In vivo complementation test of Didinium nasutum eRF1 in yeast understanding stop codon usage in this species

Aki SAKURAI¹, Oanh T.P.KIM², Terue HARUMOTO³ (¹Grad.Shcool Human Cul.,Nara Women's Univ., ²Chem.Sci.,Nara Women's Uni.,(JSPS)., ³ Bio.Sci.,Nara Women's Univ.)

In some ciliates, the specificity of the stop codon recognition by eRF1 has been altered, and it does not respond to all universal stop codons. In *Didinium nasutum*, eRF1 is known to recognize UAA. However, it is still unknown whether *Didinium* eRF1 recognizes UAG and/or UGA as stop codons. In this study, we aim to examine the release activity of *Didinium* eRF1. The crystal structure of human eRF1 has been determined, and it is composed of three domains. The stop codon recognition sites have been located in domain 1 of eRF1. We have constructed a chimeric eRF1 from *Didinium* domain 1 and human domain 2–3. An *in vivo* complementation in yeast was carried out to examine whether *Didinium* eRF1 domain 1 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. The chimeric eRF1 was able to complement a defect in yeast eRF1 *in vivo*. Our result suggests that *Didinium* eRF1 can recognize all three stop codons (UAA, UAG and UGA).