

Investigation of amino acid residues of eRF1 which are responsible for stop codon recognition in ciliates

Ying LEE¹, Oanh T. P. KIM² and Terue HARUMOTO³

(¹Department of Biological Science, Graduate School of Human Culture, Nara Women's University,
²Applied DNA Technology Lab., Institute of Biotechnology, Vietnamese Academy of Science and Technology, Vietnam, ³Department of Biological Science, Faculty of Science, Nara Women's University)

SUMMARY

The genetic code of nuclear genes in some ciliates differs from that 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, and is believed to play an important role in stop codon reassignment in ciliates. eRF1 is composed of three domains, and the stop codon recognition site is located in domain 1. It is commonly assumed that changes in domain 1 of ciliate eRF1s are responsible for altered stop codon recognition. To identify the residues that are responsible for specific recognition of ciliate eRF1, we made hybrid proteins that contained mutated eRF1 domain 1 from *Dileptus* fused to eRF1 domains 2 and 3 from *Saccharomyces cerevisiae*. An *in vivo* complementation in yeast was carried out to examine whether mutated eRF1 domain 1 of *Dileptus* recognizes all three stop codons. The chimeric eRF1 was cloned into a yeast expression vector, and then transformed to yeast strains containing the mutated eRF1. Our result suggests that, in particular, R₁₂₈ of the *Dileptus* eRF1 plays an important role in stop codon recognition.