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Translation Termination performed by ciliate's eRF1s: Implications for Stop Codon Reassignment

Научный руководитель – Алкалаева Елена Зиновьевна

Salman A.[1], *Бизяев Н.С.*[2]

1 - Московский физико-технический институт, Москва, Россия, *E-mail: ali.mahmoud.salman1996@gmail.com*; 2 - Институт молекулярной биологии им. В.А.Энгельгардта РАН, Москва, Россия, *E-mail: nikita.biz@mail.ru*

Translation termination is a crucial process in protein synthesis, orchestrated by release factor 1 (eRF1) recognizing stop codons on mRNA. Ciliates, a group of unicellular eukaryotic organisms, exhibit intriguing variations in their translation termination machinery, particularly in the codon-recognizing N-domain of eRF1 and the surprisingly short length of mRNA's 3'UTR. These variations raise questions about their mechanistic significance and potential implications for stop codon dynamics. By investigating these phenomena, we aim to advance our understanding of regulation of translation termination.

We employed Termi-Luc assay and fluorescent toe-print analysis of pre-termination complexes assembled in the reconstituted system to evaluate translation termination efficiency, termination complex formation, and the effect of eRF1 competition with tRNA. In Termi-Luc analysis, luminescent signal emitted by Nanoluciferase was utilized as indicator for peptide release events. Toe-print analysis utilized fluorescently labeled primers in reverse transcription to determine ribosome position and termination complex conformation on mRNA based on length of obtained cDNA. Chimeric eRF1 variants, containing N-domains from various ciliate species and M/C-domains from humans, were expressed and purified for *in vitro* studies. Model mRNA constructs, featuring different stop codons (UGA, UAA, UAG) and 3'UTR lengths (0, 10, 30, 160 nt), were synthesized to mimic diverse termination scenarios.

The toe-print analysis revealed that the chimeric release factor 1 derived from *Euplotes* did not recognize UGA as a stop codon at the long 3' UTRs, unlike the human eRF1, indicating a lack of termination under these conditions. In contrast, the toe-print profile of *Blepharisma* showed a comparable level of stop codon recognition and termination occurrence. These findings align with the literature suggesting that UGA functions as a sense codon in *Euplotes* and do not contradict the idea that it has an intermediate state (sense/nonsense) in *Blepharisma*. Termi-luc results showed the minimal termination efficiency at long 3' UTR length for UGA stop codon , surprisingly with comparable increasing of termination efficiency at short 3 ' UTR, for both ciliates. These finding proofs the concept of context-dependent termination coding in such organisms postulating efficient termination only on short 3' UTR due to proximity on stop codon and poly (A) tail [1]. Meanwhile, we do not observed such dependence for other stop codons (UAA and UAG), that are not annotated as sense codons for these organisms.

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References

1) 1. Alkalaeva, Elena, and Tatiana Mikhailova. "Reassigning stop codons via translation termination: how a few eukaryotes broke the dogma." BioEssays 39.3 (2017): 1600213.