# Polypeptide release factor eRF1 from *Tetrahymena thermophila*: cDNA cloning, purification and complex formation with yeast eRF3

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**Abstract The first cDNA for the translational release factor eRF1 of ciliates was cloned from *Tetrahymena thermophila*. The coding frame contained one UAG and nine UAA codons that are reassigned for glutamine in *Tetrahymena*. The deduced protein sequence is 57% identical to human eRF1. The recombinant *Tetrahymena* eRF1 purified from a yeast expression system was able to bind to yeast eRF3 as do other yeast or mammalian eRF1s as a prerequisite step for protein termination. The recombinant *Tetrahymena* eRF1, nevertheless, failed to catalyze polypeptide termination in vitro with rat or *Artemia* ribosomes, at least in part, due to less efficient binding to the heterologous ribosomes. Stop codon specificity and phylogenetic significance of *Tetrahymena* eRF1 are discussed from the conservative protein feature.**



*Key words:* Translation termination; UGA; Polypeptide release factor; eRF1-eRF3 complex; *Tetrahymena thermophila*

## 1. Introduction

Newly synthesized polypeptide chains are released from peptidyl-tRNA when the ribosome encounters a stop signal on mRNA. Translation termination requires two classes of polypeptide release factors (RFs): one, codon-specific (RF1 and RF2 in prokaryotes; eRF1 in eukaryotes) and the other, non-specific to codon (RF3 in prokaryotes; eRF3 in eukaryotes) [1,2]. RF1 and RF2 recognize UAG/UAA and UGA/UAA, respectively [1,3], and known eukaryotic eRF1s recognize all three stop codons [1,4]. It is suggested that the codon-specific RF interacts directly with the stop codon of mRNA, and the non-specific RF mediates GTP hydrolysis in this process [1,2,5]. However, the mechanism of how RF recognizes the stop codon has long been unknown in spite of the discovery of RF activity in vitro in the late 1960s. We have shed light on this problem by findings of conserved protein motifs in prokaryotic and eukaryotic RFs, as well as in the C-terminal portion of bacterial elongation factor EF-G [6]. Since the C-terminal part of EF-G appears to mimic the shape of tRNA [7–9], we have proposed the 'RF-tRNA mimicry' hypothesis in which codon-specific RFs mimic a tRNA shape for binding to the ribosome as well as an anticodon for reading the stop codon [1,6].

Determination of polypeptide region(s) and residues of RF that are responsible for recognition and discrimination of stop codons is a clue to understanding the mechanism underlying this triplet decoding. In prokaryotes, the selective recognition of UAG and UGA by RF1 and RF2 provides us with an experimental clue to studying the RF domain(s) and amino acids that affect the selectivity by mutational and biochemical means [5,10].

Contrary to the bacterial RFs, the omnipotence of eRF1s in reading the stop codons impedes the identification of the anticodon moiety of eRF1s in eukaryotes. It could facilitate the study if we were able to deal with an eRF1 protein that harbors selectivity or preference in stop codon recognition. Potentially two ways might be possible: one, to find such an eRF1 in nature; the other, to create it by genetic means. It might be useful to screen for a putative eRF1 responsive to specific codon(s) in ciliates (unicellular protozoa) because some of them are known to have reassigned UAA and UAG (or UGA) as a sense codon instead of a stop codon during evolution. For example, in *Euplotes octacarinatus*, UGA is decoded as cysteine [11] and UAA/UAG are decoded as glutamine in *Tetrahymena thermophila* [12–15]. It is tempting to speculate that these eRF1s possess selective recognition of UAA/UAG and UGA, respectively, and do not recognize the other(s). Unless they possess selectivity or preference in reading these triplets, there must be a putative mechanism behind that to have cysteinyl- or glutaminyl-tRNA win the competition with eRF1.

Toward this aim, we cloned the eRF1 gene from *T. thermophila* and purified the recombinant *Tetrahymena* eRF1 (referred to as Tt-eRF1) using the heterologous expression system in the budding yeast, *Saccharomyces cerevisiae*, for the initial characterization of Tt-eRF1. This is the first report of cloning of an eRF1 gene from ciliates.

## 2. Materials and methods

### 2.1. Strains and media

Strains used are: *T. thermophila* strain CU427 [16], *S. cerevisiae* YPH499 [17]. *Tetrahymena* was grown at 25°C in liquid medium containing 2% (w/v) Bacto peptone, 0.2% (w/v) yeast extract, and 0.5% (w/v) glucose. Yeast cells were grown in YPD or synthetic complete (SC) media [18].

### 2.2. Gene manipulation

The Tt-eRF1 segment was first cloned from *Tetrahymena* DNA by polymerase chain reaction (PCR) using primer 4 (5′-TTGAATTCTTGAA(A/T)CCATTTTCACCACC-3′) and primer 5 (5′-TAGAATTCGAGTATGG(C/A)ACTGC(C/A)TCTAACATTAA-3′). The N-terminal and C-terminal coding sequences were amplified by the RACE (Rapid amplification of cDNA ends) method from *Tetrahymena* RNA using the 5′ RACE System (Gibco BRL, Gaithersburg, MD, USA) according to the manufacturer's instructions. The 5′

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*Abbreviations:* RF, release factor; Tt-eRF1, *Tetrahymena thermophila* release factor 1


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RACE products were synthesized using primer 11 (5′-ACCTCT-ACCGTGCTTCTTG-3′) for first cDNA strand synthesis, the pair of primer 9 (5′-TTGAATTCTTTGTATTTCCTTGGAGATTGG-3′) and 5′-RACE Abridged Anchor Primer (5′-GGCCACGCGTCGAC-TAGTACGGGIIGGGIIGGGIIG-3′; Gibco BRL) for first PCR, and the pair of primer 9 and AUAP primer (5′-GGCCACGCGTCGAC-TAGTAC-3′; Gibco BRL) for nested PCR. The 3′ RACE products were synthesized using oligo(dT) primer (5′-GAGGATCCGGGTAC-CATTTTTTTTTTTTTTTT-3′) for first cDNA strand synthesis, and the pair of primer 8 (5′-TTGAATTCAAAGAACTCCCAATAAT-GGTTTG-3′) and adapter primer 15 (5′-GAGGATCCGGGTAC-CATTT-3′) for PCR. Correct 5′ and 3′ coding parts confirmed by DNA sequencing were used to reconstruct the cDNA for Tt-eRF1 in plasmid pALTER-1 (Promega). Site-directed substitutions of the glutamine codon for 10 UAA/UAG codons within the coding sequence were performed using Altered Sites II in vitro Mutagenesis System (Promega) and designed oligonucleotides. Other DNA or RNA manipulations were conducted according to standard methods [19].

### 2.3. Anti-Tt-eRF1 antibody

Rabbit antibody against Tt-eRF1 was prepared using the recombinant Tt-eRF1 protein solubilized with 8 M urea from the aggregates formed in *Escherichia coli* (to be published elsewhere).

### 2.4. Purification of soluble Tt-eRF1 expressed in yeast

The soluble form of the recombinant Tt-eRF1 protein was purified from *S. cerevisiae* strain YPH499 transformed with the Tt-eRF1 expression plasmid pTHeRF1-4/12, a derivative of pYES2 (Invitrogen) that contained the sense codon-manipulated Tt-eRF1 cDNA. Tt-eRF1 was marked with a histidine ($His_6$) tag at its N-terminus. Transformants were grown at 25°C in SC medium free of uracil supplemented with galactose (for induction of the *GAL1* promoter), lysed by French press in lysis buffer containing 50 mM $NaH_2PO_4$, pH 7.7, 300 mM NaCl, 10 mM $MgCl_2$, 20 mM imidazole, 5 mM 2-mercaptoethanol and 1 mM phenylmethylsulfonyl fluoride, and cell lysates were prepared by low speed centrifugation. Two separate purification procedures were employed: one, to purify directly from the lysates by affinity chromatography using Ni-NTA Agarose (Qiagen) column ac-

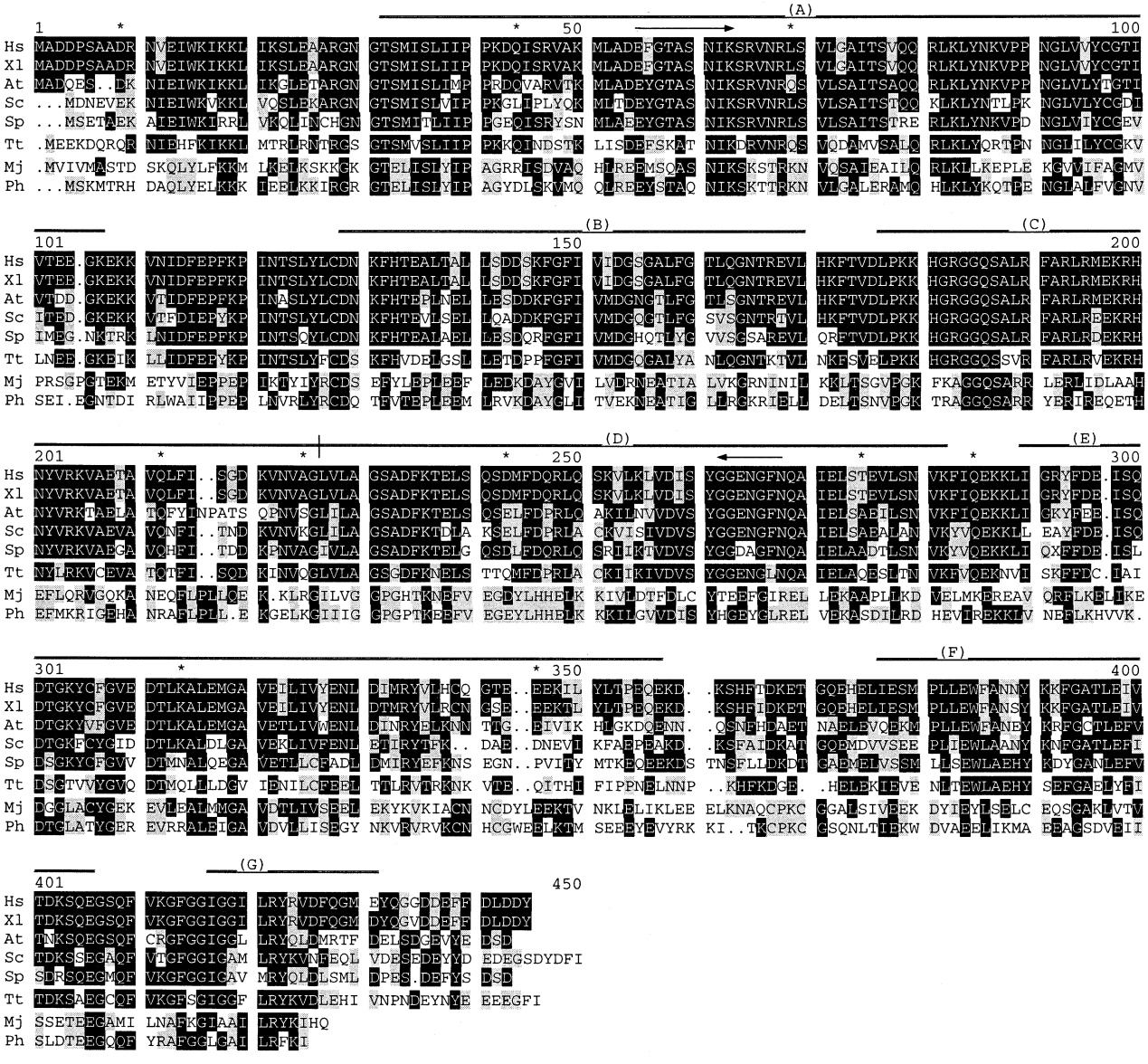


Fig. 1. Comparison of the amino acid sequences of eukaryotic and archaebacterial release factors. The number of the amino acid position is counted from the N-terminal Met of the human eRF1. Eukaryotic eRF1s: Hs, *Homo sapiens* (accession number X81625); Xl, *Xenopus laevis* (Z14253); At, *Arabidopsis thaliana* (X69375); Sc, *S. cerevisiae* (X04082); Sp, *S. pombe* (D63883); Tt, *T. thermophila* (AB026195, this study). Archaebacterial RFs: Mj, *Methanococcus jannaschii* (U67526); Ph, *Pyrococcus horikoshii* (AP000006). Identical amino acids conserved in the majority of eRF1s are shown in black; those conserved in the second majority of eRF1s are shown in gray. Seven conserved domains A–G [6] are shown. Asterisks indicate positions of UAA/UAG manipulated to the glutamine codon in Tt-eRF1. Positions of primers 4 and 5 are shown by arrows.

cording to the manufacturer's instruction; the other, to purify from the post-ribosomal supernatant (see below) by the same Ni-NTA affinity chromatography, followed by two sequential chromatographies using AF-Heparin Toyopearl 650 M (Tosoh Co.) and ÄKTA Mono Q (Pharmacia) columns. The details of purification conditions will be published elsewhere.

*2.5. In vitro eRF1 and eRF3 binding assay*

*Schizosaccharomyces pombe* eRF1 and eRF3 derivatives, $His_6$-Sp-eRF1 (histidine-tagged) and GST-Sp-eRF3* (glutathione *S*-transferase fusion to the C-terminal two-thirds) were isolated and used for in vitro binding assays with $His_6$-Tt-eRF1 essentially as described previously [20].

*2.6. Preparation of ribosomal and post-ribosomal fractions*

The ribosomal fraction and post-ribosomal supernatants were prepared according to the method by Stansfield et al. [21] except that *Tetrahymena* and yeast cells were lysed using a French press and by sonication coupled with freezing-thawing, respectively.

## 3. Results

*3.1. Cloning of cDNA for Tetrahymena eRF1*

Based on the multiple sequence alignment of eRF1s, we designed three sense and four antisense degenerated primers at conservative regions for PCR amplification. Of 12 possible combinations of primer sets tested, the combination of primers 4 and 5 (see Fig. 1) gave rise to a 1268 bp product from *Tetrahymena* DNA (data not shown). DNA sequence analysis of this PCR product revealed that the amplified sequence shares high polypeptide sequence homology with yeast and mammalian eRF1s, and is interrupted by two intronic sequences of 160 and 464 bp in length as well as by three in-frame UAA and one in-frame UAG within the predicted exon sequences (data not shown).

Based on the above exon information, whole cDNAs for Tt-eRF1 were first synthesized in two overlapping 5′ (603 bp) and 3′ (1305 bp) fragments from *Tetrahymena* RNA by 5′ and 3′ RACE method, and the complete sequence of putative Tt-eRF1 was constructed by ligating them at the overlapping unique *Hin*dIII site. The DNA sequence contained a 1308 bp open reading frame (ORF) flanked by AUG initiator and UGA terminator codons, given that the in-frame UAA

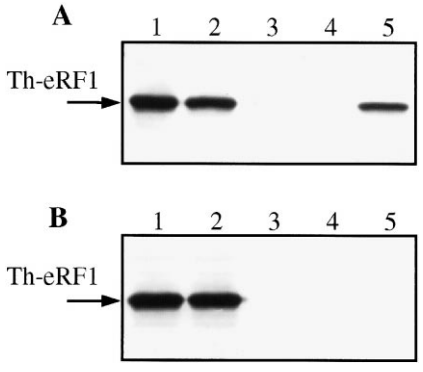


Fig. 2. Complex formation between *Tetrahymena* eRF1 and *S. pombe* eRF3 fused to GST. Proteins were mixed in equimolar amounts, and those bound to the immobilized-eRF3* beads were eluted from the beads by adding excess glutathione. A: Binding of Tt-eRF1 to GST-Sp-eRF3*. B: Control experiment on binding of Tt-eRF1 to glutathione Sepharose in the absence of Sp-eRF3*. Lanes: 1, samples before binding to glutathione Sepharose; 2, unbound protein; 3 and 4, wash; 5, eluted protein. Samples were analyzed by SDS-PAGE and Western blotting using anti-Tt-eRF1 antibody.

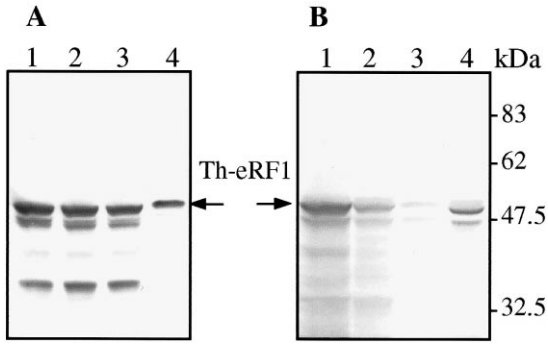


Fig. 3. Subcellular fractionation of *Tetrahymena* eRF1 expressed in *S. cerevisiae* (A) and *T. thermophila* (B). Experimental procedures are described in Section 2. Total cell lysates were subjected to low-speed centrifugation, and the resulting cleared lysates were fractionated by high-speed centrifugation at $130\,000 \times g$, giving rise to the ribosomal pellet and the post-ribosomal supernatant. Lanes: 1, total lysate; 2, cleared lysate; 3, post-ribosomal supernatant; 4, ribosomes. The equal volume of fractions derived from the same cell samples was analyzed by SDS-PAGE and Western blot using anti-Tt-eRF1 antibody.

and UAG codons are assigned as glutamine in *Tetrahymena*. The putative Tt-eRF1 coding sequence contained one UAG at codon position 239, and nine UAA at positions 8, 43, 68, 210, 221, 271, 281, 309 and 339 (Fig. 1). The deduced protein sequence is composed of 435 amino acids (calculated molecular mass 49.5 kDa) and 57% identical (and 66% similar) to human and *Xenopus* eRF1 sequences.

Similarity alignments of multiple eRF1 sequences clearly indicated that the conservative amino acids in known eRF1s are also highly conserved in this putative Tt-eRF1 except for some residues that are unique to this sequence (see Fig. 1). Southern blot analyses of restriction digests of *Tetrahymena* DNA showed a unique band hybridized to this cDNA probe, showing that it is the single gene encoded in the *Tetrahymena* chromosome (data not shown). Moreover, this recombinant protein is able to bind to yeast eRF3 and associated with the ribosome (see below). For these reasons, we believe that the cloned cDNA encodes the unique *Tetrahymena* eRF1. The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with accession number AB026195.

*3.2. Expression and purification of Tetrahymena eRF1*

To express and purify the recombinant Tt-eRF1 protein under heterologous expression conditions, 10 UAA/UAG triplets within the coding sequence were changed to the glutamine codon CAA or CAG by site-directed mutagenesis. Overexpression of Tt-eRF1 was first tested in *E. coli* using the T7 promoter-driven expression system. Though the amount of synthesized Tt-eRF1 reached one third of the total proteins, the recombinant Tt-eRF1 was fully insoluble in spite of any trials (data not shown). The aggregated Tt-eRF1 was solubilized and isolated in denaturing conditions to homogeneity and used to prepare the rabbit antibody. The resulting anti-Tt-eRF1 antibody detected a single major protein band with a molecular mass of 49–50 kDa by Western blot analysis of *Tetrahymena* total protein (see Fig. 3B, lane 1). These immunostaining data indicate that the cloned gene is expressed in *Tetrahymena* and encodes a unique protein whose mass is consistent with that predicted by the DNA sequence.

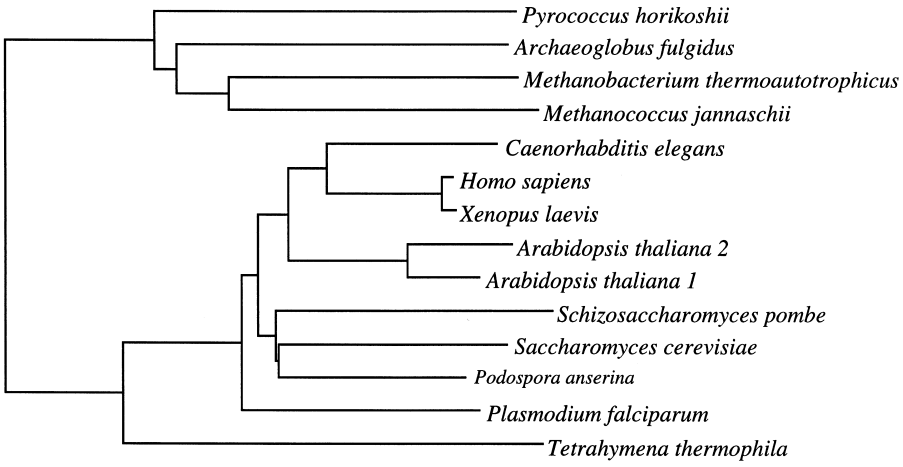


Fig. 4. Phylogenetic tree of the eukaryotic and archaebacterial release factors adopted from the public databases and this study.

In the course of screening for a soluble form of Tt-eRF1, we found that the heterologous expression of Tt-eRF1 in *S. cerevisiae* yielded a soluble fraction of Tt-eRF1 in cell lysates though the expression level was very low. The protein was purified to homogeneity by three steps of affinity and anion exchange chromatography using Ni-NTA agarose, heparin Toyopearl and Mono Q columns. Purified soluble recombinant Tt-eRF1 showed a molecular mass of 49–50 kDa and comigrated identically with the authentic Tt-eRF1.

The ability to terminate protein synthesis of the purified Tt-eRF1 was monitored by the rate of *N*-formylmethionine (fMet) release at the UGA codon from the in vitro termination complex composed of f[$^3$H]Met-tRNA$^f$, AUG, UGAAAAAA, and the ribosome isolated from rat or *Artemia* according to the published procedures [22]. While the *S. pombe* or rabbit eRF1 protein exerted release activity in this system, Tt-eRF1 failed to catalyze fMet release (data not shown).

### 3.3. Heterodimer complex between Tetrahymena eRF1 and S. pombe eRF3

To assess the reason for disability of Tt-eRF1 in the in vitro termination reaction, we examined the protein activity to interact with other components. Recombinant eRF1 and eRF3 (or its N-terminally truncated EF-1α-like polypeptide, referred to as eRF3* [20]) from different eukaryotes are known to bind in vivo and in vitro [20,21,23,24]. To test if the cloned Tt-eRF1 possesses the activity to bind to heterologous eRF3, pull-down analysis was carried out in vitro using the purified recombinant eRF3* of *S. pombe* (referred to as Sp-eRF3*) and Tt-eRF1. Protein complexes with Sp-eRF3* fusion to GST were immobilized onto glutathione-agarose beads, precipitated, and washed with buffer to remove non-specific proteins. Bound proteins were eluted and analyzed by Western blotting using anti-Tt-eRF1 antibody. When the two components, Tt-eRF1 and Sp-eRF3*, were mixed, immobilized Sp-eRF3* efficiently precipitated Tt-eRF1 as shown in the Western blot (Fig. 2A, lane 5). In the absence of eRF3*, the resin failed to precipitate Tt-eRF1 (negative control; Fig. 2B, lane 5). The binding activity of Tt-eRF1 to *S. pombe* eRF3 seemed to be strong since its binding was not efficiently competed by 20–25 molar excess amount of an N-terminally truncated eRF1 polypeptide, Sp-eRF1-ΔN2 [20], that retains sufficient activity for binding to Sp-eRF3* in vivo and in vitro (data not shown).

### 3.4. Subcellular localization of Tetrahymena eRF1 in yeast and Tetrahymena cells

It is known that de novo synthesized eRF1 and eRF3 proteins of *S. cerevisiae* as well as *Xenopus* eRF1 expressed in *S. cerevisiae* are found almost exclusively bound to the ribosome [25–27]. Based on the subcellular fractionation and the detection by anti-Tt-eRF1 antibody, we also found that the majority of de novo Tt-eRF1 was bound to the ribosome in *Tetrahymena* cells and little Tt-eRF1 was detected in the post-ribosomal supernatant (Fig. 3B; compare lanes 3 and 4). The Tt-eRF1 was tightly associated with the *Tetrahymena* ribosome and released only partly even by washing with 1 M $NH_4Cl$ (data not shown). In contrast to the de novo Tt-eRF1, the recombinant Tt-eRF1 expressed in *S. cerevisiae* was bound to the ribosome only partly (Fig. 3A; compare lanes 3 and 4), suggesting that Tt-eRF1 binds much less efficiently to heterologous ribosomes such as yeast ribosomes. We assumed that the lack of effective binding to the heterologous ribosome of Tt-eRF1 might, at least in part, account for the inability of *Tetrahymena* eRF1 to catalyze polypeptide release in vitro.

The lack of efficient interaction of Tt-eRF1 with the heterologous ribosome is conceivable from the large phylogenetic distance of Tt-eRF1 from other eukaryotic eRF1s (Fig. 4). It is quite remarkable that Tt-eRF1 is very isolated from most other eRF1s in the phylogenetic tree, showing only 53–57% identity to mammalian and yeast eRF1s. Therefore, one could argue that Tt-eRF1 might have evolved rapidly compared with other eRF1s. It remains to be examined whether eRF1 genes from other ciliates are also distantly related to the mammalian release factor genes.

## 4. Discussion

The first release factor gene of ciliates was cloned from *Tetrahymena* in this work. Several lines of evidence support that the cloned sequence encodes eRF1 and originated from *Tetrahymena*. First, the predicted ORF contains multiple UAA and UAG triplets that are assigned as a glutamine codon in *Tetrahymena*. Second, the cloned sequence uniquely

hybridized to *Tetrahymena* DNA but not to others. Third, the raised antibody detected a single major protein of *Tetrahymena*. Fourth, the deduced protein sequence is highly homologous to other eukaryotic and archaebacterial release factors (see Fig. 1). Fifth, the recombinant protein is able to bind to yeast eRF3 in vitro (see Fig. 2) and associated with the ribosome in vivo (see Fig. 3).

Sequence comparison of Tt-eRF1 with known eRF1s demonstrates its conservative primary sequence throughout domains A–G that have been assigned as seven conserved domains in prokaryotic and eukaryotic RFs [6] (see Fig. 1). We have predicted that domains C and D play important roles in stop codon recognition [5,6,10]. In view of this prediction, it is rather surprising that these two domains of Tt-eRF1 are highly conservative and contain only a few residues that are unique to Tt-eRF1. This limited number of distinct residues would not be sufficient to confer the strict and sole specificity to UGA on Tt-eRF1, although they might be able to confer some (relaxed) preference to UGA compared with the other two if any.

In spite of the overall conservative protein structure of Tt-eRF1 compared with mammalian and yeast eRF1s, the soluble recombinant Tt-eRF1 did not show any polypeptide release activity in vitro using rat or *Artemia* ribosomes. As already mentioned above, this disability may be due to the heterologous translation system using heterologous ribosomes, with which Tt-eRF1 is associated much less efficiently compared with *Tetrahymena* ribosome (see Fig. 3). This view is supported by the distant phylogenetic position of Tt-eRF1 compared with other eRF1s (see Fig. 4). It is noteworthy that most known eRF1s from different eukaryotic organisms including *Xenopus*, human and yeast are functionally exchangeable in vivo and in vitro and that Tt-eRF1 is the first exception to this property. There are several cases of functional disability or limited ability of translational components in heterologous systems [28–30]. It is also known that mutational defects in binding to the ribosome of *S. cerevisiae* release factors led to termination defects [31] and gave rise to an allosuppressor phenotype [27,32]. The observed weak binding of Tt-eRF1 to heterologous ribosomes might be correlated with the sequence diversity of Tt-eRF1 in its N-terminal (domains A/B) and C-terminal (domains E/F) regions (see Fig. 1). Recently, it has been suggested that initial (primary) binding to the ribosome of bacterial RF1 and RF2 is catalyzed by N-terminal domains A/B [33]. We assume that the less conservative nature of the N-terminal of Tt-eRF1 could be due to its binding site property of *Tetrahymena* ribosomes, which might be more diverse than others. This prediction might account for the reduced affinity of Tt-eRF1 to heterologous ribosomes (see Fig. 3).

Given that Tt-eRF1 possesses potentially omnipotent recognition capacity, other element(s) in Tt-eRF1 domains or in the *Tetrahymena* ribosome might function to confer UGA specificity on Tt-eRF1 or to block termination at UAA and UAG. One could speculate that some mRNA context around in-frame UAA and UAG may function as such a *cis* element. It is known that three stop codons are not used randomly or equitably in the database and the base following the triplet greatly influences the usage frequency in both prokaryotes and eukaryotes [34,35]. For example, six of eight possible tetranucleotides starting with UAA and UAG are frequently used, but the two sequences, UAGC and UAGU, are used very rarely if at all in eukaryotes [35]. The rarely used stop signals represent those that can be recognized weakly by release factors [36,37], thus providing a clue to weaken polypeptide termination and increase suppression by tRNA by competition. We scored the usage of UAAN and UAGN coding tetranucleotides in 20 known cDNA sequences of *Tetrahymena*. Of 87 UAGN cases, there is no apparent tetranucleotide bias: 32 UAGAs, 22 UAGGs, 20 UAGCs, and 13 UAGUs. Therefore, we assume that frequent use of the rare (and weak) tetranucleotides UAGC and UAGU might contribute, at least in part, to enhanced assignment of glutamine by two glutaminyl-tRNAs cognate to UAA and/or UAG [14,15] in *Tetrahymena* even if Tt-eRF1 is capable of reading UAA and UAG.

Apparently, the in vitro termination system directed by the *Tetrahymena* ribosome remains to be established to answer the key question of whether Tt-eRF1 reads only UGA or UAA/UAG as well. Alternatively, genetic manipulation or selection for a Tt-eRF1 derivative that is active with heterologous ribosomes in vitro or complements a temperature-sensitive eRF1 mutation of *S. cerevisiae* should provide us with a powerful tool to facilitate the analysis.

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## References

[1] Nakamura, Y., Ito, K. and Isaksson, L.A. (1996) Cell 87, 147–150.
[2] Buckingham, R.H., Grentzmann, G. and Kisselev, L. (1997) Mol. Microbiol. 24, 449–456.
[3] Scolnick, E., Tompkins, R., Caskey, T. and Nirenberg, M. (1968) Proc. Natl. Acad. Sci. USA 61, 768–774.
[4] Frolova, L., Le Goff, X., Rasmussen, H.H., Cheperegin, S., Drugeon, G., Kress, M., Arman, I., Haenni, A.L., Celis, J.E., Philippe, M., Justesen, K. and Kisselev, L. (1994) Nature 372, 701–703.
[5] Nakamura, Y. and Ito, K. (1998) Genes Cells 3, 265–278.
[6] Ito, K., Ebihara, K., Uno, M. and Nakamura, Y. (1996) Proc. Natl. Acad. Sci. USA 93, 5443–5448.
[7] Ævarsson, A., Brazhnikov, E., Garber, M., Zheltonosova, J., Chirgadze, Y., al-Karadaghi, S., Svensson, L.A. and Liljas, A. (1994) EMBO J. 13, 3669–3677.
[8] Czworkowski, J., Wang, J., Steitz, T.A. and Moore, P.B. (1994) EMBO J. 13, 3661–3668.
[9] Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F. and Nyborg, J. (1995) Science 270, 1464–1472.
[10] Ito, K., Uno, M. and Nakamura, Y. (1998) Proc. Natl. Acad. Sci. USA 95, 8165–8169.
[11] Meyer, F., Schmidt, H.J., Plumper, E., Hasilik, A., Mersmann, G., Meyer, H.E., Engstrom, A. and Heckmann, K. (1991) Proc. Natl. Acad. Sci. USA 88, 3758–3761.
[12] Horowitz, S. and Gorovsky, M.A. (1985) Proc. Natl. Acad. Sci. USA 82, 2452–2455.
[13] Cupples, C. and Pearlman, R.E. (1986) Proc. Natl. Acad. Sci. USA 83, 5160–5164.

[14] Kuchino, Y., Hanyu, N., Tashiro, F. and Nishimura, S. (1985) Proc. Natl. Acad. Sci. USA 82, 4758–4762.
[15] Hanyu, N., Kuchino, Y. and Nishimura, S. (1986) EMBO J. 5, 1307–1311.
[16] Fujishima, M., Katsu, Y., Ogawa, E., Sakimura, M., Yamashita, M. and Nagahama, Y. (1992) J. Protozool. 39, 683–690.
[17] Sikorski, R.S. and Hieter, P. (1989) Genetics 122, 19–27.
[18] Sherman, F. (1991) Methods Enzymol. 194, 3–20.
[19] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, 2nd edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[20] Ito, K., Ebihara, K. and Nakamura, Y. (1998) RNA 4, 958–972.
[21] Stansfield, I., Jones, K.M., Kushnirov, V.V., Dagkesamanskaya, A.R., Poznyakovski, A.I., Paushkin, S.V., Nierras, C.R., Cox, B.S., Ter-Avanesyan, M.D. and Tuite, M.F. (1995) EMBO J. 14, 4365–4373.
[22] Andjelkovic, N., Zolnierowicz, S., Van Hoof, C., Goris, J. and Hemmings, B.A. (1996) EMBO J. 15, 7156–7167.
[23] Zhouravleva, G., Frolova, L., Le Goff, X., Le Guellec, R., Inge-Vechtomov, S., Kisselev, L. and Philippe, M. (1995) EMBO J. 14, 4065–4072.
[24] Frolova, L.Y., Simonsen, J.L., Merkulova, T.I., Litvinov, D.Y., Martensen, P.M., Rechinsky, V.O., Camonis, J.H., Kisselev, L.L. and Justesen, J. (1998) Eur. J. Biochem. 256, 36–44.
[25] Stansfield, I., Grant, G.M., Akhmaloka and Tuite, M.F.(1992) Mol. Microbiol. 6, 3469–3478.
[26] Didichenko, S.A., Ter-Avanesyan, M.D. and Smirnov, V.N. (1991) Eur. J. Biochem. 198, 705–711.
[27] Urbero, B., Eurwilaichitr, L., Stansfield, I., Tassan, J.P., Le Goff, X., Kress, M. and Tuite, M.F. (1997) Biochimie 79, 27–36.
[28] Eberly, S.L., Locklear, V. and Spremulli, L.L. (1985) J. Biol. Chem. 260, 8721–8725.
[29] Lee, C.C., Timms, K.M., Trotman, C.N. and Tate, W.P. (1987) J. Biol. Chem. 262, 3548–3552.
[30] Kumazawa, Y., Himeno, H., Miura, K. and Watanabe, K. (1991) J. Biochem. (Tokyo) 109, 421–427.
[31] Pel, H.J., Rep, M., Dubbink, H.J. and Grivell, L.A. (1993) Nucleic Acids Res. 25, 5308–5315.
[32] Stansfield, I., Eurwilaichitr, Akhmaloka and Tuite, M.F. (1996) Mol. Microbiol. 20, 1135–1143.
[33] Yoshimura, K., Ito, K. and Nakamura, Y. (1999) Genes Cells 4, 253–266.
[34] Tate, W.P., Poole, E.S., Horsfield, J.A., Mannering, S.A., Brown, C.M., Moffat, J.G., Dalphin, M.E., McCaughan, K.K., Major, L.L. and Wilson, D.N. (1995) Biochem. Cell. Biol. 73, 1095–1103.
[35] Tate, W.P. and Mannering, S.A. (1996) Mol. Microbiol. 21, 213–219.
[36] Poole, E.S., Brown, C.M. and Tate, W.P. (1995) EMBO J. 14, 151–158.
[37] McCaughan, K.K., Brown, C.M., Dalphin, M.E., Berry, M.J. and Tate, W.P. (1995) Proc. Natl. Acad. Sci. USA 92, 5431–5435.