Supplementary Materials for:

Horizontal transfer of post-translational modifiers brings evolutionary opportunity and challenges to a conserved translation factor

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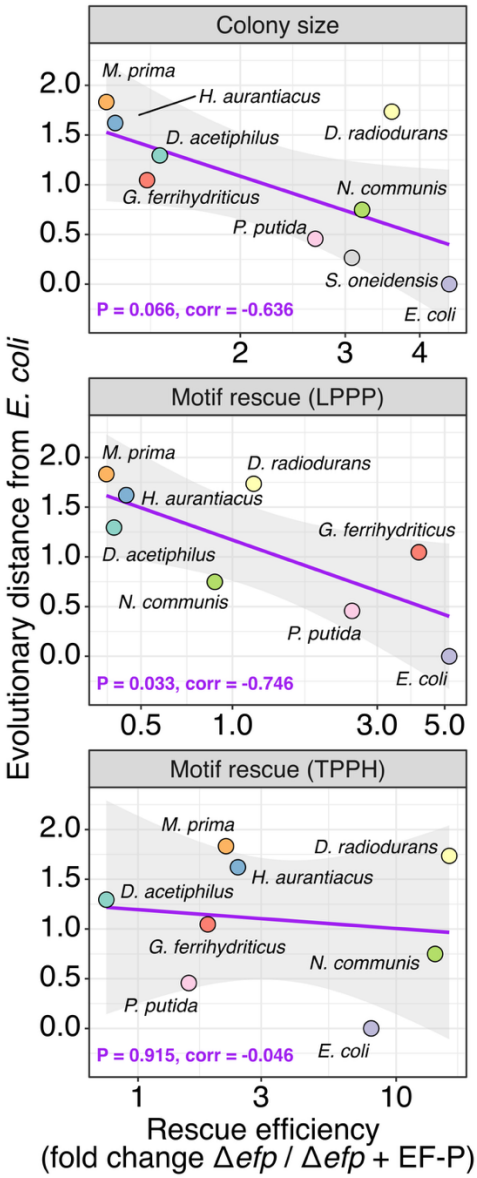
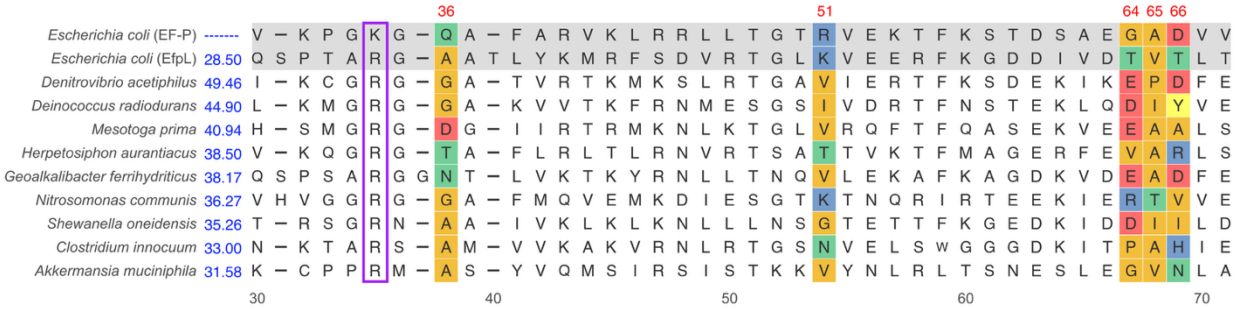
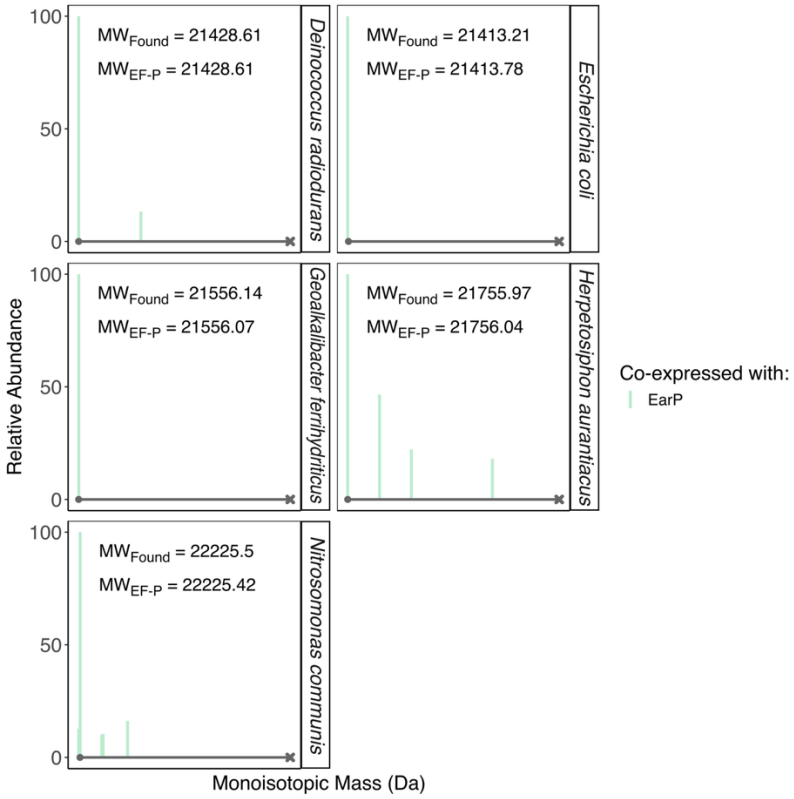
Contains:
Supplemental Figures 1-6



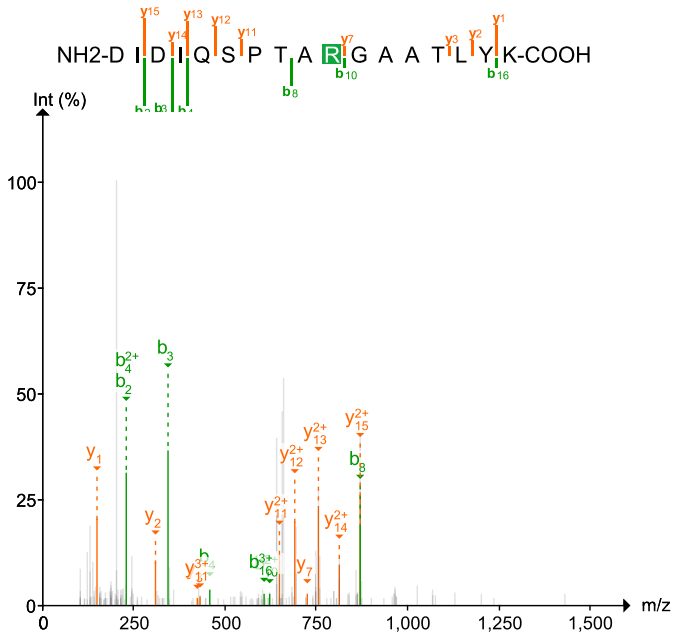
Supplemental Figure 1) EF-P from more closely related species generally function better in *E. coli* than those from distantly related species. Evolutionary distance represents the phylogenetic distance between the labelled species and *E. coli* on a phylogenetic tree based on concatenated amino acid sequences of 40 marker genes (see Methods for further details). Rescue efficiency refers to the fold change between the Δ*efp* strain carrying a control plasmid (although in the case of the colony size experiments the control strains was Δ*efp*Δ*uup*), and the trans-complemented strain carrying an EF-P expression plasmid. Correlations are Pearson's correlations, purple lines indicate the linear regression line, and the shaded area represents the 95% confidence area. The horizontal axis is plotted on a logarithmic scale. For *S. oneidensis* and *P. putida* only the values for the rhamnosylated form of EF-P were included.



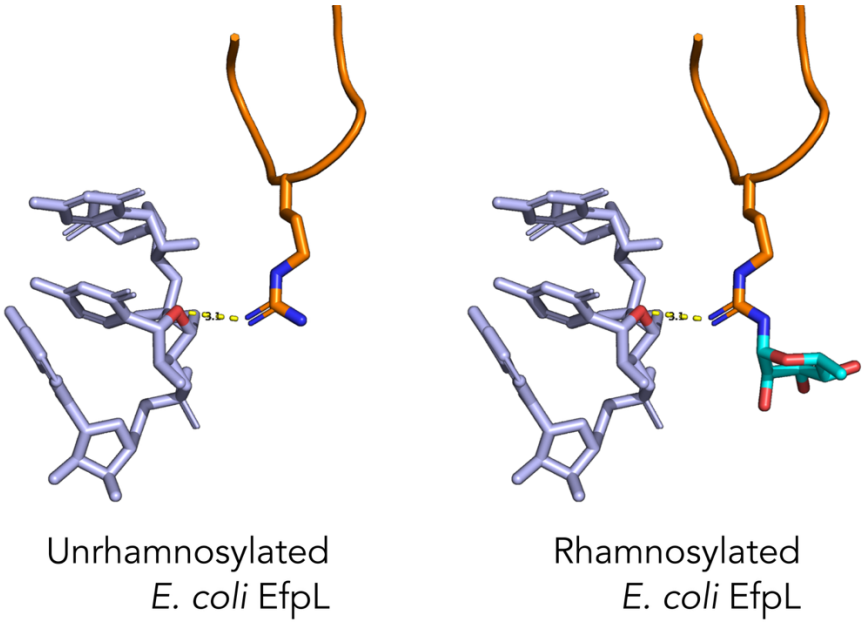
Supplemental Figure 2) Alignment of selected regions of tested EF-P. Numbers in blue indicate the percent similarity of each EF-P amino acid sequence to the *E. coli* (non EfpL) version. Identity of amino acids in colored positions had an impact on function in a different set of heterologous EF-P expressed in *E. coli* in Tomasiunaite, U et al 2024. Colors correspond to amino acid chemistry (blue = positively charged AAs: K, R; red = negatively charged AAs: D, E; green = polar side changes: T, Q; yellow = aromatic: Y; gold = others). Purple outline highlights the amino acid position that is post-translationally modified and red numbers at top indicate relative position in the *E. coli* EF-P.



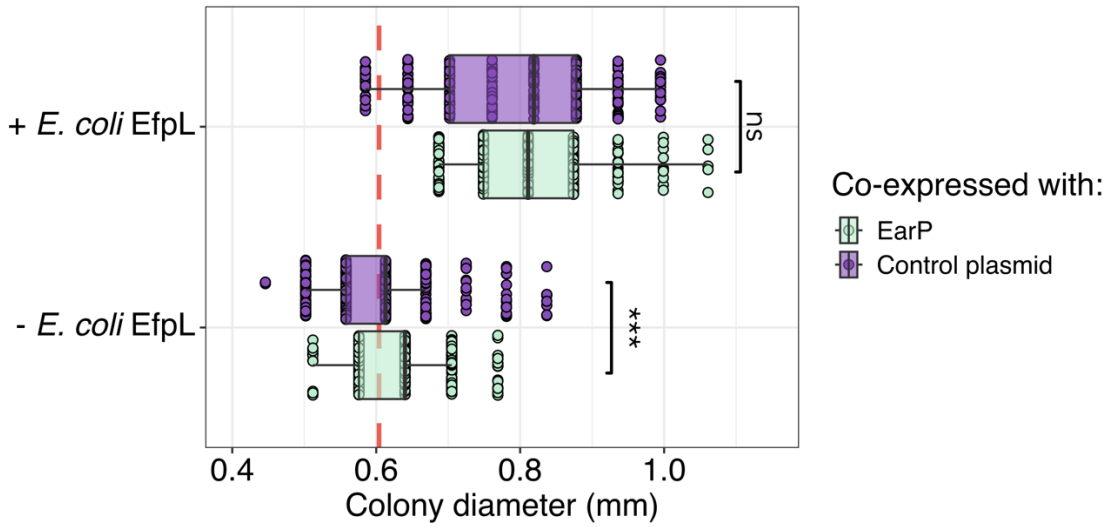
Supplemental Figure 3) Deconvoluted intact protein mass spectra of the indicated His-tagged EF-P co-overproduced with EarP from *Shewanella oneidensis* in *E. coli* LMG194. MW_{found} refers to the molecular weight (Da) of the dominant peak, while MW_{EF-P} refers to the calculated monoisotopic mass of each EF-P based on the sequence of the expressed gene (the masses for the *E. coli* and *N. communis* EF-P do not include the N-terminal methionine, a common modification for EF-P[1]). The X indicates the increase in MW expected if each EF-P were rhamnosylated. Only dominant peaks are labelled.



Supplemental Figure 4) After overproduction of EarP$_{Ppu}$ in *E. coli*, we detected a modified EfpL peptide through MS-based proteomics, indicating rhamnosylation. Annotated fragment ion mass spectrum of the Arg33-modified EfpL peptide (Δm = 146.058 Da, Rhamnose-H_2O, $C_6H_{10}O_4$).



Supplemental Figure 5) Two models showing possible alignments of EF-P with the tRNA CCA supporting the functional observations. The *E. coli* EfpL is shown in either an unrhamnosylated state (left) or rhamnosylated state (right). The tRNA CCA is colored light blue. Dashed lines highlight polar contacts between protein and RNA with involved atoms colored by element. Structures were created with Pymol.



Supplemental Figure 6) Complementation assay of *E. coli* BW23113 mutant strain lacking *efp* and *uup* (Δ*efp*Δ*uup*). Same general experimental set-up as in **Figure 6.** The Δ*efp*Δ*uup* control shows slight significant differences upon EarP expression (mean colony size with EarP co-expression = 0.63 mm ± 0.06, without EarP = 0.60 mm ± 0.08), while the EfpL complementation shows no significant difference. P-values are from Wilcoxon Rank Sum tests corrected for multiple comparisons with the Bonferroni method.

Supplemental References

1. Tomasiunaite, U., Kielkowski, P., Krafczyk, R., Forné, I., Imhof, A. & Jung, K. Decrypting the functional design of unmodified translation elongation factor P. *Cell Rep* 43, 114063 (2024).