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EF-P Is Essential for Rapid Synthesis of Proteins Containing Consecutive Proline Residues

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Elongation factor P (EF-P) is a translation factor of unknown function that has been implicated in a great variety of cellular processes. Here, we show that EF-P prevents ribosome from stalling during synthesis of proteins containing consecutive prolines, such as PPG, PPP, or longer proline strings, in natural and engineered model proteins. EF-P promotes peptide-bond formation and stabilizes the peptidyl–transfer RNA in the catalytic center of the ribosome. EF-P is posttranslationally modified by a hydroxylated β-lysine attached to a lysine residue. The modification enhances the catalytic proficiency of the factor mainly by increasing its affinity to the ribosome. We propose that EF-P and its eukaryotic homolog, elf5A, are essential for the synthesis of a subset of proteins containing proline stretches in all cells.

Bacterial elongation factor P (EF-P) and its archaeal/eukaryotic homolog, initiation factor 5A (a/elf5A), are universally conserved proteins with unknown cellular function. EF-P was reported to modulate cell viability, growth, virulence, motility, and sensitivity to low osmolarity, detergents, and antibiotics (1, 2). The EF-P binding site on the ribosome is located at the interface of the 30S and 50S ribosomal subunits between the binding sites for peptidyl-tRNA (P site) and the exiting tRNA (E site). The N-terminal domain of EF-P interacts with the acceptor stem of the P site–bound tRNA near the peptidyl transferase center (3). Both EF-P and elf5A are posttranslationally modified (4–8). In Escherichia coli EF-P, Lys91 is modified by the action of three enzymes, YjeK, YjeA, and YcfM (5, 7–9). The proposed cellular function of EF-P/elf5A is to optimize the ribosomes for more productive interactions with tRNA and release factors (3, 10–13). However, the magnitude of the reported effects appeared too small (less than twofold) for a universal function. We clarify the functional role of EF-P by investigating the role of the factor in the translation of various mRNAs sequences.

The activity of EF-P in accelerating peptide bond formation was originally identified by its ability to increase the yield of formylmethionyl-puromycin (fMet-Pnn) synthesis (1/2, 13). Because reaction rates vary by ~1000-fold for different C-terminal amino acids in the peptidyl-tRNA (14), we examined whether EF-P may specifically accelerate the product formation with poorly reactive peptidyl-tRNAs, such as those containing C-terminal proline residues (Fig. 1A and fig. S1). The formation of the tripeptide fMetPro-Pnn was accelerated by almost 90-fold in the presence of EF-P, whereas in the other cases the reaction was stimulated by less than fivefold. To explore the effect of the amino acid following a proline residue, we tested Gly- and Pro-tRNAs that are known to be slow in peptide bond formation and can contribute to ribosome stalling (15–17). Whereas EF-P enhanced peptide-bond formation by less than twofold for most combinations, large effects were observed for the formation of fMetProGly (fMPG, eightfold) and fMetProPro (16-fold) (Fig. 1B). When longer strings of Pro and Gly residues, such as fMPPg, fMPPPG, or fMPPPPF, were examined, practically no correct product was formed in the absence of EF-P, whereas the addition of EF-P rescued the rapid production of the respective peptide (Fig. 1C). The low yield of product formation in the absence of EF-P can be explained by the loss of peptidyl-tRNA from the stalled ribosomes (e.g., fMPPP-rtRNA; fig. S2A). These results suggested a specific effect of EF-P in accelerating the reaction with poorly reactive substrates that otherwise cause ribosome stalling.

To verify whether proline-containing sequences as such induce ribosome stalling that can be rescued by EF-P, we engineered PG, PP, and PPP sequences into an N-terminal fragment of protein PrmC, which originally does not contain such sequences and is rapidly synthesized independent of the presence of EF-P (Fig. 2). Introducing a glycine residue after Pro20 resulted in the ribosome pausing, as shown by the accumulation of a peptide of about 20 amino acids; however, pausing was transient and not affected by EF-P. When a second proline residue preceding Pro20 was engineered, the ribosome paused at the ProPro sequence, and EF-P reduced the pause time from 20 to 10 s. When PPG or PPP sequences were introduced, the ribosomes were stalled, and essentially no full-length product was produced. The ribosomes stalled upon translation of the PPG sequence that contained a ProPro-ending peptidyl-tRNA in the P site and a Gly-tRNA in the A site (fig. S2B). Addition of EF-P rescued the synthesis of full-length product by preventing or alleviating ribosome stalling (Fig. 2 and fig. S2C).

References and Notes
19. Material and methods are available as supplementary materials on Science Online.

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Supplementary Materials
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Fig. 1. EF-P promotes the synthesis of Pro- and Gly-containing peptides on the ribosome. (A) Rates ($k_{obs}$) of peptide-bond formation between $f$Met-tRNA$^{fMet}$ or $f$MetX-tRNA$^X$ and Pmn, where $X$ stands for different amino acids, as indicated, in the absence (white) or presence (black) of EF-P (3 μM). Error bars indicate standard deviations. Single-letter abbreviations for the amino acid residues are as follows: D, Asp; E, Glu; F, Phe; G, Gly; K, Lys; M, Met; P, Pro; Q, Gln; R, Arg; V, Val; and W, Trp. (B) Rates of peptide-bond formation between $f$Met-tRNA($f$M) or $f$MetPro-tRNA($f$MP) in the P site and Gly-tRNA$^{Gly}$, Phe-tRNA$^{Phe}$, or Pro-tRNA$^{Pro}$ (G, F, and P, respectively) in the A site. (C) Formation of model oligopeptides in a reconstituted translation system in the absence (open) and presence (solid) of EF-P.

Fig. 2. EF-P prevents ribosome stalling on PPG and PPP sequences engineered into PrmC. Translation of the N-terminal domain of PrmC (75 amino acids) with wild-type (WT) or mutant sequences containing PG, PP, PPG, or PPP in the absence or presence of EF-P. Peptides were separated by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by the fluorescence of BODIPY-FL attached to the N terminus of the peptides. M1 and M2 are peptide markers for PrmC fragments of the indicated number of amino acids. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
The sequences PPG and PPP, as well as longer proline stretches, are found in a large number of cellular proteins, raising the question of whether they require EF-P for their synthesis. We tested the effect of EF-P on the synthesis of some of these proteins, such as TonB, YafD, Rz1, and AmiB (Fig. 3). In the absence of EF-P, translation was stalled at the consecutive prolines, and the addition of EF-P alleviated ribosome stalling, resulting in the rapid synthesis of the respective full-length peptide. When the Pro stretches were particularly long, as in AmiB or Rz1, very little full-length product was synthesized in the absence of EF-P, because translation was halted at the stalling site. In all cases, the addition of EF-P resulted in the efficient synthesis of full-length protein. Transient pauses that occurred at sequences other than PPP and PPG were not alleviated by EF-P (Fig. 3).

Posttranslational modification of EF-P appears crucial for the factor’s function (9, 18). Translation of the fMPPG sequence, which was abolished in the absence of EF-P, was fully restored with EF-P carrying the β-lysine modification at Lys34; further hydroxylation did not alter the activity, whereas the absence of modifications reduced the activity of the factor (Fig. 4). A similar effect was observed for the translation of the PPP sequence in PrmC (fig. S4). Analysis of the reaction kinetics indicated that the modification increased the affinity of EF-P binding to the ribosome 30-fold (Fig. 4). The maximum rate of fMPPG synthesis decreased ~fourfold with unmodified compared with modified EF-P, resulting in a >100-fold difference in $k_{\text{cat}}/K_M$ (where $k_{\text{cat}}$ is the maximum reaction rate obtained by hyperbolic fitting and $K_M$ is the Michaelis constant). The low catalytic proficiency of unmodified EF-P may explain why deletions of the yjeA or yjeK genes that code for the modification enzymes lead to phenotypes that are similar to, or only somewhat milder than, the deletion of $efp$, the gene coding for EF-P (1, 2). On the other hand, even in the absence of active EF-P, small amounts of proteins with proline stretches are slowly formed (Fig. 3); this may be sufficient to support the viability of strains in which the $efp$, $yieA$, and $yieK$ genes are deleted.

Our data indicate that EF-P is a translation factor that promotes the synthesis of proteins containing PPG, PPP, and longer polyproline stretches by preventing ribosome stalling during translation.
Para-Aminosalicylic Acid Acts as an Alternative Substrate of Folate Metabolism in Mycobacterium tuberculosis

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Folate biosynthesis is an established anti-infective target, and the antifolate para-aminosalicylic acid (PAS) was one of the first anti-infectives introduced into clinical practice on the basis of target-based drug discovery. Fifty years later, PAS continues to be used to treat tuberculosis. PAS is assumed to inhibit dihydropteroate synthase (DHS) in Mycobacterium tuberculosis by mimicking the substrate p-aminobenzoate (PABA). However, we found that sulfonamide inhibitors of DHPS inhibited growth of M. tuberculosis only weakly because of their intracellular metabolism. In contrast, PAS served as a replacement substrate for DHPS. Products of PAS metabolism at this and subsequent steps in folate metabolism inhibited those enzymes, competing with their substrates. PAS is thus a prodrug that blocks growth of M. tuberculosis when its active forms are generated by enzymes in the pathway they poison.

However, sulfonamides had little activity against Mycobacterium tuberculosis, the single leading cause of death from bacterial infection (1). Shortly after Waksman’s and Schatz’s discovery of streptomycin, Lehmann developed para-aminosalicylic acid (PAS), a close structural analog of the folate precursor p-aminobenzoate (PABA) (2). PAS became the second drug for tuberculosis (TB). Hinchings subsequently developed antibacterial inhibitors of dihydrofolate reductase, validating a second enzyme of folate metabolism as a therapeutically selective target (3). In parallel, studies of PAS in combination with streptomycin and eventually other anti-TB drugs laid the foundation for modern TB chemotherapy.

References and Notes

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