

Amino acid residues involved in stop codon recognition in ciliate eRF1

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SUMMARY

The genetic code of nuclear genes in some ciliates was found to differ from those of other organisms in the assignment of UGA, UAG, and UAA codons, which are normally assigned as stop codons. However, *Dileptus margaritifer* was found to use universal stop codons. Eukaryotic release factor 1 (eRF1) is a key protein in stop codon recognition, thereby, the protein is believed to play an important role in the stop codon reassignment in ciliates. In fact, eRF1 comprises three domains; the stop codon recognition site is located in domain 1. It is commonly assumed that changes in the domain 1 of ciliate eRF1s are responsible for altered stop codon recognition. To identify the residues responsible for specific recognition of ciliate eRF1, in this study, we constructed a hybrid gene that contained mutated eRF1 domain 1 from *Dileptus* fused to eRF1 domain2 and 3 from *Saccharomyces cerevisiae*. An *in vivo* complementation test in yeast was conducted to examine whether mutated eRF1 domain 1 of *Dileptus* recognizes all three stop codons. The chimeric eRF1 was cloned into a yeast expression vector, then transformed to yeast strains containing the mutated eRF1. Our result suggests that R₁₂₈ of the *Dileptus* eRF1 plays an important role in stop codon recognition.