondrial ribosomes, owing to their endosymbiotic origins, often exhibit reduced fidelity compared to cytoplasmic ribosomes (Liu et al., 2024). Stress conditions, such as oxidative damage or nutrient limitation, can furthermore modulate mistranslation rates, suggesting that translation fidelity itself is a dynamic trait under environmental regulation (Ling and Söll, 2010, Netzer et al., 2009).

Prokaryotic translation operates within a tightly integrated system in which transcription and translation are frequently coupled on the same mRNA. This co-transcriptional translation creates a high-throughput, resource-efficient setting that favors speed and economy, so within this minimalist framework, fidelity is entrusted to a small number of strategically placed checkpoints (Rodnina, 2016, Proshkin et al., 2010, Keiler, 2015). These can be summarized as follows: pre-ribosomal quality control, which conditions that tRNAs reach the ribosome; ribosomal decoding and frame maintenance that decide which tRNAs are accepted and how the frame is kept; and post-ribosomal rescue and proteostasis surveillance systems that mitigate the consequences when errors slip through (Keiler, 2015, Ling et al., 2009).

Before ribosomal decoding and peptide synthesis aminoacyl-tRNA synthetases (aaRSs) enforce a major fidelity barrier via double-sieve chemistry, combining pre-transfer and post-transfer editing to clear misactivated amino acids and misacylated tRNAs. As a result, the ribosome is presented with correct substrates, as in a correct amino acid linked to the corresponding tRNA (Nureki et al., 1998, Ling et al., 2009). Environment matters as well: severe oxidative stress can impair aaRS editing and increase misacylation, shifting the error balance before decoding even starts (Ling and Söll, 2010).

Once an aminoacyl-tRNA arrives at the ribosome, two general checks dominate: an initial codon–anticodon fit followed by a GTPase-powered proofreading step that disfavors near-cognate tRNAs; in parallel, orderly

tRNA movement through the P and E sites helps preserve the reading frame (Devaraj et al., 2009, Thompson and Stone, 1977). The 70S ribosome safeguards decoding fidelity primarily through the 30S decoding center, where conserved 16S rRNA residues A1492, A1493, and G530 monitor Watson-Crick geometry and trigger an induced-fit conformational change that licenses EF-Tu-GTP hydrolysis only for cognate aminoacyl-tRNAs (Ogle et al., 2001, Zaher and Green, 2009, Rodnina, 2016). Mutations within 16S rRNA can likewise alter translational fidelity; for example, changes in helices h8 and h14 decrease the stringency of aminoacyl-tRNA selection and increase miscoding and stop-codon read-through (McClory et al., 2010). Small-subunit proteins uS12, uS4, and uS5 tune this checkpoint: mutations can yield hyperaccurate or ribosomal-ambiguity (Ram) phenotypes by shifting the speed-accuracy trade-off (Demirci et al., 2013, Kirthi et al., 2006, Agarwal et al., 2015).

Beyond selection at the ribosome, bacteria depend on efficient "damage-control" to keep mistranslation from cascading into growth-limiting proteotoxicity. Conceptually, this is accomplished by two interconnected systems: firstly, freeing and recycling stalled ribosomes so that energy is not wasted on defective messages; secondly, rounding up incomplete or misfolded nascent chains so that they are refolded if possible and eliminated if not (Buskirk and Green, 2017). Together, these actions limit both the opportunity cost (idle ribosomes) and the material cost (toxic protein species) of residual errors.

2.2. Key Factors Affecting Translational Fidelity

Translation errors can be broadly categorized into misincorporations, frameshifts and stop codon readthrough events. Each arises from distinct molecular causes, yet all share a common theme: perturbations in the decoding process that allow for an incorrect amino acid, reading frame or stop signal to be processed as correct. Errors are also typically classified as programmed, when used beneficially by cells or viruses to regulate gene expression, or non-programmed, when taking place as a byproduct of molecular imperfections, environmental stress or just stochastic fluctuation of the protein synthesis process (Drummond and Wilke, 2009).

As the network of molecular machinery participating in protein synthesis is complex, it's no surprise that maintenance of translational fidelity is multifaceted. The molecular components that maintain translational fidelity by acting at every stage of the protein synthesis cycle, from the loading of amino acids onto tRNAs to the post-ribosomal handling of the released proteins, are summarized in Figure 1.

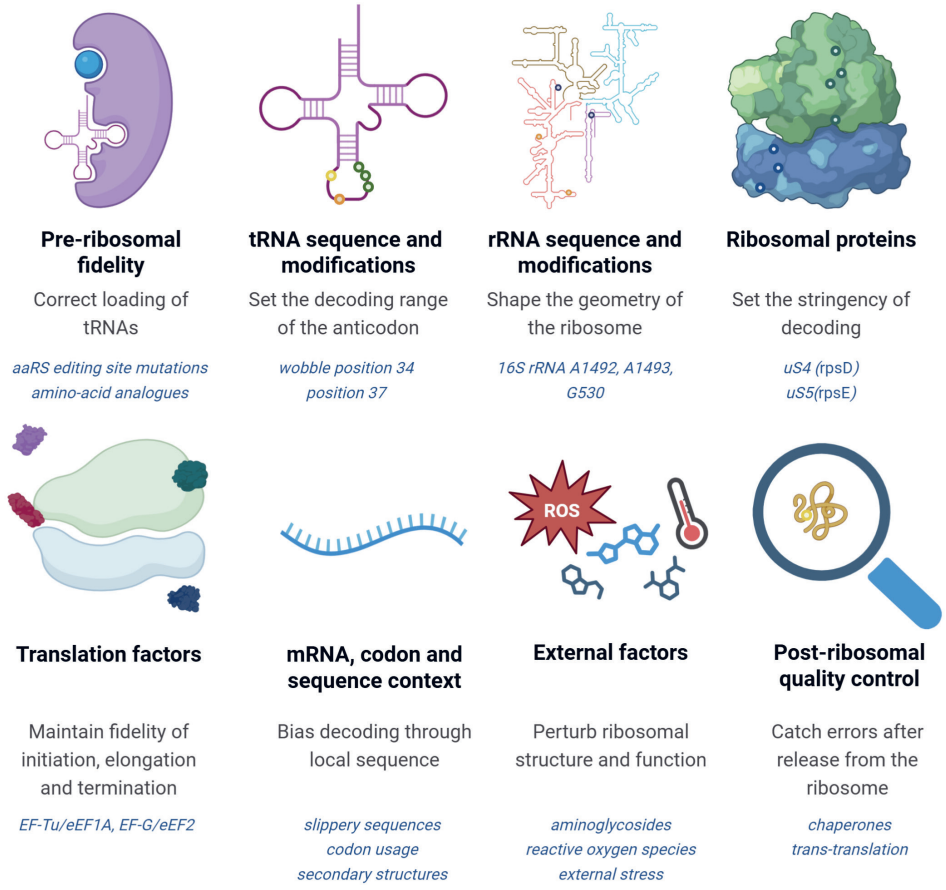

Figure 1. Translational fidelity is maintained by the coordinated function of multiple molecular components, organised here into eight categories that span the translation cycle from the loading of amino acids onto tRNA to the post-ribosomal handling of the released protein. Within each category, representative examples are given of well-characterized targets whose perturbation has been shown to elevate mistranslation. Categories and representative examples reviewed in Farabaugh, 1996; Decatur and Fournier, 2002; Drummond and Wilke, 2009; Ling et al., 2009; Keiler, 2015; Rodnina, 2016; Agris et al., 2017. Created with BioRender.com.

The main "actors" in this play are RNAs and ribosomal proteins. Among RNA species, rRNA is the most abundant and structurally conserved, with relatively limited diversity within a species. This stability reflects its essential role in the ribosome's core functions. In contrast, tRNAs, though also highly conserved in structure, exhibit greater diversity at the sequence level, owing to the requirement to be discriminable from each other. This diversity is, in part, achieved through post-transcriptional chemical modifications, with tRNAs being the most heavily modified nucleic acids in cells. Messenger RNAs (mRNAs), on the other hand, represent the most

diverse RNA class in terms of sequence, length, and structure. While their primary role is to convey coding information, regulatory elements embedded in mRNAs can affect translational outcomes by altering ribosome dynamics. Other non-coding RNA species, such as transfer messenger RNAs (tmRNAs), play more auxiliary roles in translation regulation or rescue.

Post-transcriptional RNA modifications are different covalent modifications to the standard nucleotides – A, U, G, C. This repertoire of chemical moieties added to various positions of nucleobase or ribose can range from the addition of a single methyl group to multi-step enzymatic transformations of the nucleobase. All in all, across all kingdoms of life, more than 170 RNA modifications have been described in the literature, although current databases such as MODOMICS list over 350 distinct chemical entities and continue to expand (Sordyl et al., 2026). They are commonly positioned in functionally relevant regions (Decatur and Fournier, 2002, Ofengand and Del Campo, 2004) and linked with a wide range of functions, for example, assisting with proper folding, stabilizing key structural elements (Vögele et al., 2023) or serving as signals for quality control (Alexandrov et al., 2006).

In translation, RNA modifications act as tunable fidelity factors. In tRNAs, modifications around the anticodon loop are crucial, as they affect base-pairing dynamics and maintenance of the reading frame (Agris et al., 2017). Particularly critical are modifications at the wobble position (position 34 of tRNA), which basepair with the third position of a codon on mRNA, and at position 37 of tRNA, which is directly adjacent to the anticodon. For example, $tRNA^{Pro}$ of *Salmonella enterica* and *Escherichia coli* carry cmo^5U at their 34 position, which actually decreases decoding accuracy to allow for near-cognate codon recognition, thus expanding the decoding capacity of this tRNA (O'Connor, 2002, Kothe and Rodnina, 2007). Conversely, a $tRNA^{Pro}$ missing m^1G modification in the 37th position is more prone to +1 frameshifts and induces ribosomal stalling (Masuda et al., 2021, Gamper et al., 2015).

Among the modifications bracketing the anticodon, pseudouridine (Ψ) holds a central status. Ψ is the most abundant modified nucleoside in cellular RNA and is formed when a pseudouridine synthase isomerises uridine, replacing the N-glycosidic bond with a carbon-carbon linkage while leaving the base otherwise unchanged. The freed imino group permits an additional water-mediated hydrogen bond and improves base stacking, so that Ψ rigidifies and stabilises local RNA structure relative to uridine (Agris et al., 2017, Vögele et al., 2023). In the tRNA anticodon stem-loop, pseudouridines are placed at positions 38–40 by TruA (Kammen

et al., 1988) and at position 32 by RluA (Wrzesinski et al., 1995), sites that flank the anticodon and can shape the geometry of codon–anticodon pairing and the maintenance of the reading frame.

RNA modifications at critical sites of rRNA also shape translational accuracy. The rRNAs of the ribosome (16S in the small subunit, 23S in the large subunit in bacteria) carry numerous modifications concentrated in functional centers such as the decoding center (in the small subunit) and the peptidyl transferase center (in the large subunit). In the small subunit, methyltransferase KsgA adds methyl groups to two adjacent adenines A1518 and A1519, which are located near the decoding center. Ribosomes lacking these modifications show increased stop codon readthrough and frameshift errors indicating the importance of these modifications to accurate decoding (Kyuma et al., 2015).

RNA alterations affecting translational fidelity are not limited to post-transcriptional modifications. In the 23S rRNA, it has been shown that when a conserved base adjacent to the A site of peptidyl transferase center, U2555, is replaced by adenine or guanine, stop-codon readthrough and +1/–1 frameshifting are increased (O'Connor and Dahlberg, 1993). This indicates that changes to the primary structure of RNA can markedly alter ribosome function and, furthermore, although decoding is carried out in the small subunit by 16S rRNA, alterations in 23S rRNA in the large subunit can also influence translation fidelity.

In prokaryotes, rRNA accounts for roughly 2/3 of the mass of ribosomes, with the rest being ribosomal proteins (Kurland, 1960). Although the catalytic activity is performed by RNA, ribosomal proteins are critical components that support and stabilize the RNA framework and assist its functions. In the 30S subunit, three proteins in particular – uS4, uS5, and uS12 – stand out as key modulators of decoding accuracy (Agarwal et al., 2015, Agarwal et al., 2011). These proteins lie near the mRNA entry and decoding center, and influence how the ribosome discriminates between correct and incorrect codon-anticodon pairing (Nikolay et al., 2015). Mutants of uS4 and uS5 that have increased translation errors are collectively referred to as having ribosomal ambiguity (Ram) phenotype (Rosset and Gorini, 1969, Zimmermann et al., 1971), and uS12 mutants typically exhibit hyperaccurate, restrictive phenotypes (Zaher and Green, 2010, Bohman et al., 1984). Several well-studied mutants highlight how alterations in these proteins affect the translation accuracy. For example, Agarwal et al. (2011) applied targeted mutagenesis to the uS12 protein (*rpsL*) and identified dozens of substitutions distributed throughout the protein, rather than confined to a specific structural region. Many of these mutations measurably altered decoding fidelity. In a related study,

Agarwal et al. (2015) performed random mutagenesis of the uS4 and uS5 proteins (*rpsD* and *rpsE*) and similarly recovered more than ten unique amino-acid substitutions per protein, again scattered across the polypeptide. Most of these uS4 and uS5 variants reduced translational accuracy, although to widely differing extents. Together, these studies demonstrate that fidelity modulation is not restricted to a small set of "hotspot" or critical residues: changes at many positions across uS12, uS4, and uS5 can influence the precision of decoding, highlighting the sensitivity of the accuracy-center to perturbations.

On the opposite side of the fidelity spectrum, these same three proteins also harbor variants known as restrictive or hyperaccurate mutants. These substitutions increase translational accuracy by tightening discrimination against near-cognate tRNAs, a change that often slows growth due to reduced decoding efficiency, thereby illustrating the classical accuracy–efficiency trade-off. Notable examples include substitutions at conserved residues in uS12, such as K42T or P90A, which reduce miscoding and suppress readthrough (Sharma et al., 2007, Agarwal et al., 2011). Restrictive phenotypes have also been reported for specific mutations in uS4 and uS5, although they are less common than error-prone variants (Agarwal et al., 2015). Importantly, restrictive and ram-type mutations need not act in isolation: combinations of accuracy-increasing and accuracy-decreasing alleles can partially compensate for one another. In *E. coli*, such epistatic interactions between uS12 and uS4/uS5 mutants have been shown to rebalance growth and decoding fidelity, underscoring that decoding accuracy is an inherent property of the entire protein cluster rather than any single protein (Björkman et al., 1999). Notably, mutations conferring resistance to antibiotics such as streptomycin and spectinomycin often co-occur with translational accuracy phenotypes: uS12 mutants conferring streptomycin resistance typically yield restrictive decoding (Bohman et al., 1984), while certain uS5 mutants conferring spectinomycin resistance can lead to ram-like ambiguity phenotypes while simultaneously affecting streptomycin tolerance (Kamath et al., 2017).

Modulating decoding accuracy is not limited to these three proteins. Notably, a mutation in the large-subunit protein uL4 increases frame-shifting, miscoding and stop-codon readthrough, demonstrating that fidelity modulation can also arise from structural perturbations of the 50S subunit, not only from proteins located near the decoding center (O'Connor et al., 2004).

2.3. Functional Consequences of Altered Fidelity

Changes in translation error rates can have profound effects on the organism, whether they increase (more errors) or decrease (fewer errors). Hyperaccurate mutants that tolerate fewer errors tend to have slower protein synthesis, which leads to reduced growth rate and fitness in rich conditions (Ruusala et al., 1984, Kurland, 1992). In such strains, the ribosome is more cautious, spending more time checking the validity of tRNAs and codon-anticodon pairings and is more likely to reject near-cognate or even cognate tRNAs. As a result of sacrificing elongation speed for accuracy, the cellular proteome, while containing fewer erroneous amino acids and misfolded proteins, is unable to sustain a growth rate comparable to cells with less accurate but faster protein synthesis. That said, owning a nearly errorless repertoire of proteins may provide longer-term benefits. For example, if DNA polymerases or repair enzymes have been produced with fewer errors, they may be less likely to produce errors themselves, meaning that fixed errors – mutations – become rarer. The question that remains is whether this transgenerational benefit is sufficient or whether these hyperaccurate strains will be quickly outcompeted by more moderate counterparts in competitive environments.

As an absolutely accurate protein synthesis is energetically unfeasible, translation errors must be tolerated to a certain degree. At the extreme, however, cells start to suffer the consequences. Misincorporated amino acids can hinder proper protein folding, leading to the accumulation of proteotoxic aggregates, which, in turn, trigger heat-shock and stress responses (Fredriksson et al., 2006). In addition to global effects, when errors strike critical components of specific proteins, for example, the catalytic residue of an enzyme, the functionality of the cellular proteome starts to degrade. This can initiate a snowball effect, as proposed by Orgel (first in 1963 and a correction in 1970), known as the “error catastrophe”. This theoretical model posits that as mistakes occur during protein production, the resulting proteins are more likely to amplify the effect, and this cycle continues until cells are no longer viable (Orgel, 1970).

Not all “errors” are accidental. Programmed recoding events purposefully redirect decoding to modulate gene expression (e.g., to control protein ratios or to change the length of a protein’s C-terminus), often by employing “slippery” sequences, mRNA structures, or rare codons (Farabaugh, 1996). By contrast, non-programmed errors arise stochastically from molecular noise, imperfect editing or environmental stress.

In bacteria, a well-described example of programmed frameshifting occurs at the *dnaX* gene in *E. coli*. *DnaX* codes for the 71.1 kDa τ-subunit

of DNA polymerase III, which shares its N-terminal amino acid sequence with the 56.5 kDa γ-subunit. The production of γ-subunit is dependent on a –1 programmed frameshift followed by an encounter of a stop codon in the new frame. The translational reprogramming event operates with substantial efficiency (~40%), resulting in a close to 1:1 ratio of the subunits (Flower and McHenry, 1990, Blinkowa and Walker, 1990). Also, transposase synthesis in bacterial IS1 and IS3 insertion sequences families requires a –1 programmed frameshift to produce the full-length active protein from two overlapping reading frames (Escoubas et al., 1991, Mahillon and Chandler, 1998).Programmed frameshifting is especially common in viruses, where overlapping reading frames allow for more compact genomes.

In retroviruses, the same mechanistic logic underlies the production of the viral enzyme complement. In HIV-1, for example, the *pol* open reading frame, encoding the viral reverse transcriptase and integrase, is located in the –1 reading frame relative to the upstream *gag* ORF. As the ribosome translates *gag*, a subset of elongating ribosomes slips backward by a single nucleotide at a slippery heptanucleotide sequence (U UUU UUA in HIV-1) and continues translation in the –1 frame, producing a Gag-Pol fusion protein (Jacks et al., 1988, Jacks and Varmus, 1985). The slippage is stimulated by a downstream RNA pseudoknot located approximately eight nucleotides 3′ of the slippery site; this structure transiently stalls the ribosome at the slip site, giving the tRNAs in the A and P sites time to realign on the upstream codon in the –1 frame before translocation resumes (Brierley et al., 1989, Plant et al., 2003). Crucially, retroviral frameshifting is calibrated to a low efficiency, typically 5–10%, which means that for every ten to twenty Gag structural proteins produced, only one Gag-Pol fusion protein is synthesised. This ratio is not incidental; it reflects the stoichiometric requirements of viral particle assembly, and experimental perturbation of frameshift efficiency in either direction disrupts capsid formation and abolishes infectivity (Jacks et al., 1988).

The *dnaX* frameshift and retroviral *gag-pol* frameshifting thus share the same two-component architecture: a slippery heptanucleotide sequence that permits tRNA realignment, combined with a downstream RNA stimulatory element that pauses the ribosome and promotes slippage, a stem-loop in the case of *dnaX* and a pseudoknot in the retroviral case. The key difference between them is one of efficiency. The *dnaX* frameshift generates roughly equimolar amounts of the τ- and γ-subunits required for stoichiometric assembly of the clamp loader complex (Flower and McHenry, 1990, Blinkowa and Walker, 1990). Retroviral frameshifting, by contrast, is tuned lower, reflecting the comparatively small enzymatic

complement that each virion requires relative to its structural proteins. This comparison illustrates a broader principle: the efficiency of a programmed frameshift is not a fixed mechanistic property but an evolvable parameter, set by the identity of the slippery sequence and the architecture of the RNA, to match the stoichiometric demands of the protein complex being produced.

Functionally, both classes matter for evolvability: programmed recoding extends the regulatory toolkit, while non-programmed errors seed phenotypic heterogeneity and, under certain stresses, can transiently enhance survival, even as they impose costs in proteostasis burden and growth (Drummond and Wilke, 2009, Samhita et al., 2020).

Although protein synthesis and DNA replication are mechanistically distinct, translational fidelity can influence mutation rates. In bacteria, low-fidelity ribosomes (e.g., ram mutants) have been linked to increased mutagenesis, potentially via mistranslation-induced stress responses, impaired DNA repair, or accumulation of reactive oxygen species. For instance, uS4 mutants in *E. coli* have been shown to activate the translational stress-induced mutagenesis (TSM) pathway, increasing genome instability (Balashov and Humayun, 2003).

The TSM model proposes a specific indirect pathway connecting translational errors to elevated DNA mutation rates (Balashov and Humayun, 2003, Balashov and Humayun, 2002). The core mechanism is as follows: when translational fidelity is reduced, whether by aberrant tRNAs, ribosomal mutations, or other perturbations, aberrant copies of every cellular protein are produced at some low frequency, including subunits of DNA polymerases and mismatch repair proteins (MutS, MutL). These mistranslated enzymes carry random amino acid substitutions that reduce their catalytic accuracy or stability in a fraction of molecules. Because the fidelity of DNA replication and repair depends on the cumulative accuracy of these enzyme populations, even a modest increase in the proportion of aberrant copies is predicted to elevate the overall mutation rate. The model further predicts a potential self-amplifying feedback: if mutations accumulate in genes encoding translational components, further translational deterioration could ensue; however, growth dilution and protein quality control are thought to prevent such cascades under normal conditions. The TSM model received direct experimental support from *in vitro* studies showing that DNA polymerase III purified from *mutA* mistranslating cells is demonstrably more error-prone than the wild-type enzyme (Al Mamun et al., 2002), and from single-cell analyses demonstrating that subpopulations of *E. coli* cells with elevated translation errors also display higher rates of replication errors (Woo et al., 2018). The mutator phenotype in TSM-

active strains is independent of SOS-induced error-prone polymerases and elevated reactive oxygen species, consistent with the model's prediction that the mutagenic effect operates through impaired replication and repair fidelity rather than through DNA damage or mutagenic bypass synthesis (Murphy and Humayun, 1997, Ren et al., 1999). The TSM cascade, together with the alternative mutagenic routes considered in this work, is summarized in Figure 2.

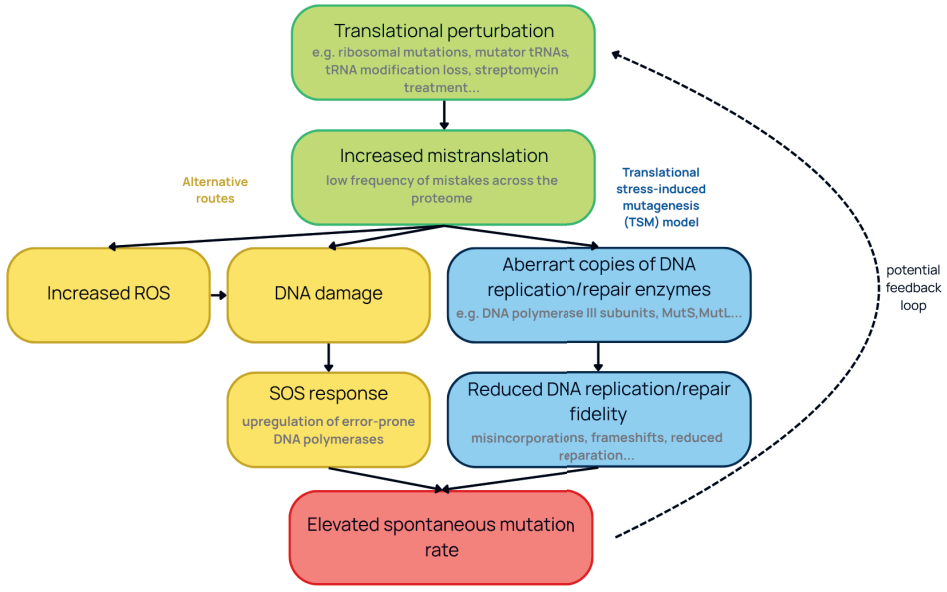

Figure 2. The cascade postulated by the TSM model, alongside the alternative mutagenic routes.

This connection may reflect an indirect pathway: translation errors produce defective proteins, some of which participate in genome maintenance. A defective DNA polymerase or repair enzyme, resulting from mistranslation, could elevate mutation rates. Conversely, error-restrictive ribosomes may suppress mutagenesis at the cost of slower growth or reduced adaptability.

Translational fidelity is not simply a passive trait; it evolves under selective pressures. Bacteria inhabiting stable environments may favor high fidelity to ensure proteome integrity, while those in fluctuating or hostile conditions, e.g., under oxidative stress or antibiotic exposure, might benefit from a controlled degree of mistranslation (Massey, 2008, de Pouplana et al., 2014, Samhita et al., 2020). The latter strategy enables phenotypic plasticity without committing to permanent genetic changes – a form of “soft” adaptation (Samhita et al., 2021).

In evolutionary terms, mistranslation can also interact with DNA mutations through epistasis. Recent models suggest that mistranslation may expose or mask the effects of deleterious DNA variants, shaping which mutations are fixed in a population (Zheng et al., 2021). This interplay between genotype and noisy gene expression creates a layered model of evolvability in which the translation error rate can itself become a selectable trait.

2.4. Approaches to measuring molecular errors

Each organism, each cell slips up every now and then. Catching it in the act, however, takes some ingenuity. Measuring rare molecular errors, whether in DNA replication or protein synthesis, demands precision, creativity, and scale. From single-nucleotide changes to "slippage" of molecular structure, the tools developed to track these events reveal the hidden instability beneath cellular robustness. Some rely on clever genetic circuits; others row through oceans of sequencing or proteomic data. Yet all share a common goal: to peel back the layers of order to glimpse at the chaos beneath.

One of the earliest and most widely used approaches for measuring translational errors relies on β-galactosidase (*lacZ*) reporter systems. In these assays, restoration or disruption of *lacZ* activity depends on a specific translational error, such as frameshifting or stop codon readthrough, allowing quantification through enzyme activity determination by colorimetry (Weiss et al., 1987, Miller, 1972). These systems proved particularly powerful in genetic screens, as their simplicity and scalability made them foundational tools for identifying factors affecting decoding accuracy, though they are limited to population-level measurements and single-error contexts.

Building on this logic, more sophisticated dual-reporter constructs were developed to improve quantification and reduce artifacts from variable expression levels. A common design in which an upstream reporter gene (serving as an internal expression control) is fused to a downstream reporter separated by a sequence that requires a frameshift, stop-codon readthrough, or missense error event for the downstream reporter to be correctly translated. The ratio of downstream to upstream reporter activity provides a quantitative measure of the error frequency (Kramer and Farabaugh, 2007, Grentzmann et al., 1998). Dual-luciferase systems, using *Renilla* and firefly luciferases, are particularly popular because of their wide dynamic range and compatibility with multiple bacterial hosts (Devaraj et al., 2009). Reporter panels targeting different error types, such as +1 and –1 frame-

shifts at various slippery sequences, readthrough of UAG, UGA, and UAA stop codons, and amino acid misincorporation, enable comprehensive profiling of translational fidelity in a given genetic background (Devaraj et al., 2009, Agarwal et al., 2015).

Fluorescent protein reporters have taken these approaches to the single-cell level, addressing a key limitation shared by *lacZ* and luciferase systems: they only measure the average behaviour of a whole population in bulk. In dual-fluorescent designs, two reporter proteins are expressed from the same construct. One acts as a stable internal control for overall protein output, while the other carries an introduced frameshift or stop codon mutation, so it becomes fluorescent only if a translational error occurs. The ratio of the two signals gives a measure of error frequency that accounts for differences in growth rate and metabolic activity between cells (Fan et al., 2017). One such system in *E. coli* pairs GFP as the reference protein with a mutated mScarlet-I as the error reporter, allowing individual cells to be measured by flow cytometry or fluorescence microscopy (Hinnu et al., 2024). This approach allows individual cells to be measured by flow cytometry or fluorescence microscopy, revealing variation in translational fidelity between single cells within a population that bulk assays would average out. The shared limitation of all reporter-based approaches remains: each construct captures only one error type at one sequence context, and results may not reflect the full error landscape of the cell.

Direct mass spectrometric identification of mistranslated proteins has emerged as a complementary approach to reporter-based methods, enabling detection of errors across the entire proteome rather than at predefined reporter sequences. In mistranslation mass spectrometry, tryptic peptides derived from cell lysates are subjected to tandem mass spectrometry analysis, and spectra are searched against databases that include not only canonical peptide sequences, but also systematic lists of substituted variants expected from each type of error. This allows simultaneous detection of missense errors, near-cognate substitutions, and, in some experimental designs, frameshifting events. Mordret et al. (2019) employed this approach in *Escherichia coli* and revealed that the global error rate is remarkably consistent at approximately 4×10^{-4} per codon, a value that aligns closely with earlier reporter-based estimates. However, the sensitivity of standard database-searching approaches sets a detection floor that excludes the rarest miscoding events. Garofalo et al. (2019) improved the detection of translation errors by enriching for peptides containing amino acid substitutions prior to mass spectrometry. This allowed them to measure even very rare mistranslation events, down to frequencies of 10^{-7} and 10^{-5} per codon. Their results showed that most errors are too rare to detect with

standard methods, but some hotspots can exhibit higher error rates, up to 10^{-3} per codon (Garofalo et al., 2019). Together, these studies show that mass spectrometry not only validates reporter assays but also provides proteome-wide resolution, while also revealing that the true error landscape spans several orders of magnitude depending on codon identity and sequence context.

Measuring errors in DNA replication has also relied on reporter systems analogous to those used in translational fidelity research. The fluctuation assay, originally described by Luria and Delbrück, remains the gold standard for quantifying spontaneous mutation frequency in bacteria (Luria and Delbrück, 1943). In this approach, multiple independent cultures are grown from a small inoculum and then plated on selective medium. One of the most commonly used methods is using a solid growth medium containing rifampicin for the selection of spontaneous Rif-resistant colonies carrying mutations in the RNA polymerase β-subunit gene (*rpoB*) (Garibyan et al., 2003). The mutation spectrum, determined by sequencing the selected resistance locus across many independent mutants, provides additional resolution by revealing what types of substitutions, insertions, or deletions predominate in a given genetic background (Jatsenko et al., 2010).

Advances in sequencing technology have enabled direct, genome-wide measurements of mutagenesis. Mutation accumulation experiments, in which clonal lineages are passaged through repeated single-cell bottlenecks, followed by whole-genome sequencing, provide unbiased estimates of genome-wide mutation rates and spectra (Lee et al., 2012). These approaches have revealed that mutation rates are not uniform across the genome but vary with chromosomal position, replication timing, and local sequence context (Foster et al., 2013, Niccum et al., 2019). This approach has been applied in *Pseudomonas* species to characterize not only the rate but also the spectrum and genomic distribution of spontaneous mutations (Dettman et al., 2016).

The intersection of translational fidelity measurement and mutagenesis assays provides a powerful experimental framework for understanding how these processes are connected. Reporter-based translational fidelity assays quantify decoding accuracy under defined conditions, fluctuation assays and whole genome sequencing (WGS) can provide genome-level evidence of mutagenic outcomes. By applying these methods in combination, it becomes possible to ask not merely whether a given perturbation, such as a mutation in a ribosomal protein or the loss of a tRNA modification enzyme, increases translational error frequency, but also whether and how this translational lagging behind can incorporate into altered genomic mutation rates.

2.5. Translation errors and genome stability

The possibility that errors in protein synthesis might cascade into errors in DNA replication is not novel, but the mechanistic details and quantitative significance of this connection are yet to be resolved. The conceptual basis for a link is straightforward: because DNA replication, repair, and other genome maintenance processes are carried out by proteins, any systematic degradation of protein quality through mistranslation could plausibly impair these machines and thereby increase the rate at which mutations are incorporated into the genome.

One of the earliest types of evidence for a link between translational errors and mutagenesis came from studies of mutator tRNAs in *E. coli*. The *mutA* and *mutC* genes encode defective glycine tRNAs that misread aspartate codons, causing Asp → Gly misincorporation at a subset of codons (Slupska et al., 1996). Strains carrying these alleles exhibit a mutator phenotype that is recombination-dependent (*recA*, *recB*) but independent of the SOS-regulated error-prone polymerases (*umuD*, *umuC*, *dinB*) (Murphy and Humayun, 1997, Ren et al., 1999). Importantly, the mutagenic effect is not specific to Asp → Gly mistranslation, as mistranslating alanine tRNAs produces similar increases in mutation frequency (Dorazi et al., 2002). *In vitro* replication fidelity analyses using purified DNA polymerase III complex from *mutA* cells showed that the polymerase was more error-prone than the wild-type enzyme, although the magnitude of the effect was smaller than the *in vivo* mutator phenotype, which suggests that additional factors amplify the effect in living cells (Al Mamun et al., 2002). One proposed mechanism involves increased replication fork collapse in mistranslating strains: if critical replication or repair proteins are mistranslated, the replication fork may stall or collapse more frequently, necessitating recombinational rescue and thereby increasing the opportunity for mutagenic events (Al Mamun et al., 2006). Single-cell studies in *E. coli* have shown that subpopulations with elevated translation errors also exhibit higher rates of replication errors, further suggesting a direct functional coupling between protein synthesis quality and DNA replication fidelity (Woo et al., 2018).

Another set of experimental evidence for a translation-mutagenesis link comes from studies of ribosomal protein mutants. Ribosomal ambiguity (Ram) mutants, which carry amino acid substitutions in the small subunit proteins uS4 (*rpsD*) or uS5 (*rpsE*) that increase translational error rates, were among the first characterized mutators in *E. coli* and related enterobacteria (Rosset and Gorini, 1969). Balashov and Humayun (2003) demonstrated in *E. coli* that uS4 Ram mutants exhibit elevated rates of

both missense and frameshift errors, and that this is accompanied by significantly increased spontaneous mutagenesis (Balashov and Humayun, 2003). Importantly, the increase in mutagenesis was not attributable to direct interactions between the ribosomal proteins and DNA, as no such interactions have been detected. Instead, the evidence pointed to an indirect mechanism mediated by the proteome: misfolded or aberrant proteins produced by the error-prone ribosomes impair the function of DNA maintenance enzymes, leading to increased mutation rates downstream.

2.6. Translation errors in bacterial adaptation and disease

Translational fidelity in bacteria is a quantitative trait under selection from both directions. Higher accuracy reduces aberrant protein output but imposes metabolic costs through additional proofreading steps, while lower accuracy generates erroneous proteins that risk proteotoxic aggregation but also produce phenotypic diversity that can prove adaptive under unpredictable stress (Drummond and Wilke, 2009, de Pouplana et al., 2014). The fitness consequences of this trade-off are not confined to protein quality but extend into antibiotic susceptibility, stress-response activation, and virulence. The most direct illustration of translational fidelity as a biological tool is the mechanism of action of aminoglycoside antibiotics, a class that includes streptomycin, gentamicin, and paromomycin. Aminoglycosides bind with high affinity to the decoding site of the small ribosomal subunit – the region where the ribosome checks whether the incoming aminoacyl-tRNA matches the codon currently displayed in the messenger RNA (Fourmy et al., 1998, Ogle et al., 2001). Under normal conditions, this check is strict: tRNAs that differ from the cognate at even one position are rejected through conformational proofreading. Aminoglycoside binding distorts the geometry of the decoding site, stabilising an "open" conformation that accepts near-cognate tRNAs, thereby causing widespread amino acid misincorporation throughout the proteome (Davis, 1987, Ogle et al., 2002). However, the resulting flood of aberrant proteins is not the direct cause of cell death, rather, misfolded proteins accumulate preferentially in the bacterial inner membrane, where they disrupt the electron transport chain and cause a burst of reactive oxygen species production. These ROS then inflict lethal and self-amplifying damage on DNA, proteins, and lipids, killing the cell through an oxidative catastrophe that is mechanistically downstream of the initial translational errors (Kohanski et al., 2007, Kohanski et al., 2008). The antibacterial potency of aminoglycosides therefore depends on a chain of consequences initiated

by ribosomal infidelity, making the fidelity of the decoding site a direct factor of drug susceptibility. The ribosome of an aminoglycoside-resistant *P. aeruginosa* strain isolated directly from a cystic fibrosis patient was found to carry a deletion in ribosomal protein uL6, which causes a conformational change in 23S rRNA helix H69, an intersubunit bridge that also serves as a secondary aminoglycoside binding site. This constitutes direct structural evidence that *P. aeruginosa* can, during the course of a real cystic fibrosis infection event, evolve changes in the ribosome at the exact site these antibiotics target (Halfon et al., 2019).

The most striking example of translational infidelity as an organism-level adaptive strategy comes from *Mycobacterium tuberculosis*, which maintains ribosomes that are intrinsically error-prone as an inherent feature of its translation apparatus rather than a method of stress response (Javid et al., 2014). This elevated basal mistranslation confers a direct survival advantage against rifampicin, one of the antituberculosis antibiotics. Rifampicin kills bacteria by binding to the β-subunit of RNA polymerase and blocking transcription initiation. Cells in which a fraction of RNA polymerase copies carry mistranslated variants of this subunit possess reduced drug-binding sites, and those cells survive antibiotic challenge at concentrations lethal to the majority. Since the errors are stochastically distributed, different cells in the population carry different mistranslated variants and as a result the entire population possesses pre-existing phenotypic tolerance to the drug without any genetic mutation having occurred. Antibiotic-tolerant subpopulations of *M. tuberculosis* exist prior to the start of treatment, ready to resume infection once selective pressure is removed, and this pre-existing tolerance is a product of the organism's evolved translational strategy rather than of any treatment-induced adaptation (Javid et al., 2014).

The capacity of mistranslation to serve as a protective strategy is not confined to evolved features of pathogens. Fan et al. (2015) demonstrated that *E. coli* carrying a single substitution in ribosomal protein uS4 that raises the translational error rate approximately five-fold, survives hydrogen peroxide treatment at significantly higher rates than wild-type cells. Bacteria carrying an error-restrictive mutation in ribosomal protein uS12 showed the opposite phenotype, becoming less tolerant to oxidative effects. The protective mechanism operates through the general stress response: mistranslated proteins compete with the stress response sigma factor RpoS for the ClpP protease responsible for its degradation, causing RpoS to accumulate and activate downstream antioxidant genes including the catalase KatE and the peroxidase OsmC. Critically, the same protective effect was reproduced by treating wild-type bacteria with canavanine, a

naturally occurring arginine analogue that causes misincorporation at arginine codons, demonstrating that the response is a general consequence of elevated mistranslation rather than a specific effect of the ribosomal mutations used. Since RpoS-dependent stress responses are conserved across *Gammaproteobacteria* including *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, the capacity to exploit translational noise as a stress signal may represent a broadly available mechanism among clinically relevant organisms (Fan et al., 2015, de Pouplana et al., 2014). In pathogens, the relationship between fidelity and fitness is non-linear. For example, studies in *Salmonella enterica* have shown that ribosomal mutations shifting error rates in either direction, whether increasing or decreasing mistranslation relative to the wild-type level, impair host cell interactions and reduce fitness in zebrafish infection models, with both fidelity perturbations converging on the downregulation of invasion genes through destabilisation of the virulence regulator HilD (Fan et al., 2019).

Translational infidelity in bacteria is therefore not only a source of cellular damage to be suppressed by quality control systems but a phenotypic resource under active natural selection. The optimal error rate varies between organisms and environments rather than converging on a universal minimum, because the fitness value of phenotypic variance is itself dependent on context.

3. AIMS OF THE STUDY

Protein synthesis is markedly less accurate than DNA replication, yet most translation errors are transient and were long assumed to carry no lasting cost. Because DNA is itself replicated and repaired by proteins, a systematic decline in protein quality can, in principle, reach back to the genome and increase the mutation rate, an idea formalised in the translational stress-induced mutagenesis (TSM) model (Balashov and Humayun, 2003). The evidence for this connection comes almost entirely from *Escherichia coli*, which is exceptional in several respects, leaving open how generally translational fidelity shapes mutagenesis across bacteria.

The genus *Pseudomonas* provides an additional vantage point for assessing whether observed relationships are broadly conserved or organism-specific, as its members occupy diverse habitats and differ from *E. coli* in several finer features of their translation machinery. Even where the core machinery is highly conserved, bacterial species vary in their tRNA repertoire and modification landscape, codon usage, and finer features of rRNA and ribosomal protein composition, and such variation is known to shape decoding accuracy and the spectrum of translational errors (Agris et al., 2017, Decatur and Fournier, 2002). Translational fidelity can be perturbed at several points, and this work approaches it at two: the anticodon stem-loop pseudouridines introduced into tRNA by the synthases TruA and RluA, and the small-subunit ribosomal protein uS5, which helps set the stringency of codon recognition. Neither tRNA modification enzymes nor uS5 had previously been examined as determinants of genome stability in *Pseudomonas*.

A transposon screen conducted in our lab had initially implicated the pseudouridine synthase TruA as a determinant of mutation frequency in *P. putida*, an unexpected role for an enzyme with no direct part in DNA metabolism (Tagel et al., 2016). The first aim was therefore to confirm and characterise the effect for both TruA and the related synthase RluA, to identify which DNA repair and damage pathways potentially underlie it, and whether this confers a physiological effect. In addition, the work examined whether these observations hold in another representative of the *Pseudomonas* genus.

Secondly, we asked whether changes in translational decoding accuracy account for the observed increases in mutation frequency. To address this, we used a reporter system applicable across multiple species to quantify decoding errors as it allows for comparative analysis between different species, giving a broader look at the link between mistranslation and mutagenesis.

To investigate the link further, mistranslation was targeted specifically as a potential determinant of genomic mutations. Ribosomal protein uS5 lies at the decoding centre and is an established modulator of decoding accuracy in *E. coli*, but its influence on genome stability had not been assessed. Thus, the third aim was to isolate a panel of spontaneous uS5 variants in *P. putida*, to characterise how each alters frameshifting and stop-codon readthrough, and to determine whether these decoding defects are accompanied by elevated mutation rates, thereby testing how directly the extent of mistranslation predicts the mutagenic outcome.

4. MATERIALS AND METHODS

The experimental work presented in this thesis is based on Ref. I–III and combines genetic and biochemical approaches to investigate the relationship between translational fidelity and mutagenesis. The main experimental strategies included the construction of mutant strains, reporter-based measurements of translational fidelity, and fluctuation assays to quantify spontaneous mutagenesis. Detailed experimental procedures are described in the original publications, while the following sections provide an overview of the methods used.

4.1. Construction of mutant strains

Mutant strains used in this study were constructed using two different approaches, depending on the target genes and experimental objectives.

For analysis of tRNA pseudouridylation (Ref. I and II), deletion mutants of the pseudouridine synthase genes *truA* and *rluA* were generated in *P. putida* and *P. aeruginosa*. Markerless deletions were constructed using a two-step homologous recombination strategy based on the suicide vector pEMG and the I-SceI endonuclease system (Martínez-García and de Lorenzo, 2011). For this, ~500 bp DNA fragments corresponding to the regions upstream and downstream of the target gene were assembled by fusion PCR and cloned into the pEMG vector. The resulting construct was electroporated into competent cells and introduced into the bacterial chromosome via homologous recombination. Cointegrates were selected using antibiotic resistance markers. In the second step, expression of the I-SceI endonuclease stimulated recombination between homologous regions, resulting in excision of the vector sequence and generation of a markerless deletion allele (Wong and Mekalanos, 2000). Successful deletions were verified by PCR analysis. Complementation strains were generated by introducing the corresponding genes under control of the tac promoter using a mini-Tn7 chromosomal integration system (Wong and Mekalanos, 2000). The same system was also used to construct strains carrying catalytically inactive variants of *truA* or *rluA*. In these cases, mutated gene fragments were first generated by two-step PCR and subsequently inserted into the mini-Tn7 integration vector prior to chromosomal insertion.

Deletion mutants of *truA* and *rluA* were also constructed in *Escherichia coli* MG1655. The corresponding deletion alleles (*ΔtruA::km* and *ΔrluA::km*) were obtained from the Keio collection (Baba et al., 2006) and transferred into the MG1655 background by P1 phage transduction.

Kanamycin-resistant transductants were selected, and the resistance cassette was subsequently removed using FLP recombinase expressed from the temperature-sensitive plasmid pCP20 (Datsenko and Wanner, 2000). The plasmid was later eliminated by growth at an elevated temperature. Successful deletion of the target genes was confirmed by PCR.

Mutations in the ribosomal protein gene *rpsE*, encoding ribosomal protein uS5, were obtained using a spontaneous mutant selection approach. Overnight cultures of *P. putida* were plated on LB agar containing spectinomycin (1,750 μg mL^{-1}), a concentration previously determined to inhibit growth of the wild-type strain while allowing selection of resistant mutants. After incubation, spectinomycin-resistant colonies were isolated and screened by PCR amplification of the *rpsE* locus, followed by sequencing to identify mutations in the gene. Selected isolates were subsequently subjected to whole-genome sequencing to confirm the presence of the identified *rpsE* mutations and to exclude additional mutations elsewhere in the genome.

4.2. Construction of reporter plasmids

Reporter plasmids used for translational fidelity measurements were based on a dual-luciferase reporter system in which *Renilla* luciferase (Rluc) and firefly luciferase (Fluc) are expressed as a fusion protein separated by a sequence containing a translational error signal. In this configuration, production of the downstream firefly luciferase depends on frameshifting or stop-codon readthrough events. *Renilla* luciferase serves as an internal control for reporter expression, enabling normalization of firefly luciferase activity and improving the robustness of translational fidelity measurements.

The reporter constructs were generated in a broad-host-range pSEVA/lacItac vector enabling reporter transcription under control of the IPTG-inducible tac promoter and allowing their use in multiple bacterial species. Reporter variants were assembled using circular polymerase extension cloning (CPEC) and introduced into bacterial strains by transformation (Quan and Tian, 2011). The reporter panel included previously described frameshift and stop-codon reporters (AD2, AD5, AD7, 304UAG, and 417UGA) (Devaraj et al., 2009). In addition, two UUC-based frameshift reporters (UUC+ and UUC−) were constructed in this work by inserting oligonucleotide sequences containing the UUC signal into a modified pSEVA RF plasmid that allows cloning of sequences between the *rluc* and *fluc* genes. These reporter constructs were subsequently used to quantify translational fidelity in different genetic backgrounds.

4.3. Analysis of spontaneous mutagenesis

Spontaneous mutagenesis was quantified using a fluctuation assay based on selection for rifampicin resistance, as described in Ref. I and III. In this assay, bacterial cultures were first grown overnight under non-selective conditions and subsequently diluted into fresh medium to establish multiple independent cultures. These cultures were grown to the late exponential phase to allow spontaneous mutations to accumulate before plating on medium containing rifampicin to select for resistant mutants. In parallel, aliquots were plated on non-selective medium to determine viable cell counts (Jatsenko et al., 2010).

Mutant frequency was calculated as the number of rifampicin-resistant colonies relative to the number of viable cells. In experiments where calculation of mutation rates was required, fluctuation data from independent cultures were analyzed using maximum likelihood estimation implemented in the *mlemur* software package (Łazowski, 2023). To characterize mutation spectra, the *rpoB* locus of rifampicin-resistant clones was amplified by PCR and sequenced to identify the mutations responsible for rifampicin resistance. These measurements were used to assess the impact of translational fidelity perturbations on mutation frequency and rate.

4.4. Translational fidelity measurements

Translational fidelity was measured using the dual-luciferase reporter system described above. Bacterial strains carrying reporter plasmids were grown overnight in selective medium, then diluted into fresh medium. Reporter expression was induced with IPTG, and cultures were grown further into the early exponential phase under standardized conditions. Cells were harvested by centrifugation, lysed in Promega passive lysis buffer (E1941), and luciferase activity was measured sequentially using a luminometer.

Translational error frequencies were calculated as the ratio of firefly to *Renilla* luciferase activity (Fluc/Rluc), reflecting the frequency of frameshifting or stop-codon readthrough events. Reporter signals were normalized either to a control reporter construct or to the corresponding wild-type strain in order to account for differences in reporter expression between strains and experimental conditions.

4.5. RNA modification analysis

The pseudouridylation status of the tRNA anticodon stem-loop was examined by chemical probing combined with primer extension (Ref. I). RNA was isolated from exponentially growing wild-type and mutant strains, including the *truA* and *rluA* deletion strains and strains complemented with either functional or catalytically inactive enzyme. The RNA was treated with the carbodiimide CMCT, which forms an adduct at pseudouridine that resists the subsequent alkaline treatment, whereas adducts at uridine and guanosine are removed. Modified positions were mapped by reverse transcription with primers specific for individual tRNAs ($tRNA^{Ser}$, $tRNA^{Cys}$, $tRNA^{Leu}$ and $tRNA^{Tyr}$), and the products were separated on 7% polyacrylamide gels.

4.6. Proteome analysis

To examine the consequences of pseudouridylation loss on the cellular proteome, label-free quantitative mass spectrometry was performed on whole-cell lysates from exponentially growing cultures of *P. putida* wild type, Δ*truA*, and Δ*rluA* strains. Cells were harvested by centrifugation during mid-log phase (OD_{580} ~1.0). Quantitative proteomics was performed by LC-MS/MS using an LTQ-Orbitrap XL (Thermo Fisher Scientific) coupled to an Agilent 1200 nanoflow LC via a nanoelectrospray ion source (Proxeon) at the Proteomics Core Facility, Insitute of Technology, University of Tartu. Data were analysed using MaxQuant and Perseus software (Tyanova et al., 2016). Pairwise comparisons were made from a total of 3027 identified proteins: *P. putida* wild-type versus Δ*truA* (2856 proteins) and wild-type versus Δ*rluA* (2842 proteins). Proteins detected in all three biological replicates of at least one group were retained, missing values were imputed, and differential abundance was assessed by Student's t-test with Benjamini-Hochberg correction (FDR = 0.05).

4.7. Whole genome sequencing

To verify the presence of intended mutations and to exclude the possibility of additional mutations elsewhere in the genome, whole-genome sequencing (WGS) was performed on strains from Ref. III. Genomic DNA was extracted using the Thermo Scientific GeneJET Genomic DNA Purification Kit according to the manufacturer's instructions. Library preparation and sequencing were performed on the Illumina MiSeq platform with 100×

coverage. Raw sequencing reads were cleaned and filtered using fastp (Chen et al., 2018) and mapped to the *P. putida* KT2440 reference genome (GenBank accession NC_002947). Mutations were identified using breseq v.0.35.0 (Deatherage and Barrick, 2014).

4.8. Growth analysis and stress tolerance assays

Growth and stress tolerance were compared between wild-type and mutant strains (Ref. I and III). Growth was monitored in 96-well plates: overnight cultures were diluted to a low optical density (OD600 ~0.1) in fresh medium, and optical density was recorded automatically at short intervals throughout the experiment. Growth parameters, including the duration of the lag phase, the maximum growth rate, and the generation time, were extracted from the growth curves using curve-fitting software QurvE (Wirth et al., 2023). Stress tolerance was assessed mainly by dilution spot assays, in which serial dilutions of overnight cultures were spotted onto LB agar incubated under the conditions of interest. For the uS5 (*rpsE*) mutants, this included incubation at 30 °C and 20 °C and on medium supplemented with streptomycin (Ref. III). For the tRNA pseudouridylation mutants, growth and viability were additionally tested in the presence of the antibiotics tetracycline and ampicillin and of the reactive oxygen species-generating agent 4-nitroquinoline 1-oxide (NQO) (Ref. I).

4.9. Statistical analysis

Statistical analyses were performed using Prism 10 (GraphPad Software) and/or Statistica software. Data distribution was assessed using the Shapiro–Wilk test. Because most datasets did not follow a normal distribution, non-parametric tests were applied. Differences between groups were evaluated using the Kruskal–Wallis's test followed by Dunn's post hoc test where appropriate.

5. RESULTS AND DISCUSSION

5.1. Pseudouridines at positions 38–40 and 32 of the tRNA anticodon stem-loop contribute to mutation frequency in *Pseudomonas* sp. (Ref. I)

A transposon mutagenesis screen in *P. putida* PaW85 revealed that insertions in *truA* increased Rif^R mutant frequency among exponentially growing cells (Tagel et al., 2016). TruA is a tRNA pseudouridine (Ψ) synthase that incorporates Ψ at positions 38, 39, and 40 of the tRNA anticodon stem-loop (ASL). Primer extension analysis and CMCT/alkali treatment confirmed that TruA modifies uridine at positions 38–40 in all four tRNA species in *P. putida* PaW85 that were tested ($tRNA^{Ser}$(CGA), $tRNA^{Cys}$(GCA), $tRNA^{Leu}$(CAA), and $tRNA^{Leu}$(CAG)), and that the catalytic residue required for pseudouridylation is Asp70 (Ref. I, Fig. 1). Substitution of Asp70 with alanine (D70A) abolished pseudouridylation activity, and the D70A TruA variant retained the mutator phenotype of the deletion strain. These results confirmed that the elevated mutation frequency depends specifically on U38-40 pseudouridylation and not on the presence of the TruA protein itself.

Deletion of *truA* caused an approximately 5-fold increase in Rif^R mutant frequency in *P. putida* PaW85 (Ref. I, Fig. 2). RluA, a second ASL pseudouridine synthase responsible for Ψ at position 32, was also characterized. Deletion of *rluA* increased mutant frequency approximately 3-fold, and its pseudouridylation activity, rather than any non-catalytic function of the protein, was required for the effect, as confirmed by catalytically inactive RluA (D57A). The Δ*truA* Δ*rluA* double mutant exhibited a mutator phenotype comparable in magnitude to the Δ*truA* single mutant, suggesting convergent rather than additive contributions from the two enzymes (Ref. I, Fig. 2). Mutant frequency measurements in *P. aeruginosa* PAO1 showed that Δ*truA* also elevated mutation frequency in this species, although the effect was markedly smaller than in *P. putida*, and deletion of *rluA* had no detectable effect on mutation frequency in *P. aeruginosa* (Ref. I, Fig. 2).

A series of genetic epistasis experiments was conducted to identify the pathway through which Ψ38–40 and Ψ32 influence mutagenesis. Double-mutant analyses with *uvrD* (required for both mismatch repair and nucleotide excision repair) confirmed that malfunction of these DNA repair pathways is not the primary cause of the mutator phenotype, as *uvrD truA* and *uvrD rluA* double mutants exhibited elevated but qualitatively similar mutant frequencies compared to the *uvrD* single deletion (Ref. I, Fig. 3A).

The elevated mutant frequency persisted in a Δ*pol* background in which the inducible error-prone DNA polymerases Pol II (*polB*), Pol IV (*dinB*), and DnaE2 (*imuABC*) were all deleted, ruling out SOS-dependent mutagenesis and translesion synthesis as causal factors (Ref. I, Fig. 3B). Sequencing of Rif^R mutant *rpoB* alleles from wild-type, Δ*truA*, and Δ*rluA* strains revealed no significant differences in the spectrum of base substitutions, further excluding altered polymerase specificity or lesion-biased synthesis as an explanation (Ref. I, Table S6). The addition of the reactive oxygen species (ROS) scavenger thiourea to *P. putida* Δ*truA* and Δ*rluA* cultures had no effect on mutation frequency, suggesting that endogenously elevated ROS is an unlikely primary cause (Ref. I, Fig. 3C).

The Δ*truA* phenotype in *P. putida* extends well beyond mutagenesis. Label-free proteome analysis of exponentially growing Δ*truA* cells identified 158 proteins with statistically significant abundance changes relative to wild type, of which 18 differed by at least 2-fold; among these, the catalase KatE was downregulated, consistent with the hypersensitivity of Δ*truA* to the ROS-generating compounds NQO (Ref. I, Fig. 3D, 4). Further stress tolerance assays showed that *truA* deficiency reduced growth in the presence of antibiotics tetracycline and ampicillin, and ROS-inducing 4-Nitroquinoline 1-oxide (NQO) in *P. putida*, whereas Δ*rluA* strains showed no such changes in any of the three species examined (Ref. I, Fig. 3D). The NQO hypersensitivity is readily explained by the reduced KatE expression seen from proteome data (Ref. I, Fig. 4): impaired catalase activity compromises neutralization of exogenously generated hydrogen peroxide. This mechanism could be distinct from the endogenous metabolic ROS that thiourea scavenging showed not to be responsible for the elevated mutation frequency (Ref. I, Fig. 3C). Thus, TruA-mediated pseudouridylation is required for the normal translation of diverse cellular proteins including stress response enzymes, but the primary mutagenic consequence of pseudouridylation loss operates through a mechanism independent of intracellular ROS levels.

The identification of *truA* and *rluA* as determinants of mutation frequency is notable in that tRNA modification enzymes had not previously been associated with DNA mutagenesis. Classical mutator genes either directly encode components of the DNA replication or repair machinery (e.g., *mutD*, encoding the proofreading ε-subunit of DNA polymerase III) (Echols et al., 1983), or encode tRNAs that alter amino acid identity during decoding (e.g., *mutA*, *mutC*) (Slupska et al., 1996). In contrast, TruA and RluA act upstream by modifying specific uridines in tRNA anticodon stem-loops without altering tRNA charging specificity. Thus, a mechanistically distinct position in the information flow from gene to protein. This places

truA and *rluA* in a new category of mutator genes: enzymes whose role in maintaining translational accuracy is required for genome stability, even though they do not directly interact with DNA.

Several mechanistic routes could connect loss of tRNA pseudouridylation to elevated mutagenesis. Under a TSM-type model, the relevant question is whether Ψ38–40 or Ψ32 loss specifically impairs translation of genome-maintenance enzymes, such as mismatch repair components (MutS, MutL) or DNA polymerase III subunits, at levels sufficient to compromise their function without being detectable by standard reporter assays. The *dnaX* frameshift, well established as a TSM-relevant target in *E. coli*, is unlikely to play an equivalent role in *P. putida*, where the *dnaX* gene lacks an obvious -1 frameshift signal and proteomic data suggest that the τ- and γ-subunits are produced at comparable stoichiometry (unpublished data).

5.2. Translational fidelity is affected by tRNA anticodon stem-loop pseudouridines in a species- and context-dependent manner (Ref. II)

Modifications at the tRNA ASL directly influence codon–anticodon pairing geometry and the dynamics of tRNA accommodation at the ribosomal A-site, providing a mechanistic basis for context-specific effects on decoding accuracy. Given that disruption of tRNA pseudouridylation increases mutagenesis, it is important to determine whether these effects are associated with changes in translational fidelity, as alterations in decoding accuracy may provide a mechanistic link between translation and genome stability.

Translational fidelity is not necessarily uniform across bacterial species, as differences in the composition and regulation of the translation machinery can yield distinct decoding accuracies. However, direct comparison between species has been limited by the lack of standardized reporter systems applicable across multiple hosts. To enable such comparisons, a dual-luciferase reporter system was adapted to a broad-host-range plasmid, allowing quantitative assessment of translational fidelity in *P. putida* PaW85, *P. aeruginosa* PAO1, and *E. coli* MG1655 under comparable conditions (Ref. II, Fig. 1). In this system, firefly luciferase (Fluc) production depends on a translational error event, while Renilla luciferase (Rluc) serves as an internal expression control.

The use of identical reporter constructs across species also enables a direct, standardized comparison of absolute translational error frequencies

under equivalent conditions, a capability that has been lacking in comparative microbiology owing to the host-specificity of previous reporter systems. The cross-species data thereby provide a novel quantitative benchmark of translational fidelity across these three bacterial species.

In total, five frameshift and two stop codon readthrough events were analysed. Comparison of translational fidelity across the three species revealed distinct species-specific error profiles (Ref. II, Fig. 2). Translational errors were strongly dependent on reporter context, with substantial variation between different frameshift signals and between frameshifting and stop codon readthrough. No single species consistently exhibited higher or lower error frequencies across all reporters; instead, each organism displayed a distinct combination of error tendencies.

For example, +1 frameshifting in the AD5 context in *P. putida* was measured at 17%, indicating that approximately one in six ribosomes failed to maintain the correct reading frame in this context, highlighting that certain signals can promote very high levels of translational errors. In contrast, other frameshift signals in *P. putida* produced substantially lower error frequencies, demonstrating that frameshifting is highly dependent on the local sequence context. Consistent with this, the same AD5 reporter yielded markedly lower error frequencies in both *P. aeruginosa* and *E. coli*, with mistranslation rates of 2–3%, indicating that the same decoding signal can be interpreted very differently across host organisms.

More broadly, frameshifting patterns differed between species. While *P. putida* and *P. aeruginosa* displayed considerable variation across different frameshift reporters, *E. coli* maintained relatively low and more uniform frameshifting frequencies through all tested contexts. This suggests that *E. coli* exhibits a more robust maintenance of the reading frame, whereas translational accuracy in the *Pseudomonas* species is more sensitive to sequence context.

A different pattern was observed for stop codon readthrough. For the UAG codon, readthrough levels were low across all three species, with only minor differences between *Pseudomonas* species and *E. coli*. In contrast, readthrough of the UGA codon showed a pronounced species-specific effect. While both *Pseudomonas* species maintained low UGA readthrough frequencies (0.04–0.08%), *E. coli* exhibited substantially higher readthrough, reaching approximately 12%. This indicates that termination efficiency at UGA differs markedly between species and suggests that distinct components of translational fidelity are differentially regulated.

The markedly elevated UGA readthrough in *E. coli* compared to both *Pseudomonas* species may reflect differences in the abundance or modi-

fication status of near-cognate suppressor tRNAs. In *E. coli*, $tRNA^{Trp}$ (anticodon CCA) is a well-characterized near-cognate UGA suppressor, and its suppression efficiency is sensitive to anticodon-adjacent modifications, including modifications at position 37 of the tRNA anticodon stem-loop. In *E. coli*, $tRNA^{Trp}$ carries ms^2i^6A at position 37, immediately adjacent to the anticodon, and this modification is required for efficient near-cognate decoding of UGA, as strains lacking it show substantially reduced UGA readthrough (Petrullo et al., 1983, Vacher et al., 1984). *P. putida* encodes the additional enzyme MiaE, which converts ms^2i^6A37 to the hydroxylated derivative ms^2io^6A37, giving *P. putida* $tRNA^{Trp}$ a chemically distinct modification at this position that may support near-cognate UGA suppression less efficiently (Carpentier et al., 2020). Species-specific differences in the modification landscape of tRNAs positioned at or near the UGA codon during translation could therefore account for the divergent readthrough frequencies observed, independently of differences in ribosomal decoding stringency.

To assess the contribution of tRNA pseudouridylation to translational fidelity, *truA* and *rluA* deletion mutants of *P. putida*, *P. aeruginosa*, and *E. coli* were analysed using the same reporter panel (Ref. II, Fig. 1).

In *P. putida* PaW85, Δ*truA* significantly increased frameshifting in the AD2 reporter (2.2-fold increase) but had no statistically significant effect on AD5, AD7, or either UUC-based reporter (Ref. II, Fig. 3). Expression of the wild-type *truA* gene in the deletion mutant restored −1 frameshifting in the AD2 context to near wild-type levels, whereas expression of a catalytically inactive variant had no effect, confirming that the observed increase in frameshifting was directly attributable to loss of TruA activity (Ref. II, Fig. 4). Stop codon readthrough was differentially affected: 304UAG readthrough was elevated in Δ*truA* cells, whereas 417UGA readthrough was unchanged, indicating that the decoding consequences of Ψ38–40 loss are codon-specific rather than global (Ref. II, Fig. 3). Deletion of *rluA* had no detectable effect on any of the seven reporters in *P. putida*, a result that is particularly significant given that Δ*rluA* cells exhibit an elevated mutant frequency in this organism (Ref. I). Thus, the mechanism underlying the Δ*rluA* mutator phenotype cannot be attributed to detectable alterations in translational accuracy at the tested sequence contexts.

The species comparison produced a markedly different picture. In *P. aeruginosa* PAO1, neither Δ*truA* nor Δ*rluA* produced any statistically significant change in translational fidelity across any of the reporters tested, despite the documented mutator phenotype of Δ*truA* in this organism (Ref. II, Fig. 5, Ref. I). In *E. coli* MG1655, by contrast, Δ*truA* affected almost all reporters in the panel, representing the most pronounced

translational fidelity changes observed across the three species; Δ*rluA* again had minimal impact in *E. coli* (Ref. II, Fig. 6). The observation that *truA* deficiency causes the largest translational accuracy defects in *E. coli* yet no mutator phenotype in that organism, while producing comparatively modest fidelity changes but a robust 5-fold mutator phenotype in *P. putida*, is inconsistent with a straightforward translational stress-induced mutagenesis (TSM) model as a sufficient explanation. The absence of any fidelity effect for Δ*rluA* across all three species, despite its contribution to mutagenesis in *P. putida*, further decouples the two phenotypes for this enzyme.

The reporter-specificity of translational errors in Δ*truA P. putida*, detectable at AD2 and 304UAG but not at other frameshifting sequences or at 417UGA, reflects the context-dependence of tRNA modification effects on decoding, consistent with prior observations in *Salmonella enterica* where Ψ38 influenced frameshifting selectively depending on the tRNA species positioned at the ribosomal P-site (Urbonavičius et al., 2001, Urbonavičius et al., 2003). The divergent fidelity phenotypes across three species with near-identical TruA and RluA substrate patterns indicate that codon usage frequency, tRNA pool composition, and the competitive landscape of cognate and near-cognate tRNA accommodation determine whether a given ASL modification loss manifests as a measurable decoding defect. Collectively, the data from Ref. II establish that Ψ38–40 and Ψ32 modulate translational fidelity in a reporter-, context-, and species-specific manner, but that the pattern of translational errors does not straightforwardly account for the mutagenesis phenotypes characterized in Ref. I.

5.3. Mutations in ribosomal protein uS5 alter translational accuracy and mutagenesis in *Pseudomonas putida* (Ref. III)

Ribosomal protein uS5, encoded by *rpsE*, is positioned near the decoding center of the 30S subunit and, together with uS4 and uS12, constitutes a principal modulator of codon–anticodon discrimination stringency. Mutations in *rpsE* have been shown in *E. coli* to generate either ribosomal ambiguity (ram) alleles that increase acceptance of near-cognate aminoacyl-tRNAs, or restrictive alleles that tighten decoding selectivity, with the ram phenotype arising from disrupted interactions between uS5 and uS4 near the A-site (Rosset and Gorini, 1969, Zimmermann et al., 1971, Agarwal et al., 2015). Spectinomycin resistance in bacteria is associated with specific *rpsE* mutations, providing a phenotypic marker for functional

changes at the decoding center. In *P. putida*, a total panel of 7 spontaneous spectinomycin-resistant *rpsE* mutants was isolated and sequenced, consisting of substitutions V22I, K24E, G28D, G29R, T33I, T33P, and a Δ34–35 deletion (Ref. III, Fig. 1).

Translational fidelity was characterized for each *rpsE* allele using the dual-luciferase reporter panel employed in Ref. II. Analysis of translational fidelity revealed mutation-specific effects across the reporter panel. The G29R mutant showed the most pronounced deviations, with increased −1 frameshifting in reporters such as AD7 and elevated stop codon readthrough (Ref. III, Fig. 3 and 4). In contrast, mutants such as K24E and T33I exhibited more limited and context-dependent changes, with several reporters remaining at levels comparable to the wild type. Other variants, including T33P and Δ34–35, affected only a subset of reporters, further indicating that the impact of uS5 mutations depends on the decoding context (Ref. III, Fig. 3 and 4). The translational fidelity changes conferred by individual *rpsE* alleles were confirmed to be attributable to the ribosomal protein mutation itself, as whole-genome sequencing of selected isolates excluded additional mutations elsewhere in the chromosome.

Spontaneous mutation frequencies were determined by Rif^R fluctuation analysis for each *rpsE* allele. Results indicated that all examined uS5 mutants except G29R showed significantly elevated mutation rates relative to the wild type. The strongest effect was observed for the Δ34–35 mutant, which displayed an approximately threefold increase, whereas V22I, K24E, and G28D showed roughly twofold increases and T33I and T33P about 2.5-fold increases (Ref. III, Fig. 5). G29R was the only mutant that did not differ from the wild type in mutation rate, despite showing one of the strongest translational fidelity phenotypes. This indicates that pronounced mistranslation does not automatically result in increased mutagenesis, and that the relationship depends on the specific nature of the translational perturbation.

The G29R allele, which produced the most pronounced frameshifting and elevated stop codon readthrough across the reporter panel, was paradoxically the only allele that did not elevate mutagenesis, whereas alleles with more modest and context-limited fidelity changes consistently did. This suggests that the magnitude of translational errors per se does not predict mutagenic potential. Under the TSM model, only mistranslation of specific genome-maintenance enzymes, DNA polymerase subunits, mismatch repair components etc., would be expected to elevate mutation rates. It is therefore possible that the error signature of G29R does not efficiently corrupt these critical targets while registering strongly in our reporter system, while in the meantime, the subtler but more broadly distributed

fidelity changes of other alleles exhibit inverse action. Identifying which translational targets mediate the mutagenic effect remains an important open question.

The results presented in these publications demonstrate that the translational apparatus influences genome stability, but the relationship between translational fidelity and mutagenesis is not direct or proportional. Disruption of tRNA pseudouridylation increases mutation frequency, yet these effects are not consistently reflected in measurable changes in translational fidelity. Conversely, mutations in the ribosomal protein uS5 produce clear and mutation-specific alterations in decoding accuracy, but only a subset of these changes are associated with elevated mutagenesis. Taken together, these findings indicate that increased mistranslation alone is not sufficient to predict mutagenesis. Instead, the impact of translational perturbations on genome stability depends on the specific nature of the decoding errors and the cellular context in which they occur. This suggests that distinct types of translational errors may differentially influence downstream processes linked to mutation formation.

Overall, the results support a model in which translation contributes to genome stability through multiple, context-dependent mechanisms, rather than through a simple linear relationship between mistranslation and mutagenesis.

6. CONCLUSIONS

The accurate transmission of genetic information depends not only on the fidelity of DNA replication and repair, but also on the broader accuracy of cellular information processing. It has long been recognized that translational errors can feed back to influence genome stability, a concept formalized in the translational stress-induced mutagenesis (TSM) model, which proposes that mistranslated DNA replication and repair enzymes introduce additional DNA errors, amplifying mutation frequency. Whether this model fully accounts for the relationship between translational fidelity and mutagenesis in bacteria beyond *E. coli* remains an open question. The studies presented in this thesis addressed this question primarily in *Pseudomonas putida*, with comparative analysis also extending to *Pseudomonas aeruginosa*, by targeting tRNA modification enzymes and ribosomal proteins to perturb translational fidelity at distinct levels of the translation machinery. The main findings can be summarized as follows:

Pseudouridines in the tRNA anticodon stem-loop contribute to genome stability in *Pseudomonas* (Ref. I)

- Loss of TruA-mediated pseudouridylation at positions 38–40 of the tRNA anticodon stem-loop elevates spontaneous mutation frequency approximately 5-fold in exponentially growing *P. putida* PaW85, while loss of RluA-mediated Ψ32 produces a ~3-fold increase. Catalytically inactive variants of both enzymes recapitulate the mutator phenotype of the respective deletion strains, establishing that the effect is a direct consequence of the absent pseudouridylation activity.
- The mutator phenotypes of *ΔtruA* and *ΔrluA* cannot be attributed to defects in mismatch repair, nucleotide excision repair, error-prone DNA polymerases, homologous recombination, or elevated intracellular ROS, indicating a novel mutagenic pathway linked to translational function.
- TruA-mediated pseudouridylation contributes to genome stability in *P. aeruginosa* PAO1 as well, though the effect is smaller than in *P. putida*. Loss of RluA has no detectable effect on mutation frequency in *P. aeruginosa*.
- TruA deficiency broadly alters the proteome of exponentially growing *P. putida*, including downregulation of the catalase KatE, which explains the hypersensitivity of *ΔtruA* cells to exogenous oxidative stress. RluA deficiency has minimal phenotypic and proteome consequences despite its contribution to mutagenesis.

Translational fidelity is modulated by tRNA pseudouridylation in a context- and species-dependent manner (Ref. II)

- A broad-host-range dual-luciferase reporter system enabled the first direct comparison of translational error frequencies across *P. putida*, *P. aeruginosa* and *E. coli* under equivalent conditions. All three species display distinct translational error profiles strongly dependent on reporter context.
- Loss of Ψ38–40 (TruA) increases −1 frameshifting in the AD2 reporter and UAG stop codon readthrough in *P. putida* but leaves most other contexts unaffected. Loss of Ψ32 (RluA) has no detectable effect on any reporter in any species, despite its contribution to mutagenesis in *P. putida*. These results show that the mutagenic consequences of pseudouridine loss are not directly explained by globally elevated translational error frequencies.

Ribosomal protein uS5 mutations alter translational accuracy and mutagenesis in a mutation-specific manner (Ref. III)

- Seven spontaneous spectinomycin-resistance-conferring mutations in *rpsE* were isolated and characterized in *P. putida* PaW85. All alleles altered translational fidelity in a reporter- and mutation-specific manner, consistent with their location near the uS4–uS5 interface affecting codon-anticodon discrimination.
- Six of the seven uS5 alleles elevated spontaneous mutation frequency 2–3-fold relative to wild type, consistent with a TSM-like mechanism. This establishes ribosomal proteins as contributors to genome stability in *P. putida*.
- The G29R substitution, which produced the most pronounced translational fidelity changes across the reporter panel, was the sole allele that did not elevate mutagenesis. This demonstrates that the magnitude of translational errors does not directly predict mutagenic outcome.

Taken together, the results of this thesis demonstrate that translational fidelity and genome stability are connected through multiple, mechanistically distinct pathways in *P. putida*. Disruption of translational accuracy – whether at the level of tRNA anticodon loop modifications or at the ribosomal decoding center – can elevate mutagenesis, but the relationship is not proportional and is not captured by a simple, linear TSM model. The specific nature of the translational perturbation, the cellular context, and species-specific differences in translation machinery and DNA repair

capacity all shape the mutagenic outcome. Comparison across *P. putida*, *P. aeruginosa* and *E. coli* reveals that *P. putida* exhibits a particularly robust functional link between translational stress and mutagenesis compared to *E. coli*, underscoring the importance of studying mutational processes in diverse bacterial species. These findings expand our understanding of the factors that shape mutation frequency in bacteria and position the translational apparatus as an integral component of the genome stability network in *P. putida*.

7. SUMMARY IN ESTONIAN

Valgusünteesi täpsuse ja genoomi stabiilsuse tingimuslik põimumine bakteriperekonnas *Pseudomonas*

Valgusüntees on rakus suure kiirusega lakkamatult kulgev protsess ning selle paratamatuks hinnaks on aeg-ajalt tekkivad vead: ribosoom lisab valku vale aminohappe või nihkub lugemisraamist välja umbes kord mõne tuhande koodoni kohta. Kuna valesti sünteesitud valgud on enamasti lühiealised ja kaovad kiiresti raku ainevahetuse voolus, on neid pikka aega peetud rakule võrdlemisi kahjutuks nähtuseks. Siiski on pandud tähele, et valesti tõlgitud geneetiline sõnum võib jätta endast ka püsivama jälje, suurendades mutatsioonide hulka DNAs. Käesolev doktoritöö otsib vastust küsimusele, kuidas ja millistel tingimustel on valgusünteesi täpsus seotud genoomi terviklikkuse püsivusega.

Suurem osa senistest teadmistest selle seose kohta pärineb soolebakterist *Escherichia coli*. See liik on aga paljude omaduste poolest erandlik ning seetõttu jääb lahtiseks, kuivõrd üldised on sellest tehtud järeldused. Käesolevas töös kasutati mudelorganismidena hoopis bakteriperekonna *Pseudomonas* liikmeid. Kuigi *Pseudomonas putida* ja oportunistlik haigustekitaja *Pseudomonas aeruginosa* jagavad *E. coli*-ga paljusid bakterile omaseid põhitunnuseid, pakuvad nende erinev ökoloogiline taust ja translatsioonisüsteemi eripärad võimaluse hinnata, kui üldine on valgusünteesi täpsuse ja mutageneesi vaheline seos.

Valgusünteesi täpsus sõltub biokeemilistest protsessidest mitmes eri etapis ning käesolevas töös uuriti seda kahel tasandil. Esimesel juhul puudusid tRNA antikoodoni läheduses paiknevad pseudouridiinid (Ψ), mida metsiktüve rakkudes sünteesivad ensüümid TruA (positsioonid 38–40) ja RluA (positsioon 32). Teisel juhul muudeti ribosoomi väikese alaühiku valku uS5, mis hõlbustab koodoni ja antikoodoni paardumist ning toimib omamoodi kvaliteedikontrolörina geneetilise sõnumi lugemisel. Mõlemal juhul hinnati paralleelselt nii dekodeerimise täpsust topelt-lutsiferaasi reporteritega kui ka spontaanset mutatsioonisagedust, mida mõõdeti rifampitsiiniresistentsusel põhineva fluktuatsioonitestiga.

Pseudouridiinide puudumine osutus mutatsioonisageduse seisukohalt ootamatult oluliseks. Geenide *truA* või *rluA* väljalülitamine suurendas *P. putida* mutatsioonisagedust vastavalt ligikaudu viis ja kolm korda. Sama toime ilmnes ka katalüütiliselt inaktiveeritud ensüümivariantide puhul, mis näitab, et määrava tähtsusega oli just modifikatsiooni kadumine, mitte valgu enda puudumine. Seda tõusu ei õnnestunud seletada ühegi seni teadaoleva DNA reparatsiooniraja, vigu tegevate polümeraaside, rekombinat-

siooni ega rakusiseste reaktiivsete hapnikuühenditega, mis annab alust viidata seni kirjeldamata mehhanismile. Laiem proteoomianalüüs näitas, et TruA puudumine mõjutab paljude valkude taset, sealhulgas vähendab katalaasi KatE hulka ning muudab rakud väliste oksüdantide suhtes tundlikumaks. *P. aeruginosa*-s suurenes *truA* puudumisel samuti mutatsioonisagedus, kuigi mõju oli väiksem, ning *rluA* puudumine mutatsioonisagedust ei mõjutanud.

Selgitamaks, kas need muutused kajastuvad ka dekodeerimise täpsuses, võrreldi translatsioonivigade sagedusi korraga kolmes bakteriliigis ning selleks viidi topelt-lutsiferaas reportersüsteem laia peremeesringiga plasmiidi, et säilitada katseandmete võrreldavus. Selgus, et igal liigil on oma iseloomulik veaprofiil ning vigade sagedus sõltub tugevalt geneetilisest kontekstist. Pseudouridiinide mõju osutus tagasihoidlikuks ja valikuliseks: TruA puudumine suurendas *P. putida*-s vaid üksikuid veatüüpe, samas kui RluA puudumisel ei olnud mõõdetavat mõju, kuigi mutatsioonisagedus oli mõlema ensüümi puudumisel suurenenud. Translatsioonivigade hulga ja mutageneesi vahel ei joonistunud seega välja sirgjoonelist vastavust.

Töö teine osa keskendus ribosoomivalgule uS5. *P. putida*-st õnnestus antibiootikumi spektinomütsiini selektsiooni abil luua seitse geeni *rpsE* mutanti, mis kõik mõjutasid mingil määral valgusünteesi täpsust. Neist kuus suurendasid mutatsioonisagedust kahe- kuni kolmekordselt, kinnitades, et genoomi terviklikkuse püsivusse panustavad ka ribosoomivalgud. Mutant G29R oli kõnekas erand, kuna sellel asendusel oli suurim mõju valgusünteesi täpsusele, kuid see oli ka uuritud paneelist ainuke, mille puhul mutatsioonisagedus jäi metsiktüvega võrreldes muutumatuks. See näitab, et mutageenne tagajärg ei sõltu pelgalt vigade arvust, vaid sellest, milliseid protsesse need rakus mõjutavad.

Kokkuvõttes näitavad käesoleva doktoritöö tulemused, et valgusünteesi täpsus ja genoomi püsivus on seotud mitme omavahel põimunud mehhanismi kaudu, mida ei saa taandada ühele lihtsale põhjus-tagajärg seosele. Oluline ei ole niivõrd vigade üldine hulk, kuivõrd nende iseloom, rakuline kontekst ja liigist tulenevad erinevused. Kõige selgemini avaldus see seos mullabakteris *P. putida*, mis tuletab meelde, kui palju võib bakterite alusbioloogiast olla veel avastamata väljaspool tavapäraseid mudelorganisme.

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REFERENCES

AGARWAL, D., GREGORY, S. T. & O'CONNOR, M. 2011. Error-prone and error-restrictive mutations affecting ribosomal protein S12. *Journal of molecular biology,* 410**,** 1–9.

AGARWAL, D., KAMATH, D., GREGORY, S. T. & O'CONNOR, M. 2015. Modulation of decoding fidelity by ribosomal proteins S4 and S5. *J Bacteriol,* 197**,** 1017–25.

AGRIS, P. F., NARENDRAN, A., SARACHAN, K., VÄRE, V. Y. P. & ERUYSAL, E. 2017. The importance of being modified: the role of RNA modifications in translational fidelity. *The Enzymes,* 41**,** 1–50.

AL MAMUN, A. A. M., GAUTAM, S. & HUMAYUN, M. Z. 2006. Hypermutagenesis in mutA cells is mediated by mistranslational corruption of polymerase, and is accompanied by replication fork collapse. *Molecular microbiology,* 62**,** 1752–1763.

AL MAMUN, A. A. M., MARIANS, K. J. & HUMAYUN, M. Z. 2002. DNA Polymerase III from Escherichia coliCells Expressing mutA Mistranslator tRNA Is Error-prone. *Journal of Biological Chemistry,* 277**,** 46319–46327.

ALEXANDROV, A., CHERNYAKOV, I., GU, W., HILEY, S. L., HUGHES, T. R., GRAYHACK, E. J. & PHIZICKY, E. M. 2006. Rapid tRNA decay can result from lack of nonessential modifications. *Molecular cell,* 21**,** 87–96.

BABA, T., ARA, T., HASEGAWA, M., TAKAI, Y., OKUMURA, Y., BABA, M., DATSENKO, K. A., TOMITA, M., WANNER, B. L. & MORI, H. 2006. Construction of Escherichia coli K-12 in-frame, single-gene knockout mutants: the Keio collection. *Molecular systems biology,* 2**,** MSB4100050.

BALASHOV, S. & HUMAYUN, M. Z. 2002. Mistranslation induced by streptomycin provokes a RecABC/RuvABC-dependent mutator phenotype in Escherichia coli cells. *Journal of molecular biology,* 315**,** 513–527.

BALASHOV, S. & HUMAYUN, M. Z. 2003. Escherichia coli cells bearing a ribosomal ambiguity mutation in rpsD have a mutator phenotype that correlates with increased mistranslation. *Journal of bacteriology,* 185**,** 5015–5018.

BJÖRKMAN, J., SAMUELSSON, P., ANDERSSON, D. I. & HUGHES, D. 1999. Novel ribosomal mutations affecting translational accuracy, antibiotic resistance and virulence of Salmonella typhimurium. *Molecular microbiology,* 31**,** 53–58.

BLINKOWA, A. L. & WALKER, J. R. 1990. Programmed ribosomal frameshifting generates the *Escherichia coli* DNA polymerase III γ subunit from within the τ subunit reading frame. *Nucleic acids research,* 18**,** 1725–1729.

BOHMAN, K., RUUSALA, T., JELENC, P. C. & KURLAND, C. G. 1984. Kinetic impairment of restrictive streptomycin-resistant ribosomes. *Molecular and General Genetics MGG,* 198**,** 90–99.

BRIERLEY, I., DIGARD, P. & INGLIS, S. C. 1989. Characterization of an efficient coronavirus ribosomal frameshifting signal: requirement for an RNA pseudoknot. *Cell,* 57**,** 537–547.

BUSKIRK, A. R. & GREEN, R. 2017. Ribosome pausing, arrest and rescue in bacteria and eukaryotes. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 372.

CARPENTIER, P., LEPRÊTRE, C., BASSET, C., DOUKI, T., TORELLI, S., DUARTE, V., HAMDANE, D., FONTECAVE, M. & ATTA, M. 2020. Structural, biochemical and functional analyses of tRNA-monooxygenase enzyme MiaE from

Pseudomonas putida provide insights into tRNA/MiaE interaction. *Nucleic Acids Research,* 48**,** 9918–9930.
CHEN, S., ZHOU, Y., CHEN, Y. & GU, J. 2018. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics,* 34**,** i884–i890.
DATSENKO, K. A. & WANNER, B. L. 2000. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. *Proceedings of the National Academy of Sciences,* 97**,** 6640–6645.
DAVIS, B. D. 1987. Mechanism of bactericidal action of aminoglycosides. *Microbiological reviews,* 51**,** 341–350.
DE POUPLANA, L. R., SANTOS, M. A. S., ZHU, J.-H., FARABAUGH, P. J. & JAVID, B. 2014. Protein mistranslation: friend or foe? *Trends in biochemical sciences,* 39**,** 355–362.
DEATHERAGE, D. E. & BARRICK, J. E. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. *Engineering and analyzing multicellular systems: methods and protocols.* Springer.
DECATUR, W. A. & FOURNIER, M. J. 2002. rRNA modifications and ribosome function. *Trends in biochemical sciences,* 27, 344–351.
DEMIRCI, H., WANG, L., MURPHY, F. V., MURPHY, E. L., CARR, J. F., BLANCHARD, S. C., JOGL, G., DAHLBERG, A. E. & GREGORY, S. T. 2013. The central role of protein S12 in organizing the structure of the decoding site of the ribosome. *Rna,* 19**,** 1791–1801.
DETTMAN, J. R., SZTEPANACZ, J. L. & KASSEN, R. 2016. The properties of spontaneous mutations in the opportunistic pathogen Pseudomonas aeruginosa. *BMC genomics,* 17**,** 27.
DEVARAJ, A., SHOJI, S., HOLBROOK, E. D. & FREDRICK, K. 2009. A role for the 30S subunit E site in maintenance of the translational reading frame. *Rna,* 15, 255–265.
DORAZI, R., LINGUTLA, J. J. & HUMAYUN, M. Z. 2002. Expression of mutant alanine tRNAs increases spontaneous mutagenesis in Escherichia coli. *Molecular microbiology,* 44, 131–141.
DRUMMOND, D. A. & WILKE, C. O. 2009. The evolutionary consequences of erroneous protein synthesis. *Nature Reviews Genetics,* 10**,** 715–724.
ECHOLS, H., LU, C. & BURGERS, P. M. 1983. Mutator strains of Escherichia coli, mutD and dnaQ, with defective exonucleolytic editing by DNA polymerase III holoenzyme. *Proceedings of the National Academy of Sciences,* 80**,** 2189–2192.
ESCOUBAS, J.-M., PRERE, M. F., FAYET, O., SALVIGNOL, I., GALAS, D., ZERBIB, D. & CHANDLER, M. 1991. Translational control of transposition activity of the bacterial insertion sequence IS1. *The EMBO Journal,* 10, 705–712.
FAN, Y., EVANS, C. R., BARBER, K. W., BANERJEE, K., WEISS, K. J., MARGOLIN, W., IGOSHIN, O. A., RINEHART, J. & LING, J. 2017. Heterogeneity of stop codon readthrough in single bacterial cells and implications for population fitness. *Molecular cell,* 67, 826–836.
FAN, Y., THOMPSON, L., LYU, Z., CAMERON, T. A., DE LAY, N. R., KRACHLER, A. M. & LING, J. 2019. Optimal translational fidelity is critical for Salmonella virulence and host interactions. *Nucleic acids research,* 47, 5356–5367.
FAN, Y., WU, J., UNG, M. H., DE LAY, N., CHENG, C. & LING, J. 2015. Protein mistranslation protects bacteria against oxidative stress. *Nucleic acids research,* 43, 1740–1748.

FARABAUGH, P. J. 1996. Programmed translational frameshifting. *Microbiological reviews,* 60**,** 103–134.
FIJALKOWSKA, I. J., SCHAAPER, R. M. & JONCZYK, P. 2012. DNA replication fidelity in Escherichia coli: a multi-DNA polymerase affair. *FEMS microbiology reviews,* 36**,** 1105–1121.
FLOWER, A. M. & MCHENRY, C. S. 1990. The gamma subunit of DNA polymerase III holoenzyme of *Escherichia coli* is produced by ribosomal frameshifting. *Proceedings of the National Academy of Sciences,* 87, 3713–3717.
FOSTER, P. L., HANSON, A. J., LEE, H., POPODI, E. M. & TANG, H. 2013. On the mutational topology of the bacterial genome. *G3: Genes, Genomes, Genetics,* 3, 399–407.
FOURMY, D., RECHT, M. I. & PUGLISI, J. D. 1998. Binding of neomycin-class aminoglycoside antibiotics to the A-site of 16 S rRNA. *Journal of molecular biology,* 277, 347–362.
FREDRIKSSON, Å., BALLESTEROS, M., DUKAN, S. & NYSTRÖM, T. 2006. Induction of the heat shock regulon in response to increased mistranslation requires oxidative modification of the malformed proteins. *Molecular microbiology,* 59, 350–359.
GAMPER, H. B., MASUDA, I., FRENKEL-MORGENSTERN, M. & HOU, Y.-M. 2015. Maintenance of protein synthesis reading frame by EF-P and m1G37-tRNA. *Nature communications,* 6, 7226.
GARIBYAN, L., HUANG, T., KIM, M., WOLFF, E., NGUYEN, A., NGUYEN, T., DIEP, A., HU, K., IVERSON, A. & YANG, H. 2003. Use of the rpoB gene to determine the specificity of base substitution mutations on the Escherichia coli chromosome. *DNA repair,* 2, 593–608.
GAROFALO, R., WOHLGEMUTH, I., PEARSON, M., LENZ, C., URLAUB, H. & RODNINA, M. V. 2019. Broad range of missense error frequencies in cellular proteins. *Nucleic acids research,* 47, 2932–2945.
GRENTZMANN, G., INGRAM, J. A., KELLY, P. J., GESTALAND, R. F. & ATKINS, J. F. 1998. A dual-luciferase reporter system for studying recoding signals. *Rna,* 4, 479–486.
HALFON, Y., JIMENEZ-FERNANDEZ, A., LA ROSA, R., ESPINOSA PORTERO, R., KROGH JOHANSEN, H., MATZOV, D., EYAL, Z., BASHAN, A., ZIMMERMAN, E. & BELOUSOFF, M. 2019. Structure of Pseudomonas aeruginosa ribosomes from an aminoglycoside-resistant clinical isolate. *Proceedings of the National Academy of Sciences,* 116, 22275–22281.
HINNU, M., PUTRINŠ, M., KOGERMANN, K., KALDALU, N. & TENSON, T. 2024. Fluorescent reporters give new insights into antibiotics-induced nonsense and frameshift mistranslation. *Scientific Reports,* 14**,** 6883.
IMASHIMIZU, M., OSHIMA, T., LUBKOWSKA, L. & KASHLEV, M. 2013. Direct assessment of transcription fidelity by high-resolution RNA sequencing. *Nucleic acids research,* 41, 9090–9104.
JACKS, T., POWER, M. D., MASIARZ, F. R., LUCIW, P. A., BARR, P. J. & VARMUS, H. E. 1988. Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. *Nature,* 331, 280–283.
JACKS, T. & VARMUS, H. E. 1985. Expression of the Rous sarcoma virus pol gene by ribosomal frameshifting. *Science,* 230**,** 1237–1242.
JATSENKO, T., TOVER, A., TEGOVA, R. & KIVISAAR, M. 2010. Molecular characterization of Rifr mutations in Pseudomonas aeruginosa and Pseudomonas putida.

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 683, 106–114.
JAVID, B., SORRENTINO, F., TOOSKY, M., ZHENG, W., PINKHAM, J. T., JAIN, N., PAN, M., DEIGHAN, P. & RUBIN, E. J. 2014. Mycobacterial mistranslation is necessary and sufficient for rifampicin phenotypic resistance. *Proceedings of the National Academy of Sciences,* 111**,** 1132–1137.
KAMATH, D., GREGORY, S. T. & O'CONNOR, M. 2017. The loop 2 region of ribosomal protein uS5 influences spectinomycin sensitivity, translational fidelity, and ribosome biogenesis. *Antimicrobial Agents and Chemotherapy,* 61**,** 10.1128/aac. 01186–16.
KAMMEN, H. O., MARVEL, C. C., HARDY, L. & PENHOET, E. E. 1988. Purification, structure, and properties of Escherichia coli tRNA pseudouridine synthase I. *Journal of Biological Chemistry,* 263**,** 2255–2263.
KEILER, K. C. 2015. Mechanisms of ribosome rescue in bacteria. *Nature Reviews Microbiology,* 13**,** 285–297.
KIRTHI, N., ROY-CHAUDHURI, B., KELLEY, T. & CULVER, G. M. 2006. A novel single amino acid change in small subunit ribosomal protein S5 has profound effects on translational fidelity. *RNA,* 12**,** 2080–2091.
KIVISAAR, M. 2020. Narrative of a versatile and adept species Pseudomonas putida. *Journal of medical microbiology,* 69**,** 324–338.
KOHANSKI, M. A., DWYER, D. J., HAYETE, B., LAWRENCE, C. A. & COLLINS, J. J. 2007. A common mechanism of cellular death induced by bactericidal antibiotics. *Cell,* 130**,** 797–810.
KOHANSKI, M. A., DWYER, D. J., WIERZBOWSKI, J., COTTAREL, G. & COLLINS, J. J. 2008. Mistranslation of membrane proteins and two-component system activation trigger antibiotic-mediated cell death. *Cell,* 135**,** 679–690.
KOTHE, U. & RODNINA, M. V. 2007. Codon reading by tRNAAla with modified uridine in the wobble position. *Molecular cell,* 25**,** 167–174.
KRAMER, E. B. & FARABAUGH, P. J. 2007. The frequency of translational misreading errors in E. coli is largely determined by tRNA competition. *RNA,* 13**,** 87–96.
KURLAND, C. G. 1960. Molecular characterization of ribonucleic acid from Escherichia coli ribosomes: I. Isolation and molecular weights. *Journal of Molecular Biology,* 2**,** 83–91.
KURLAND, C. G. 1992. Translational accuracy and the fitness of bacteria. *Annual review of genetics,* 26**,** 29–50.
KYUMA, T., KIZAKI, H., RYUNO, H., SEKIMIZU, K. & KAITO, C. 2015. 16S rRNA methyltransferase KsgA contributes to oxidative stress resistance and virulence in Staphylococcus aureus. *Biochimie,* 119**,** 166–174.
ŁAZOWSKI, K. 2023. Efficient, robust, and versatile fluctuation data analysis using MLE MUtation Rate calculator (mlemur). *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 826**,** 111816.
LEE, H., POPODI, E., TANG, H. & FOSTER, P. L. 2012. Rate and molecular spectrum of spontaneous mutations in the bacterium Escherichia coli as determined by whole-genome sequencing. *Proceedings of the National Academy of Sciences,* 109**,** E2774–E2783.
LING, J., REYNOLDS, N. & IBBA, M. 2009. Aminoacyl-tRNA synthesis and translational quality control. *Annual review of microbiology,* 63**,** 61–78.

LING, J. & SÖLL, D. 2010. Severe oxidative stress induces protein mistranslation through impairment of an aminoacyl-tRNA synthetase editing site. *Proceedings of the National Academy of Sciences,* 107**,** 4028–4033.
LISTER, P. D., WOLTER, D. J. & HANSON, N. D. 2009. Antibacterial-resistant Pseudomonas aeruginosa: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clinical microbiology reviews,* 22**,** 582–610.
LIU, L., SHAO, M., HUANG, Y., QIAN, P. & HUANG, H. 2024. Unraveling the roles and mechanisms of mitochondrial translation in normal and malignant hematopoiesis. *Journal of Hematology & Oncology,* 17**,** 95.
LURIA, S. E. & DELBRÜCK, M. 1943. Mutations of bacteria from virus sensitivity to virus resistance. *Genetics,* 28**,** 491.
MAHILLON, J. & CHANDLER, M. 1998. Insertion sequences. *Microbiology and molecular biology reviews,* 62**,** 725–774.
MARTÍNEZ-GARCÍA, E. & DE LORENZO, V. 2011. Engineering multiple genomic deletions in Gram-negative bacteria: analysis of the multi-resistant antibiotic profile of Pseudomonas putida KT2440. *Environmental microbiology,* 13**,** 2702–2716.
MASSEY, S. E. 2008. The proteomic constraint and its role in molecular evolution. *Molecular biology and evolution,* 25**,** 2557–2565.
MASUDA, I., HWANG, J.-Y., CHRISTIAN, T., MAHARJAN, S., MOHAMMAD, F., GAMPER, H., BUSKIRK, A. R. & HOU, Y.-M. 2021. Loss of N1-methylation of G37 in tRNA induces ribosome stalling and reprograms gene expression. *Elife,* 10**,** e70619.
MCCLORY, S. P., LEISRING, J. M., QIN, D. & FREDRICK, K. 2010. Missense suppressor mutations in 16S rRNA reveal the importance of helices h8 and h14 in aminoacyl-tRNA selection. *Rna,* 16**,** 1925–1934.
MILLER, J. H. 1972. Experiments in molecular genetics. *(No Title)*.
MORDRET, E., DAHAN, O., ASRAF, O., RAK, R., YEHONADAV, A., BARNABAS, G. D., COX, J., GEIGER, T., LINDNER, A. B. & PILPEL, Y. 2019. Systematic detection of amino acid substitutions in proteomes reveals mechanistic basis of ribosome errors and selection for translation fidelity. *Molecular Cell,* 75**,** 427–441.
MURPHY, H. S. & HUMAYUN, M. Z. 1997. Escherichia coli cells expressing a mutant glyV (glycine tRNA) gene have a UVM-constitutive phenotype: implications for mechanisms underlying the mutA or mutC mutator effect. *Journal of bacteriology,* 179**,** 7507–7514.
NETZER, N., GOODENBOUR, J. M., DAVID, A., DITTMAR, K. A., JONES, R. B., SCHNEIDER, J. R., BOONE, D., EVES, E. M., ROSNER, M. R. & GIBBS, J. S. 2009. Innate immune and chemically triggered oxidative stress modifies translational fidelity. *Nature,* 462**,** 522–526.
NICCUM, B. A., LEE, H., MOHAMMEDISMAIL, W., TANG, H. & FOSTER, P. L. 2019. The symmetrical wave pattern of base-pair substitution rates across the Escherichia coli chromosome has multiple causes. *MBio,* 10**,** 10–1128.
NIKOLAY, R., VAN DEN BRUCK, D., ACHENBACH, J. & NIERHAUS, K. H. 2015. Ribosomal proteins: role in ribosomal functions. *eLS***,** 1–12.
NUREKI, O., VASSYLYEV, D. G., TATENO, M., SHIMADA, A., NAKAMA, T., FUKAI, S., KONNO, M., HENDRICKSON, T. L., SCHIMMEL, P. & YOKOYAMA, S. 1998. Enzyme structure with two catalytic sites for double-sieve selection of substrate. *Science,* 280**,** 578–582.

O'CONNOR, M. & DAHLBERG, A. E. 1993. Mutations at U2555, a tRNA-protected base in 23S rRNA, affect translational fidelity. *Proceedings of the National Academy of Sciences,* 90**,** 9214–9218.
O'CONNOR, M., GREGORY, S. T. & DAHLBERG, A. E. 2004. Multiple defects in translation associated with altered ribosomal protein L4. *Nucleic acids research,* 32**,** 5750–5756.
O'CONNOR, M. 2002. Imbalance of tRNAPro isoacceptors induces+ 1 frameshifting at near-cognate codons. *Nucleic acids research,* 30**,** 759–765.
OFENGAND, J. & DEL CAMPO, M. 2004. Modified nucleosides of Escherichia coli ribosomal RNA. *EcoSal Plus,* 1**,** 10–1128.
OGLE, J. M., BRODERSEN, D. E., CLEMONS JR, W. M., TARRY, M. J., CARTER, A. P. & RAMAKRISHNAN, V. 2001. Recognition of cognate transfer RNA by the 30 S ribosomal subunit. *Science,* 292**,** 897–902.
OGLE, J. M., MURPHY, F. V., TARRY, M. J. & RAMAKRISHNAN, V. 2002. Selection of tRNA by the ribosome requires a transition from an open to a closed form. *Cell,* 111**,** 721–732.
ORGEL, L. E. 1970. The maintenance of the accuracy of protein synthesis and its relevance to ageing: a correction. *Proceedings of the National Academy of Sciences,* 67**,** 1476–1476.
PETRULLO, L. A., GALLAGHER, P. J. & ELSEVIERS, D. 1983. The role of 2-methylthio-N6-isopentenyladenosine in readthrough and suppression of nonsense codons in Escherichia coli. *Molecular and General Genetics MGG,* 190**,** 289–294.
PLANT, E. P., JACOBS, K. L. M., HARGER, J. W., MESKAUSKAS, A., JACOBS, J. L., BAXTER, J. L., PETROV, A. N. & DINMAN, J. D. 2003. The 9-Å solution: How mRNA pseudoknots promote efficient programmed− 1 ribosomal frameshifting. *Rna,* 9**,** 168–174.
PROSHKIN, S., RAHMOUNI, A. R., MIRONOV, A. & NUDLER, E. 2010. Cooperation between translating ribosomes and RNA polymerase in transcription elongation. *Science,* 328**,** 504–508.
QUAN, J. & TIAN, J. 2011. Circular polymerase extension cloning for high-throughput cloning of complex and combinatorial DNA libraries. *Nature protocols,* 6**,** 242–251.
REN, L., AL MAMUN, A. A. M. & HUMAYUN, M. Z. 1999. The mutA mistranslator tRNA-induced mutator phenotype requires recA and recB genes, but not the derepression of lexA-regulated functions. *Molecular microbiology,* 32**,** 607–615.
RODNINA, M. V. 2016. The ribosome in action: Tuning of translational efficiency and protein folding. *Protein Science,* 25**,** 1390–1406.
ROSSET, R. & GORINI, L. 1969. A ribosomal ambiguity mutation. *Journal of molecular biology,* 39**,** 95–112.
RUUSALA, T., ANDERSSON, D., EHRENBERG, M. & KURLAND, C. G. 1984. Hyper-accurate ribosomes inhibit growth. *The EMBO journal,* 3**,** 2575–2580.
SAMHITA, L., K RAVAL, P., STEPHENSON, G., THUTUPALLI, S. & AGASHE, D. 2021. The impact of mistranslation on phenotypic variability and fitness. *Evolution,* 75**,** 1201–1217.
SAMHITA, L., RAVAL, P. K. & AGASHE, D. 2020. Global mistranslation increases cell survival under stress in Escherichia coli. *PLoS genetics,* 16**,** e1008654.
SCHAAPER, R. M. & MATHEWS, C. K. 2013. Mutational consequences of dNTP pool imbalances in E. coli. *DNA repair,* 12**,** 73–79.

SHARMA, D., CUKRAS, A. R., ROGERS, E. J., SOUTHWORTH, D. R. & GREEN, R. 2007. Mutational analysis of S12 protein and implications for the accuracy of decoding by the ribosome. *Journal of molecular biology,* 374**,** 1065–1076.

SILBY, M. W., WINSTANLEY, C., GODFREY, S. A. C., LEVY, S. B. & JACKSON, R. W. 2011. Pseudomonas genomes: diverse and adaptable. *FEMS microbiology reviews,* 35**,** 652–680.

SLUPSKA, M. M., BAIKALOV, C., LLOYD, R. & MILLER, J. H. 1996. Mutator tRNAs are encoded by the Escherichia coli mutator genes mutA and mutC: a novel pathway for mutagenesis. *Proceedings of the National Academy of Sciences,* 93**,** 4380–4385.

SORDYL, D., BOILEAU, E., BERNAT, A., MAITI, S., MUKHERJEE, S., MOAFINEJAD, S. N., FARSANI, M. A., SHAVINA, A., CAPPANNINI, A. & AGOSTINI, G. 2026. MODOMICS: a database of RNA modifications and related information. 2025 update and 20th anniversary. *Nucleic Acids Research,* 54**,** D219–D225.

ZAHER, H. S. & GREEN, R. 2009. Fidelity at the molecular level: lessons from protein synthesis. *Cell,* 136**,** 746–762.

ZAHER, H. S. & GREEN, R. 2010. Hyperaccurate and error-prone ribosomes exploit distinct mechanisms during tRNA selection. *Molecular cell,* 39**,** 110–120.

ZHENG, J., GUO, N. & WAGNER, A. 2021. Mistranslation reduces mutation load in evolving proteins through negative epistasis with DNA mutations. *Molecular Biology and Evolution,* 38**,** 4792–4804.

ZIMMERMANN, R. A., GARVIN, R. T. & GORINI, L. 1971. Alteration of a 30S ribosomal protein accompanying the ram mutation in Escherichia coli. *Proceedings of the National Academy of Sciences,* 68**,** 2263–2267.

TAGEL, M., TAVITA, K., HÕRAK, R., KIVISAAR, M. & ILVES, H. 2016. A novel papillation assay for the identification of genes affecting mutation rate in Pseudomonas putida and other pseudomonads. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis,* 790**,** 41–55.

THOMPSON, R. C. & STONE, P. J. 1977. Proofreading of the codon-anticodon interaction on ribosomes. *Proceedings of the National Academy of Sciences,* 74**,** 198–202.

TRAVERSE, C. C. & OCHMAN, H. 2016. Conserved rates and patterns of transcription errors across bacterial growth states and lifestyles. *Proceedings of the National Academy of Sciences,* 113**,** 3311–3316.

TYANOVA, S., TEMU, T., SINITCYN, P., CARLSON, A., HEIN, M. Y., GEIGER, T., MANN, M. & COX, J. 2016. The Perseus computational platform for comprehensive analysis of (prote) omics data. *Nature methods,* 13**,** 731–740.

URBONAVIČIUS, J., QIAN, Q., DURAND, J., HAGERVALL, T. G. & BJÖRK, G. R. 2001. Improvement of reading frame maintenance is a common function for several tRNA modifications. *The EMBO journal,* 20**,** 4863–4873.

URBONAVIČIUS, J., STAHL, G., DURAND, J. M. B., BEN SALEM, S. N., QIAN, Q., FARABAUGH, P. J. & BJÖRK, G. R. 2003. Transfer RNA modifications that alter+ 1 frameshifting in general fail to affect− 1 frameshifting. *Rna,* 9**,** 760–768.

VACHER, J., GROSJEAN, H., HOUSSIER, C. & BUCKINGHAM, R. H. 1984. The effect of point mutations affecting Escherichia coli tryptophan tRNA on anticodon-anticodon interactions and on UGA suppression. *Journal of Molecular Biology,* 177**,** 329–342.

WEISS, R. B., DUNN, D. M., ATKINS, J. F. & GESTELAND, R. F. 1987. Slippery runs, shifty stops, backward steps, and forward hops:− 2,− 1,+ 1,+ 2,+ 5, and+ 6 ribosomal frameshifting. *Cold Spring Harbor symposia on quantitative biology,* 52**,** 687–693.

WIRTH, N. T., FUNK, J., DONATI, S. & NIKEL, P. I. 2023. QurvE: user-friendly software for the analysis of biological growth and fluorescence data. *Nature Protocols,* 18**,** 2401–2403.
WOHLGEMUTH, I., POHL, C. & RODNINA, M. V. 2010. Optimization of speed and accuracy of decoding in translation. *The EMBO journal,* 29**,** 3701–3709.
WONG, S. M. & MEKALANOS, J. J. 2000. Genetic footprinting with mariner-based transposition in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences,* 97**,** 10191–10196.
WOO, A. C., FAURE, L., DAPA, T. & MATIC, I. 2018. Heterogeneity of spontaneous DNA replication errors in single isogenic Escherichia coli cells. *Science Advances,* 4**,** eaat1608.
WRZESINSKI, J., NURSE, K., BAKIN, A., LANE, B. G. & OFENGAND, J. 1995. A dual-specificity pseudouridine synthase: an Escherichia coli synthase purified and cloned on the basis of its specificity for psi 746 in 23S RNA is also specific for psi 32 in tRNA (phe). *Rna,* 1**,** 437–448.
VÖGELE, J., DUCHARDT-FERNER, E., KRUSE, H., ZHANG, Z., SPONER, J., KREPL, M. & WÖHNERT, J. 2023. Structural and dynamic effects of pseudouridine modifications on noncanonical interactions in RNA. *RNA,* 29**,** 790–807.

PUBLICATIONS

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Jürgenstein, K., Tagel, M., Ilves, H., Leppik, M., Kivisaar, M., & Remme, J. (2022). Variance in translational fidelity of different bacterial species is affected by pseudouridines in the tRNA anticodon stem-loop. RNA biology, 19(1), 1050–1058.

Jürgenstein, K., Ilves, H., Luhaäär, C., Brauer, A., Remme, J., & Kivisaar, M. (2025). Mutations in ribosomal protein uS5 alter translation fidelity and mutagenesis in *Pseudomonas putida*. Journal of Bacteriology, 207(12), e00334–25.

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Publikatsioonide loetelu:

Tagel, M., Ilves, H., Leppik, M., **Jürgenstein, K**., Remme, J., & Kivisaar, M. (2020). Pseudouridines of tRNA Anticodon Stem-Loop Have Unexpected Role in Mutagenesis in *Pseudomonas* sp. Microorganisms, 9(1), 25.

Jürgenstein, K., Tagel, M., Ilves, H., Leppik, M., Kivisaar, M., & Remme, J. (2022). Variance in translational fidelity of different bacterial species is affected by pseudouridines in the tRNA anticodon stem-loop. RNA biology, 19(1), 1050–1058.

Jürgenstein, K., Ilves, H., Luhaäär, C., Brauer, A., Remme, J., & Kivisaar, M. (2025). Mutations in ribosomal protein uS5 alter translation fidelity and mutagenesis in *Pseudomonas putida*. Journal of Bacteriology, 207(12), e00334–25.

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