A synthetic biology approach of translational control

Kirsten Jung[1,2], Wolfram Volkwein[1,2], Bastian Viverge[1,3], Andreas Reichert[1,4], Arne Skerra[1,4], Thomas Carell[1,3], and Jürgen Lassak[1,2]

[1]Center for Integrated Protein Science Munich, Ludwig-Maximilians-Universität München, D-81377 Munich

[2]Department of Biology I, Microbiology, Ludwig-Maximilians-Universität München, D-82152 Martinsried

[3]Department of Chemistry, Ludwig-Maximilians-Universität München, D-81377 Munich

[4]Biological Chemistry, Technische Universität München, D-85350 Freising-Weihenstephan

Translation of proteins with a stretch of consecutive prolines leads to ribosome stalling. To overcome this stop, bacteria depend on a specific translation elongation factor P (EF-P), being orthologous and functional identical to eukaryotic/archaeal elongation factor e/aIF-5A (1-3). EF-P binds to the ribosome between the peptidyl-tRNA binding site (P-site) and the tRNA exiting site (E-site) and stimulates peptide bond formation. In their active form both EF-P and e/aIF-5A are post-translationally modified at a positively charged amino acid, which protrudes towards the peptidyl-transferase center. While archaeal and eukaryotic IF-5A depend on hypusination of a conserved lysine, the EF-P modification strategies in bacteria vary. In *Escherichia coli* and *Salmonella enterica* a lysine of EF-P is extended by β-lysinylation and subsequently hydroxylated, whereas in *Pseudomonas aeruginosa* and *Shewanella oneidensis* an arginine in the equivalent position is rhamnosylated (reviewed in 4). In addition to structural constrains of polyproline stretches, some EF-P dependent proteins require this motif to fine-tune the protein output.
Our studies aim to create a synthetic EF-P variant, which is standardized and constitutively active independent of species-specific posttranslational modifications. For this purpose, we replace the conserved lysine with unnatural amino acids, such as pyrrolysine, acetyl-lysine, propionyl-lysine, and butyryl-lysine by using the amber suppression system. All synthetic variants are tested for functionality *in vivo* using reporter strains. In addition, first results are presented of using the amber suppression system in combination with stalling motifs to generate a translational control tool to fine-tune the protein output.

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