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Recycling of Ribosomal Complexes Stalled at the Step of Elongation in *Escherichia coli*

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Translating ribosomes often stall during elongation. The stalled ribosomes are known to be recycled by tmRNA (SsrA)-mediated *trans*-translation. Another process that recycles the stalled ribosomes is characterized by peptidyl-tRNA release. However, the mechanism of peptidyl-tRNA release from the stalled ribosomes is not well understood. We used a defined system of an AGA-minigene containing a small open reading frame (ATG AGA AGA). Translation of the AGA-minigene mRNA is toxic to *Escherichia coli* because it stalls ribosomes during elongation and sequesters $tRNA^{Arg4}$ as a short-chain peptidyl-$tRNA^{Arg4}$ in the ribosomal P-site. We show that a ribosome recycling factor (RRF)-mediated process rescues the host from the AGA-minigene toxicity by releasing the peptidyl-$tRNA^{Arg4}$ from the ribosomes. The growth phenotypes of *E. coli* strains harboring mutant alleles of RRF and initiation factor 3 (IF3) genes and their consequences on λ*imm*P22 phage replication upon AGA-minigene expression reveal that IF3 facilitates the RRF-mediated processing of the stalled ribosomes. Additionally, we have designed a uracil DNA glycosylase gene construct, *ung*-stopless, whose expression is toxic to *E. coli*. We show that the RRF-mediated process also alleviates the *ung*-stopless construct-mediated toxicity to the host by releasing the *ung* mRNA from the ribosomes harboring long-chain peptidyl-tRNAs.

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Introduction

Cells have evolved mechanisms to maintain the fidelity and efficiency of each of the steps in protein synthesis. However, due to various reasons, the translating ribosomes often stall during elongation. It is imperative, therefore, for the cell to rescue the stalled ribosomes to maintain the steady-state supply of free tRNAs and ribosomes. The stalled ribosomes containing truncated mRNAs are known to be recycled by tmRNA (SsrA)-mediated *trans*-translation.[1] The process of *trans*-translation extends the incomplete peptide by translating the short open reading frame (ORF) of the tmRNA, and rescues the stalled ribosomes by subjecting them to a termination codon-dependent translation termination. Another process that rescues the stalled ribosomes results in release of the peptidyl-tRNA (drop-off) from them.[2–6] Although the mechanism of peptidyl-tRNA release from the ribosome is not well understood, the dropped-off peptidyl-tRNAs themselves are recycled by peptidyl-tRNA hydrolase (Pth), which cleaves the ester link between the peptide and the tRNA.[7,8] The importance of Pth in recycling peptidyl-tRNAs was demonstrated by the essential nature of the *pth* gene.[3] Notably, the free peptidyl-tRNAs but not those present within ribosomes are substrates for Pth.[9] Further, it was observed that the lambda phage fails to grow on an *Escherichia coli* (*rap*) isolate.[10] The *rap* isolate was later shown to possess a mutation in *pth* gene resulting in lower specific activity of the encoded protein.[11] Interestingly, when the highly transcribed and translated *barI* and *barII* minigenes (ATG ATA TAG and ATG ATA TAA, respectively) present in the phage genome were mutated, the mutant phage could grow on the *E. coli* (*rap*) strain.[12] The *barI* and *barII* minigenes sequester $tRNA^{Ile}$ as peptidyl-$tRNA^{Ile}$

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Abbreviations used: ORF, open reading frame; Pth, peptidyl-tRNA hydrolase.

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