How is ciliate eukaryotic release factor 1 (eRF1) different from conventional eRF1s? – *In vivo* complementary activity of *Dileptus* eRF1 –

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SUMMARY

Eukaryotic release factor 1 (eRF1) of some ciliates differs from conventional ones in stop codon recognition; this results in stop codon reassignment in these organisms. To clarify the specificity of stop codon recognition in ciliate eRF1s, we have sequenced eRF1 genes from four ciliate species [Kim, O. T. P., Yura, K., Go, N., Harumoto, T. (2005) *Gene*, 346, 277-286]. In *Dileptus margaritifer*, UAA was found to encode termination, while the usage of UAG and UGA is still unknown. In this study, we started to examine the release activity of *Dileptus* eRF1. An *in vivo* experiment in yeast has been carried out to investigate whether *Dileptus* eRF1 recognizes all three stop codons. A chimeric eRF1 was constructed from *Dileptus* domain 1 and human domains 2–3, then transformed to yeast strains whose eRF1 was mutated. The chimeric eRF1 was able to complement a defect in yeast eRF1 *in vivo*. Our results suggest that *Dileptus* eRF1 recognizes all three stop codons (UAA, UAG and UGA).

[Introduction] The genetic code of nuclear genes in some ciliates has been known to deviate from the universal genetic code. Eukaryotic RF1 is believed to play an important role in the stop codon reassignment in ciliates. In eukaryotes, a single eRF1 recognizes all three stop codons. However, in ciliates, the specificity of the stop codon recognition by eRF1 has been altered. For examples, in *Euplotes*, eRF1 recognizes UAA and UAG as stop codons, but not UGA codon, which instead encodes cysteine (Kervestin *et al*., 2001). Replacement of domain 1 of yeast eRF1, which normally recognizes all three universal stop codons with that of *Tetrahymena* eRF1, altered its specificity such that it, like the native *Tetrahymena* eRF1, only recognized UGA (Ito *et al*., 2002). It is now established that the stop codon recognition sites located in domain 1 of eRF1. However, the specific sequence and structure of stop codon-binding sites remain obscure. To clarify the specificity of stop codon recognition in ciliate eRF1s, we have sequenced eRF1 genes from four ciliate species, *Loxodes striatus*, *Blepharisma musculus*, *Didinium nasutum* and *Dileptus margaritifer*, (Kim *et al.*, 2005). In this study, we continue to analyze the differences between ciliate eRF1s and conventional eRF1s which enable us to elucidate how the genetic code deviates in ciliates and to understand further the mechanism of stop codon recognition.

[Materials and methods] **Cloning eRF1 domain1 from ciliates:** Domain 1 of eRF1 cDNA from *Dileptus* (amino acid sequence positions: 1 – 140, human eRF1 numbering) was marked with restriction enzyme sites, *Nde*I at 5'-end and *Xho*I at 3'-end. The domain fragment was amplified by PCR and cloned using a TOPO TA Cloning Kit (Invitrogen). Purified plasmid DNA containing the expected insert was subjected to sequencing reaction using a ABI PRIMS BigDye™ Terminator v 3.0 Cycle Sequencing Kit (Applied Biosystems).

Cloning eRF1 domain 2-3 from human: Domain 2-3 of eRF1 cDNA from human (amino acid sequence positions: 141 – 437) was marked with restriction enzyme sites, *Xho*I at 5'-end and *Sal*I at 3'-end. The domain fragment was amplified by PCR and cloned using pT7Blue vector (Novagen). MCS of pT7Blue vector also contains *Nde*I restriction site at upstream of the insert.

eRF1 domain swapping and cloning the chimeric eRF1: Purified plasmid DNAs of TOPO vector containing domain 1 of *Dileptus* eRF1, and pT7Blue vector containing domain 2-3 of human eRF1 were treated with *Nde*I and *Xho*I. Chimeric eRF1 was constructed by inserting domain1 of *Dileptus* eRF1 into the pT7Blue vector containing domain 2-3 of human eRF1.

Cloning chimeric eRF1 with yeast expression vectors: Three low-copy-number CEN/ARS vectors containing marker gene URA3 with three different promoters (p416CYC1, p416ADH and p416GPD) were used in this study. The chimeric eRF1 was cloned in the *Spe*I-*Sal*I site of the vectors.

***In vivo* complementary tests:** Two *Saccharomyces cerevisiae* strains were used in this study: temperature sensitive eRF1 mutant (*SUP45 ts*) strain, and tetracycline-regulated eRF1 (P_{tet}*SUP45*) strain. The wild-type yeast eRF1 (*SUP45*+) and the chimeric eRF1 were transformed into these *S. cerevisiae* strains by using Frozen_EZ yeast transformation kit (ZYMO Research). The transformed yeasts were screened by cell growth on plates lacking uracil. *In vivo* complementary test of transformants of *SUP45 ts* strain were monitored by cell growth at permissive (30°C) and non-permissive (37°C) temperatures. *In vivo* complementary test of transformants of P_{tet}*SUP45* (tet-OFF system) strain were monitored by cell growth on plates with and without Doxycyclin, a tetracycline derivative.

[Results and discussion] **Domain swapping between *Dileptus* and human eRF1:** eRF1 structure is composed of three domains: domain 1 has been known to recognize stop codons, domain 2 takes part in peptidyl-tRNA hydrolysis, and domain 3 is known to interact with eRF3. Moreover, eRF1 domain 2 is supposed to interact with ribosome since the termination of protein synthesis occurs in ribosome. Taking these findings into consideration, it is more realizable for *in vivo* system to investigate RF activity if a hybrid eRF1 composed of a ciliate domain 1 and a parental domain 2-3 is introduced. There are two advantages of using human eRF1 as a parental construct: first, the crystal structure of human eRF1 has been determined (Song *et al*., 2000); second, it has been reported that human eRF1 can be expressed in yeast cells. The topologies of the three domains differ from one another, i.e. three domains are structurally separated. Domain 1, 2 and 3 are connected by hinges 1 and 2, respectively. The restriction site *Xho*I was introduced into the hinge 1 and this site do not change any conserved amino acid, as well as topologies of the domains.

***In vivo* complementation activity of the chimeric eRF1:** The wild-type *S. cerevisae* eRF1 (*SUP45*+) and the chimeric eRF1 were cloned into yeast expression vectors (p416CYC1, p416ADH and p416GPD), and transformed into *SUP45 ts* strain and P_{tet}*SUP45* strain. URA^+ transformants were selected at permissive temperature 30°C. The *in vivo* complementation test of transformants of *SUP45 ts* strain was examined for their growth at non-permissive temperature 37°C. If the chimeric eRF1 recognizes all three stop codons, like the native *S. cerevisae* eRF1, i. e. the chimera is able to complement the defect in yeast eRF1, thus the transformants maintain their growth at 37°C. The result showed that the chimeric eRF1 transformants grew normally at 37°C as well as the wild-type *SUP45*+ transformants. The *in vivo* complementation test of transformants of P_{tet}*SUP45* strain was examined for their growth in the presence of Doxycyclin. The result of this test was compatible with that of *SUP45 ts* strain in which the chimeric eRF1 transformants grew normally in the presence of Doxycyclin.

In *Tetrahymena*, only UGA is used as stop codon, whereas UAA and UAG are translated to Gln. Ito K. *et al.* (2002) showed that a hybrid eRF1 constructed from *Tetrahymena* domain 1 and yeast domain 2-3 failed to complement a defect in yeast eRF1 *in vivo* at 37oC (*SUP45 ts* strain). This finding was consistent with the *in vitro* assay that the hybrid eRF1 responded only to UGA, thus the hybrid eRF1 was unable to recognize UAA and UAG codons in the *SUP45 ts* strain. In this study, the hybrid eRF1 containing *Dileptus* domain 1 was able to complement a defect in yeast eRF1 *in vivo*. Our result suggests that *Dileptus* eRF1 recognizes all three stop codons. To confirm the result, we plan to check the expression of the chimeric eRF1 by western blotting.

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[References]

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