

Please Release Me: Termination of Polypeptide Synthesis Unraveled

Article Review by A. Super Student

Proper termination of the translation process is critical in order to guarantee properly sized proteins. Despite its importance, the polypeptide release process remains poorly understood. During termination, one of three stop codons (UGA, UGG, or UAG) occupies the ribosomal acyltransferase site (A site). Then, interaction between the mRNA-ribosome complex and proteins known as “release factors” (RFs) leads to recognition of the stop codon and release of the polypeptide chain. However, conflicting data have raised questions over whether or not RF binding is stop codon-dependent. Now however, Chavette *et al.* [1] have applied a photoactivated 4-thiouridine probe to examine the interactions between the mRNA-ribosome complex and the eukaryotic release factor eRF1.

Class I RFs are theorized to recognize stop codons via direct mRNA-protein interactions. The crystal structure of eRF1 shows a similar overall shape to tRNA, leading to comparisons between the two. It is broken up into three domains, N-terminal (N), middle (M), and C-terminal (C), with the release activity found within domains N and M. Earlier site-directed mutagenesis had established the importance of a conserved N-I-K-S peptide sequence found within the N domain [2]. Based upon these observations, Chavette *et al.* hypothesized that eRF1 makes physical contact between the N domain and mRNA stop codons in the ribosomal A site in a two-step process: a codon-independent bimolecular step followed by a codon-dependent isomerization step.

In order to test this hypothesis, the authors examined binding constants (K_D) using an *in vitro* model incorporating eRF1, tRNA, and eukaryotic ribosomes loaded with specific mRNA sequences. They synthesized each 42-mer mRNA with a ^{32}P label and a poly-purine sequence interrupted with an Asp codon followed by the variable codon: either $s^4\text{U}$ -purine-purine or the similar $s^4\text{UCA}$ or $s^4\text{UAC}$. Because of the thiol functional group introduced at position 4, the uridine of the mRNA could be crosslinked by UV irradiation to surrounding macromolecules, including 18S rRNA and ribosomal proteins. First, they demonstrated that the mRNA was phased correctly on the ribosome following the addition of tRNA^{Asp} by incubating various mixtures of mRNA, ribosomes, and tRNA. The samples were irradiated to activate cross-linking to the surrounding subunits, and the resulting complexes analyzed using SDS-PAGE separation and phosphorimaging. Consistent mRNA-rRNA binding was demonstrated by the tRNA-dependent appearance of ^{32}P -labeled crosslink products on the gel. Next, eRF1 was added at varying levels, resulting in quenching of most mRNA-rRNA crosslinks and the appearance of a new 68kDa product, the eRF1-mRNA complex. After demonstrating that the ribosomal concentration was beyond saturation, the authors refined the method by treatment with micrococcal nuclease, further improving the resolving power for the desired eRF1 complex. Finally, crosslink reaction kinetics were determined to follow a consistent first-order rate law with $k = 0.12(\pm 0.02) \text{ min}^{-1}$.

With the experimental method firmly established, the authors then examined the formation of the 34kDa mRNA-rP crosslink product as a function of eRF1 concentration. Their results demonstrated a codon-dependent quenching of these crosslinks, with stop codons UAA/UAG/UGA delivering half-maximum quenching at [eRF1]/[mRNA] ratios as low as 5:1, and K_D of $0.06\mu\text{M}$. The near-stop codons UAC and UCA required substantially higher 25:1 ratios to produce similar quenching, and yielded a K_D of $2.3\mu\text{M}$. Interestingly, the other U-purine-purine codon UGG yielded an intermediate result, with half-maximum quenching around 10:1, and K_D of $0.44\mu\text{M}$. They then incubated eRF1 with mRNA oligonucleotides containing stop $s^4\text{UAA}$ or sense $s^4\text{UAC}$ codons to determine if it would bind directly to the mRNA, without the ribosomal complex. No binding was observed. The combination of these two results supports their proposed mechanism, whereby the ternary mRNA-tRNA-ribosome complex first binds eRF1 in a bimolecular step, followed by stop codon-dependent isomerization that results in a conformational change and interaction with the peptidyl transferase center (PTC). Also supported is the concept of a two-domain eRF1-mRNA interaction, using both the eRF1 NIKS and YCF domains, properly positioned only after binding to the ribosome. The intermediate behavior of UGG led to its designation as a “cryptic” stop codon, while supporting the idea that the two coupled steps of codon recognition and eRF1 rearrangement are involved in discrimination of near-stop codons.

Future research is likely to be directed at the exact mechanism of discrimination between the stop codons and UGG, as the roles of tRNA^{Trp} and eRF3 were not included in this study. Other related work includes finding RF1-A site dissociation constants and probing the role of other eRF1 effectors. On a broader scale, the crosslink quenching strategies developed by the authors extend the utility of photolabeling techniques, and will likely find widespread application in determination of nucleic acid-protein binding constants.

1. Chavette, L., *et al.* (2003). Stop codons and UGG promote efficient binding of the polypeptide release factor eRF1 to the ribosomal A site. *J. Mol. Biol.* **331**: 745-758.
2. Frolova, G., *et al.* (2002). Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. *RNA* **8**: 129-136.