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Breaking the silos of protein synthesis

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Abstract

Protein synthesis requires factors proposed to enhance discrete steps. eIF5A was initially thought to affect initiation; however, it was later shown to facilitate translation elongation at polyproline. Recent work by Schuller et al. demonstrates that eIF5A facilitates both general elongation and termination in yeast, challenging these steps as silos.

Traditionally, eukaryotic protein synthesis is defined as occurring in three distinct stages: initiation, elongation and termination. Each of these stages is thought to require its own set of dedicated protein factors. During initiation, the ribosome is positioned at the start codon with the initiator Met-tRNA^{Met} in the P-site through the action of a series of proteins called eukaryotic initiation factors (eIFs). Many of these factors were first described in pioneering work to dissect and fractionate the translation process in the 1970s. During the elongation phase, eukaryotic elongation factors (eEFs) facilitate the delivery of aminoacyl-tRNAs to the ribosome as well as the translocation of the ribosome on the mRNA. When the ribosome encounters a stop codon, eukaryotic release factors (eRFs) release the polypeptide chain.

During the early characterization of eukaryotic translation, eIF5A was classified as an initiation factor owing to its ability to stimulate the reaction of methionine with puromycin, an aminoacyl-tRNA analog that terminates the peptide chain. (Fig. 1, reviewed in [1]). Recent studies, however, revealed that eIF5A plays a role in translation elongation, specifically in the synthesis of polyproline stretches [2, 3]. As eIF5A was unable to stimulate other assays measuring the partial reactions of initiation, these new data suggested that the original stimulation of the Met-puromycin reaction was actually an effect on the formation of the first peptide bond. The bacterial ortholog of eIF5A, EFP, exhibits similar functions underscoring the high degree of conservation previously recognized between bacterial and eukaryotic translation elongation [4, 5].

A limited role of yeast eIF5A in promoting translation elongation at polyproline motifs is somewhat incongruous with its high abundance, its requirement *in vivo* and the effects of its depletion on global translation elongation. In order to gain a better understanding of the function of eIF5A in eukaryotic translation, Schuller and colleagues performed ribosome

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profiling on eIF5A-depleted *Saccharomyces cerevisiae* cells [6]. Ribosome profiling experiments determine the location of translating ribosomes on individual mRNAs, thus providing information on translational efficiency, alternative translation, ribosomal occupancy and ribosomal frameshifting for each mRNA. The development of this powerful technique, first described in 2009, has transformed research in the field of protein synthesis [7].

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In eIF5A-depleted yeast cells, the authors found that ribosomes were enriched at the 5' end of mRNAs and this enrichment was independent of the presence of polyproline motifs supporting a more generalized role for eIF5A in translation elongation. Further analysis yielded 29 tripeptide motifs associated with strong ribosomal pausing in eIF5A-depleted cells (>10-fold via pause score analysis). As expected, proline was found to be enriched in these peptides, however, four of these pause sites were completely devoid of proline. Strikingly, a prevalence of aspartic acid residues was noted among these pause sites. *In vitro* assays of translation elongation confirmed the global effect on elongation as addition of eIF5A stimulated the synthesis of all eight peptides examined, including those lacking the predominant proline and aspartic acid codons. This suggested that eIF5A might function on many more motifs than identified as the most critical through ribosome profiling, and even on most peptidyl transfer reactions. eIF5A is also the only protein to contain a post-translationally modified lysine, known as hypusine, which is critical for its functions [1]. Interestingly, the presence of hypusine was found to be more important for the elongation of polyproline motifs than for eIF5A's general elongation function.

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Perhaps more surprisingly, the profile of eIF5A depleted cells also showed an increase in ribosome occupancy at stop codons and in the 3' UTR consistent with a defect in translation termination. Addition of eIF5A to an *in vitro* translation termination assay led to an unexpected 17-fold stimulation of the rate of peptidyl-tRNA hydrolysis that was also dependent on the presence of hypusine. This function appears to be specific to the eukaryotic factor eIF5A as increased occupancy at stop codons is not observed in *efp* deleted bacterial cells [6] and EFP does not stimulate peptide release in translation termination assays [8].

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These data have led the authors to propose a model in which eIF5A plays an essential role in facilitating slow reactions in the peptidyl transferase center during translation. These reactions include not only polyproline synthesis and peptidyl-tRNA hydrolysis but also the tripeptide motifs identified as pause sites. These additional pause sites did not correlate with codon optimality/tRNA abundance raising the question of why these particular sequences are difficult for the ribosome to translate. Interestingly 62% of the tripeptides are rich in aspartic acid suggesting that this particular amino acid, in addition to proline, poses a problem for the peptidyl transfer center. Aspartic acid was previously shown to have a slower rate of peptide bond formation with puromycin [9]. Additional experiments will be required to determine the reason for these pauses such as whether the slow reaction is due to steric effects or a consequence of the negative charge of aspartic acid in the peptidyl transferase center.

The work of Schuller and colleagues expands our understanding of the factors essential for efficient translation elongation and termination in eukaryotes and highlights important differences between bacteria and eukaryotes in this highly conserved process. EFP appears to be specific for accelerating the elongation through polyproline motifs and structural studies suggest this specificity may come from interaction with the P-site tRNA [10]. In comparison, eIF5A has acquired more generalized functions in both elongation and termination while retaining its role in facilitating polyproline synthesis. These observations raise several important questions about the evolution of protein synthesis. For example, is there a fundamental difference between bacterial and eukaryotic ribosomes that requires eIF5A for efficient elongation, especially at the pause sites identified in this work? Are the dual roles of eIF5A in translation elongation and termination conserved in higher eukaryotes as fungal elongation is known to have some unique requirements including the E-site factor eEF3? How does eIF5A interact with the E- and P-sites of the ribosome and other translation factors? How does the hypusine modification facilitate the functions of eIF5A? While more mechanistic detail on eIF5A functions remains to be determined, it is clear that descriptions of general eukaryotic translation elongation and termination should now include the likely misnamed eIF5A.

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Figure 1. eIF5A functions at multiple steps of protein synthesis. While originally identified as enhancing the met-puromycin reaction *in vitro*, recent work demonstrates eIF5A stimulates general elongation as well as translation termination.