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Getting Past Polyproline Pauses

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The ribosome is a versatile enzyme, but it cannot synthesize all proteins equally well—certain combinations of amino acids pose problems. Ribosome stalling by fairly long amino acid motifs can regulate gene expression in a variety of organisms, from bacteria to humans (1, 2). In these cases, the nascent peptide interacts with the peptide exit channel on the ribosome to induce a conformation that prevents peptide bond formation. Two papers in this issue, by Doerfel et al. on page XXX and Ude et al. on page YYY, reveal that stalling is common and fundamental: short, proline (Pro)-rich motifs impede protein synthesis, and stalling is alleviated by a poorly understood elongation factor, EF-P. These studies offer a model of the biological function and mechanism of EF-P through a convergence of biochemical and genetic methods.

EF-P was identified biochemically nearly forty years ago (3); its name reflects its ability to enhance the peptidyl-transferase (P) activity of the ribosome. While clues to its function trickled in over the years, a compelling biological role remained elusive. Following up on the original biochemical findings, Doerfel et al. took a broader look at substrate specificity; in testing all 20 amino acids, it became clear that the stimulatory effects on catalysis with Pro were unique. Pro is both a poor peptidyl donor and a poor peptidyl acceptor (4–6). Indeed, Doerfel et al. find that the synthesis of peptides with consecutive Pro residues is severely inhibited, possibly because Pro is a secondary amine (an imino acid) or from the conformational constraints imposed by its cyclic side chain. These biochemical studies reveal that EF-P supports rapid and robust synthesis of polyproline sequences.

Ude et al. independently arrived at the same conclusion through genetic analysis of the *cad* genes involved in acid resistance in *E. coli*. Using transposon mutagenesis, they identified *yjeK* as essential for robust expression of *cadA* and *cadC*. YjeK is one of two proteins that make essential covalent modifications of EF-P (7, 8); deletion of the other, YjeA, or EF-P itself results in loss of CadC function as well. It turns out that the *cadC* gene has a string of three prolines in its coding region; the authors pinpoint this sequence as the site of EF-P action. Deletion or mutation of the polyproline motif makes CadC synthesis independent of EF-P.

Additional findings from both groups further delineate the scope of EF-P activity. One observation is that three Pro residues are required for robust stalling, and longer polyproline sequences do not appear to have any additional effect. Of more than 10 examples tested in these two studies, ribosome pausing was detected at the polyproline motif whether it is engineered or naturally occurring. This suggests that the phenomenon is context-independent. The amino acid—not the tRNA that it is tethered to during translation—is proposed to be the critical player, as different Pro codons elicited similar results. Also, the role of EF-P appears to be limited to polyproline stretches; other translational intermediates that can be detected *in vitro* are not resolved by EF-P.

Structural studies show that the three domains of EF-P mimic the structure of tRNA (9). This shape allows EF-P to bind between the canonical E and P tRNA binding sites. One domain contacts the small ribosomal subunit near the mRNA channel, and another domain contacts the large subunit near the peptidyl-transferase center (10). Of particular interest is a conserved loop in domain I of EF-P, corresponding to the 3'-end of the tRNA, where the amino acid is tethered. A conserved lysine in this loop, Lys34, lies close to the ribosomal peptidyl-transferase center and the peptidyl-tRNA linkage. This Lys residue is modified by β -lysine by the enzymes YjeK and YjeA (7, 8), which creates a long, flexible cofactor that could reach into the ribosome's active site and alter its conformation or the chemistry of peptide bond formation. Biochemical and genetic data show that this modification is essential for EF-P activity (8).

From these varied approaches, a clarifying model of EF-P function emerges. A ribosome stutters on encountering a string of Pro codons; specifically, when two consecutive Pro residues are found at the end of a nascent peptide chain, the rate of peptidyl transfer to subsequent Pro-tRNAs in the A site decreases, presumably because the geometry of peptidyl-transfer is perturbed. EF-P enters the ribosome through the empty E site and binds close to the peptidyl-tRNA, allowing the β -lysyl moiety on Lys34 to reach into the active site of the ribosome and restore productive catalysis. Following peptide bond formation, EF-P dissociates and protein synthesis resumes as usual (Figure 1).

The importance of this mechanism is underscored by the universal conservation of EF-P and its orthologs. Although some bacteria can tolerate deletion of the *efp* gene, its loss induces pleiotropic phenotypes, including loss of virulence in *Salmonella* (8). The findings of Doerfel et al and Ude et al suggest that these phenotypes can be traced to defects in the translation of proteins containing polyproline motifs (roughly 100 *E. coli* proteins contain such a motif.) In eukaryotes, the orthologous protein eIF5A likely plays a similar function (11). Like EF-P, it is modified at a Lys residue, but with a unique hypusine moiety derived from spermidine (12). eIF5A and its modifying enzymes are essential in eukaryotes, perhaps because eukaryotes have many proteins rich in polyproline stretches. Differences in the modifying enzymes for EF-P and eIF5A suggest that this pathway may be a viable target for future antimicrobials.

This model of EF-P function raises additional questions. Mechanistically, it is unclear how polyproline stretches stall ribosomes and how EF-P alleviates stalling. Even though modification of Lys34 is essential for EF-P activity, 70% of bacterial species lack homologs of YjeA and YjeK (13). It is also unknown how EF-P and its modifying enzymes are regulated. Perhaps their expression is coupled to regulation of specific genes in the cell, controlling the copy number of target proteins in response to various cellular inputs. More broadly, these studies call to mind other motifs that trigger translational stalling (e.g., positively charged amino acids, structured mRNA, and rare codons), raising the possibility that the cell has evolved special mechanisms for dealing with them as well. The ribosome profiling technique will undoubtedly be instrumental in sorting out these stalling events and the mechanisms for their resolution (14).

References

1. Ito K, Chiba S, Pogliano K. Biochemical and biophysical research communications. Feb 26.2010 393:1. [PubMed: 20117091]
2. Yanagitani K, Kimata Y, Kadokura H, Kohno K. Science. Feb 4.2011 331:586. [PubMed: 21233347]
3. Glick BR, Ganoza MC. Proc Natl Acad Sci U S A. Nov.1975 72:4257. [PubMed: 1105576]

4. Tanner DR, Cariello DA, Woolstenhulme CJ, Broadbent MA, Buskirk AR. J Biol Chem. Dec 11.2009 284:34809. [PubMed: 19840930]
5. Wohlgemuth I, Brenner S, Beringer M, Rodnina MV. J Biol Chem. Nov 21.2008 283:32229. [PubMed: 18809677]
6. Pavlov MY, et al. Proc Natl Acad Sci U S A. Jan 6.2009 106:50. [PubMed: 19104062]
7. Yanagisawa T, Sumida T, Ishii R, Takemoto C, Yokoyama S. Nature structural & molecular biology. Sep.2010 17:1136.
8. Navarre WW, et al. Mol Cell. Jul 30.2010 39:209. [PubMed: 20670890]
9. Hanawa-Suetsugu K, et al. Proc Natl Acad Sci U S A. Jun 29.2004 101:9595. [PubMed: 15210970]
10. Blaha G, Stanley RE, Steitz TA. Science. Aug 21.2009 325:966. [PubMed: 19696344]
11. Saini P, Eyler DE, Green R, Dever TE. Nature. May 7.2009 459:118. [PubMed: 19424157]
12. Park MH, Nishimura K, Zanelli CF, Valentini SR. Amino Acids. Feb.2010 38:491. [PubMed: 19997760]
13. Bailly M, de Crecy-Lagard V. Biol Direct. 2010; 5:3. [PubMed: 20070887]
14. Ingolia NT, Ghaemmaghami S, Newman JR, Weissman JS. Science. Apr 10.2009 324:218. [PubMed: 19213877]

Figure 1.

No pausing at prolines. A) A ribosome stalls during translation when a peptidyl-tRNA ending in two proline residues is in the P site and tRNA^{Pro} is in the A site. B) The universal translation factor EF-P relieves stalling through interactions with the catalytic center of the ribosome. After peptide bond formation, EF-P dissociates and protein synthesis resumes.