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Cloning and expression of a mammalian peptide chain release factor with sequence similarity to tryptophanyl-tRNA synthetases

(stop codons/translation termination/cDNA)

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ABSTRACT **The termination of protein synthesis is encoded by in-frame nonsense (stop) codons. Most organisms use three nonsense codons: UGA, UAG, and UAA. In contrast to sense codons, which are decoded by specific tRNAs, nonsense codons are decoded by proteins called release factors (RFs). Here we report the cloning of a mammalian RF cDNA by the use of monoclonal antibodies specific for rabbit RF. Functional studies showed that, when expressed in *Escherichia coli*, the protein encoded by this cDNA has *in vitro* biochemical characteristics similar to those of previously characterized mammalian RFs. DNA sequencing of this eukaryotic RF cDNA revealed a remarkable sequence similarity to bacterial and mitochondrial tryptophanyl-tRNA synthetases, with the greatest similarity confined to the synthetase active site, and no obvious similarity to bacterial RFs.**

The genetic code is the means by which information is translated from DNA into protein. Two levels of coding occur in all organisms; the classic triplet codons that specify the linear arrangement of amino acids in a protein (1) and a recently described "second genetic code" that imparts aminoacylation specificity to the tRNAs (2). While the translation of sense codons involves specific tRNA recognition via RNA·RNA interactions, the termination of polypeptide translation involves the decoding of in-frame nonsense codons (UAA, UAG, and UGA) by stop-codon-specific proteins (3).

Release factors (RFs) have been purified from both prokaryotes and eukaryotes. A single RF (eRF) that recognizes all three nonsense codons has been described in eukaryotes (4, 5). The best characterized eRF is the rabbit RF, which has a subunit molecular weight of 54,000–56,000 and a ribosome-dependent GTPase activity (4–7). In *Escherichia coli*, two RFs (RF1 and RF2) mediate peptide chain termination (8–10). RF1 is required for UAG- and UAA-directed termination, while RF2 is required for UGA- and UAA-directed termination. Three separate activities can be independently assessed for both prokaryotic and eukaryotic RFs: ribosomal binding, stop-codon recognition, and peptidyl-tRNA hydrolysis (3). While the primary structures of the *E. coli* RFs have been elucidated (11), little is known of how codon specificity is maintained.

To address this question of stop-codon recognition we have used RF-specific monoclonal antibodies to isolate a cDNA encoding the rabbit RF.§ We demonstrate that the protein encoded by this cDNA has functional characteristics of a mammalian RF. We also show that the rabbit RF protein has sequence similarity to the prokaryotic and mitochondrial tryptophanyl-tRNA synthetases, suggesting that a conserved structural domain is required for both synthetase and RF function and/or that the tryptophanyl-tRNA synthetases and RF are derived from a common evolutionary lineage. Despite the obvious functional similarities, there appears to be no evolutionary or structural conservation between the bacterial and eukaryotic RFs.

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MATERIALS AND METHODS

Preparation of eRF Monoclonal Antibodies. Purified rabbit RF was used to immunize BALB/c mice. Hybridomas were prepared by standard techniques (12). Three lines of monoclonal antibodies were generated by these protocols.

Expression of eRF in *E. coli* and Monkey COS Cells. The eRF clones were rescued from λZAP (Stratagene) by the automated excision procedure (13). Expression of eRF in *E. coli* was achieved by induction with 10 mM isopropyl β-D-thiogalactopyranoside (IPTG) for 2 hr prior to Western immunoblot analysis.

The eRF A and B cDNAs were subcloned into p91023b (14) by ligation into the *Eco*RI cloning site, selection on tetracycline plates, and restriction mapping. The plasmids were transfected into COS cells as described (15). Cells were harvested after 72 hr by trypsin treatment and analyzed by Western blotting.

Release Assay and eRF Activity. The f[^{3}H]Met-tRNA/AUG/80S ribosome substrate intermediates were prepared as described (7). The peptide chain release assay was performed as described (7), except that 10 mM Pipes (pH 7.0) was substituted for 20 mM Tris and 10% (vol/vol) ethanol was added.

For the eRF A activity assay, IPTG-induced cells were ground in an equal amount of alumina for 30 min and a soluble extract was made by diluting the ground paste with ice-cold RF buffer (10 mM Pipes, pH 7.0/100 mM KCl/1 mM 2-mercaptoethanol/0.1 mM EDTA). The aqueous fraction was separated from the debris by centrifugation at 10,000 × *g* for 15 min. A postribosomal fraction was obtained from the supernatant by centrifugation at 100,000 × *g* for 4 hr. The supernatant was then purified by a 0–65% ammonium sulfate precipitation. The precipitate from the ammonium sulfate step was dialyzed in RF buffer for 5 hr before 5 ml was loaded onto a Sephadex G-75 gel permeation column (2 cm × 100 cm) equilibrated with RF buffer. The eluted fractions were collected in 2.5-ml aliquots. The expression and analysis of the control pUO-p clone were carried out under identical conditions.

Construction and Screening of a λZAP Expression Library. A rabbit liver cDNA expression library was constructed in λZAP (Stratagene) from 5 μg of rabbit liver poly(A)$^+$ RNA according to a published procedure (16). Antibody screening

Abbreviations: RF, release factor; eRF, eukaryotic RF; IPTG, isopropyl β-D-thiogalactopyranoside.

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§The sequence reported in this paper has been deposited in the GenBank data base (accession no. M33460).

of the rabbit liver cDNA expression was carried out as described (17).

Sequencing of eRF cDNA. The nucleotide sequence of the rabbit eRF B cDNA was determined by subcloning the cDNA into the vectors pTZ18 and pTZ19 (Pharmacia). Sequential deletions were prepared by the exonuclease III method (18). The single-stranded DNA was sequenced by the dideoxy chain-termination method (19) with the manual Klenow polymerase procedure (Boehringer Mannheim) as well as an automated fluorescence procedure (Applied Biosystems), as described (20).

RESULTS

Isolation of the Rabbit RF cDNA. A total of 500,000 primary recombinants were screened with the three antibodies in combination, and positive clones identified by using ^{125}I-labeled anti-mouse immunoglobulin as second antibody (17). Three clones specific to the antibodies were identified. The clones were designated eRF A, B, and C, following the current convention for eukaryotic translation factors.

Restriction enzyme mapping of the three cDNA clones revealed that the inserts are 2.5 kilobases (kb) long and have identical restriction maps (data not shown). Northern analysis using the eRF A cDNA insert as a ^{32}P-labeled probe detected a single transcript of ≈2.5 kb (Fig. 1*A*), indicating that the eRF cDNA represents virtually the entire eRF mRNA. Expression of the putative eRF cDNAs in *E. coli* demonstrated that the polypeptide produced by eRF A is larger than that of eRF B (Fig. 1*B*, lanes b and e), suggesting that in *E. coli* eRF A is expressed as a fusion protein with the amino-terminal end of β-galactosidase. In addition, eRF A was expressed in bacteria at a higher level than clones B and C. The basal level of eRF, as estimated by autoradiograph densitometry, was at least 30-fold greater for eRF A than for eRF B or C. The addition of IPTG, an inducer of β-galactosidase gene expression, effected a 5-fold increase in eRF protein expression in both eRF A and eRF B (Fig. 1*B*, lanes b and c and lanes e and f). The proteins expressed by eRF B and eRF C were identical in size (data not shown) and migrated at the size consistent with translation initiation from the authentic eRF AUG codon. When the cDNAs from eRF A and eRF B were transfected into monkey COS cells under the transcriptional control of the adenovirus major late promoter, the polypeptides expressed were of identical size (Fig. 1*B*, lanes d and g) and were the same size as the authentic eRF from rabbit reticulocytes (Fig. 1*B*, lane a). This suggests that the increase in eRF protein in bacteria harboring eRF A is a result of more efficient translation initiation from the β-galactosidase initiation AUG as compared to the authentic eRF AUG used by eRF B.

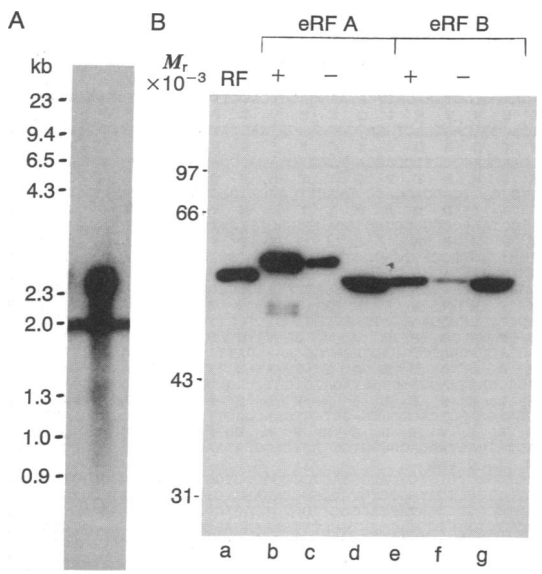

FIG. 1. The eRF mRNA transcript and cDNA expression in bacteria and COS cells. (*A*) Northern analysis of 10 μg of rabbit liver poly(A)$^+$ RNA probed with ^{32}P-labeled rabbit eRF cDNA. (*B*) Western analysis of eRF expression in *E. coli* and COS cells. Lanes: a, partially purified RF from rabbit reticulocytes; b and c, extract (5 μl) of *E. coli* carrying eRF A and grown in the presence (+) or absence (−) of 10 mM IPTG, respectively; d, extract (25 μl) prepared from eRF A-transfected COS cells; e and f, extract (25 μl) of *E. coli* carrying eRF B and grown in the presence (+) or absence (−) of 10 mM IPTG, respectively; g, extract (25 μl) of eRF B-transfected COS cells.

Expression of eRF Activity in *E. coli*. To demonstrate that the eRF clones encode a mammalian peptide chain termination factor, we carried out functional studies of the protein encoded by eRF A. We chose to study eRF A, rather than eRF B or eRF expressed in tissue culture cells, because of the high level of eRF A expression in bacteria. eRFs do not function with bacterial 70S ribosomes under any conditions, and likewise, bacterial RFs do not function with 80S ribosomes (4, 21).

A whole cell extract was prepared from bacteria harboring eRF A after induction with IPTG, and the extract was fractionated on a Sephadex G-75 gel permeation column. The peptide chain release assay was performed on these fractions using rabbit reticulocyte ribosomes. Nonsense codon (UAAA)-dependent peptide chain release activity was detected from protein fractions of eRF A (Fig. 2*A*). Western analysis showed that this activity coincided with an increasing amount of eRF protein, indicating that the codon-dependent peptide chain release activity was directly related to the presence of eRF (Fig. 2*A Inset*).

No codon-dependent peptide chain release activity was observed from a control extract prepared from bacteria harboring the plasmid pUO-p (Fig. 2*B*), a pBluescript SK(−) vector (Stratagene) containing the porcine urate oxidase cDNA (22). These functional studies show that the eRF cDNAs encode a mammalian RF.

***In Vitro* Assay Requirements for eRF Activity.** Various parameters affecting the *in vitro* termination assay that are characteristic of a mammalian RF were analyzed. In contrast to the bacterial and rat mitochondrial RFs, mammalian RF activity functions only on eukaryotic 80S ribosome, is stimulated by GTP, and requires nonsense codons to be in the form of tetranucleotides rather than triplets (4, 7, 21). In addition, the termination reaction catalyzed by rabbit RF on rabbit ribosomes is stimulated by the presence of 10% (vol/vol) ethanol. For heterologous reactions involving ribosomes and RFs from different species, the addition of ethanol is essential for RF-directed codon-dependent hydrolysis of the ribosome-bound model peptidyl-tRNA (fMet-tRNA) (21).

The addition of ethanol was required for codon-dependent peptidyl-tRNA hydrolysis by eRF A. The *in vitro* termination activity of eRF A with rabbit reticulocyte ribosomes was dependent upon the presence of 10% ethanol and was stimulated 3-fold by the addition of both GTP and stop codon (Table 1). The removal of either GTP or stop codon from the reaction decreased peptide chain termination activity by at least 50%. By comparison, rat liver RF showed some termination activity in the absence of ethanol, but, in the presence of stop codon and GTP, rat RF activity was stimulated 3-fold by the addition of ethanol. A small amount of GTP- and stop-codon-independent activity was observed for the rat RF in the presence of ethanol. The absolute requirement for ethanol by the eRF A extract may reflect the fact that eRF A produces a fusion protein, and this fusion protein may not bind 80S ribosomes efficiently. Although the mechanism of ethanol-stimulated RF activity is unknown, the addition of

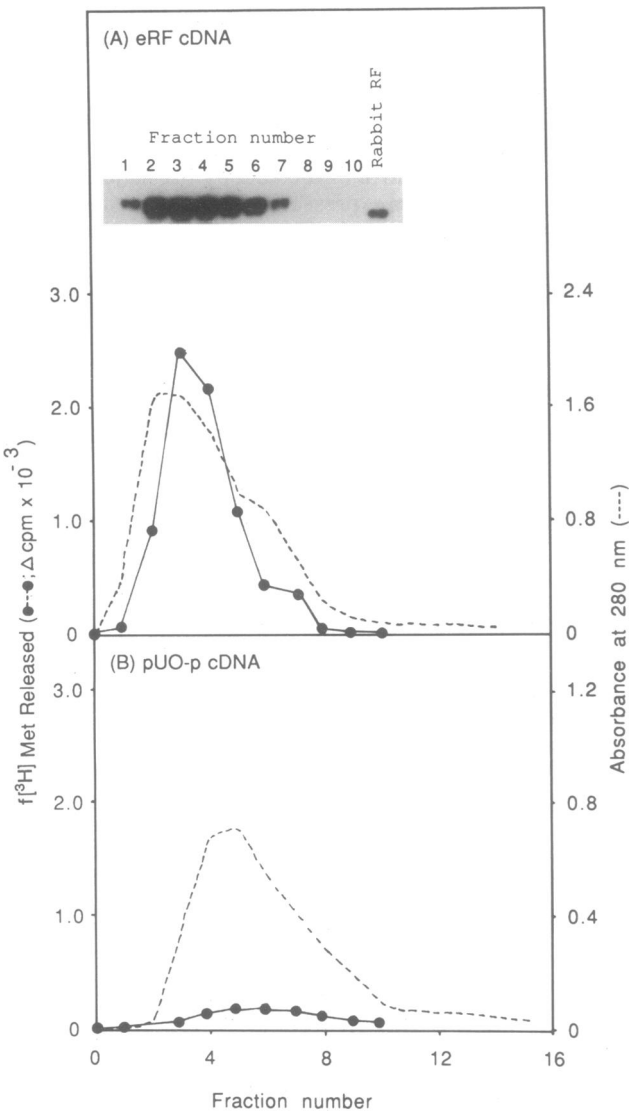

FIG. 2. Functional expression of eRF in bacteria. Whole cell extracts prepared from bacteria harboring eRF A (*A*) or the control plasmid pUO-p (*B*) were fractionated on a Sephadex G-75 gel permeation column, and the fractions were assayed for protein content (---) and peptide chain release activity (●). *Inset* in *A* shows a Western analysis of the corresponding fractions with the anti-RF antibodies.

ethanol has been shown to enhance the formation of a RF/ribosome/codon complex in both eukaryotic and prokaryotic RF binding assays, and this may have contributed to the enhanced hydrolysis of the model peptidyl-tRNA in the above experiments and in prior studies using heterologous ribosomes and RFs (8, 21, 23). These results are also consistent with previous studies demonstrating that RF-dependent hydrolysis of ribosome-bound model peptidyl-tRNA can occur in the absence of stop codons or GTP if the *in vitro* assay reaction contains a sufficient level of ethanol (23, 24). The eRF A fraction showed peptide chain termination activity with the tetramers but not the triplet nonsense codons (data not shown). These results demonstrate that the expressed eRF is similar in character to the mammalian RF.

Table 1. Substrate requirements of eRF

Substrate mixture	f[^{3}H]Met released, Δ cpm	
	eRF A	Rat liver RF
Complete	3054	3594
Minus GTP	1460	891
Minus UAAA	1389	282
Minus GTP and UAAA	885	385
Minus ethanol	0	1350

The substrates for eRF termination activity were at the following final concentrations: 0.2 mM GTP; 2 A_{260} units of UAAA per ml; and 10% (vol/vol) ethanol. Background activity in the absence of stop codon (UAAA), GTP, and ethanol has been subtracted from each.

Primary Structure of the Rabbit RF. The complete nucleotide sequence of the rabbit eRF B cDNA was determined for both strands by manual and automated DNA sequencing (18, 19). The DNA sequence and its predicted protein sequence are shown in Fig. 3. The rabbit eRF cDNA contains a 143-base-pair (bp) 5′ untranslated region followed by a single ATG codon that begins an uninterrupted open reading frame of 1425 bp, corresponding to a protein length of 475 amino acids. The TAA stop codon at position 1571 is followed by 876 bp of 3′ untranslated sequence, including the conventional polyadenylylation signal AATAAA at position 2427. The deduced polypeptide of 475 amino acids has a calculated molecular weight of 53,964, a value consistent with the subunit molecular weight of 54,000–56,000 predicted by SDS/polyacrylamide gel electrophoresis (5, 7).

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1     AATTCGGGTAAAGACGCCGCGGGCCGGGCAGCGCGTCCTCGGCTCTGCAGCGGCCCCTCG
61    GCGCGTCCGCCAGACCCGGGCTTGCCTTGACCCTGGACAGCTCCTCCTCCTCCCTAGCCA
121   GCTGTGAGCGTCAGTGCTGGAAGATGGCGGACGTGACCAACGGCGAGCGGTGTGCCTCTC
1                       M  A  D  V  T  N  G  E  R  C  A  S
181   CACAGGAGCTGTTCAGCAGCATCGCGGCGCAGGGCGAGCTCGTGAAGTCCCTCAAAGCTA
13    P  Q  E  L  F  S  S  I  A  A  Q  G  E  L  V  K  S  L  K  A
241   GAAAGGCCCCGAAGGAGGAAATTGATTCTGCAGTAAAGATGTTGCTGTCGTTAAAAACGA
33    R  K  A  P  K  E  E  I  D  S  A  V  K  M  L  L  S  L  K  T
301   GCTACAAGGAGGCCATGGGGGAGGACTACAAGGCTGACTGCCCTCCAGGGAACTCGACCC
53    S  Y  K  E  A  M  G  E  D  Y  K  A  D  C  P  P  G  N  S  T
361   CCGACAGCCACGGTGACCCAGAAGCCGTAGATGACAAAGAGGATTTTGTGGACCCGTGGA
73    P  D  S  H  G  D  P  E  A  V  D  D  K  E  D  F  V  D  P  W
421   CAGTACGGACGAGCAGTGCGAAAGGCATTGACTACGACAAGCTCATCGTTCAGTTCGGAA
93    T  V  R  T  S  S  A  K  G  I  D  Y  D  K  L  I  V  Q  F  G
481   GCAGTAAAATCGACAAGGAGCTGGTGAACCGGATCGAGAGAGCCACCGGCCAGAGACCGC
113   S  S  K  I  D  K  E  L  V  N  R  I  E  R  A  T  G  Q  R  P
541   ACCGCTTCCTGCGCAGGGGCATCTTCTTCTCACACAGAGATATGAACCAAGTTCTTGATG
133   H  R  F  L  R  R  G  I  F  F  S  H  R  D  M  N  Q  V  L  D
601   CCTACGAGAACAAGAAGCCGTTTTACCTGTACACGGGCAGGGGCCCTCTTCTGAAGCAAT
153   A  Y  E  N  K  K  P  F  Y  L  Y  T  G  R  G  P  L  L  K  Q
661   GCAATGTAGGTCACCTCATCCCGTTTATTTTCACAAAGTGGCTGCAGGATGTGTTTGACG
173   C  N  V  G  H  L  I  P  F  I  F  T  K  W  L  Q  D  V  F  D
721   TGCCGCTGGTGGTGCAGATGTCGGATGACGAGAAGTACCTGTGGAAGGACCTCACGCTGG
193   V  P  L  V  V  Q  M  S  D  D  E  K  Y  L  W  K  D  L  T  L
781   AGCAGGTCTATGGCTACACCCTGGAGAACGCCAAGGACATCATGCCCTGTGGCTTCGATG
213   E  Q  V  Y  G  Y  T  L  E  N  A  K  D  I  M  P  C  G  F  D
841   TCAACAAGACGTTCATCTTCTCTGACCTTGACTACATGGGGATGAGCCCAGGCTTCTACA
233   V  N  K  T  F  I  F  S  D  L  D  Y  M  G  M  S  P  G  F  Y
901   AGAATGTGGTGAAGATTCAGAAGCACGTCACCTTCAACCAAGTGAAAGGCATCTTCGGCT
253   K  N  V  V  K  I  Q  K  H  V  T  F  N  Q  V  K  G  I  F  G
961   TCACCGACAGTGACTGCATCGGGAAGATCAGTTTTCCTGCCATCCAGGCTGCGCCTTCCT
273   F  T  D  S  D  C  I  G  K  I  S  F  P  A  I  Q  A  A  P  S
1021  TCAGCAACTCCTTCCCCCAGATCTTCCATGGCCAGGCAGACATCCAGTGCCTCATCCCCT
293   F  S  N  S  F  P  Q  I  F  H  G  Q  A  D  I  Q  C  L  I  P
1081  GTGCCATTGACCAGGACCCCTACTTCAGGATGACCAGGGACGTCGCGCCCAGGATCGGCT
313   C  A  I  D  Q  D  P  Y  F  R  M  T  R  D  V  A  P  R  I  G
1141  ATCCTAAACCGGCCCTGCTGCACTCGACCTTCTTCCCTGCTCTGCAGGACGCCCAGACCA
333   Y  P  K  P  A  L  L  H  S  T  F  F  P  A  L  Q  D  A  Q  T
1201  AGATGAGTGCCAGTGACCCCAACTCCTCCATCTTCCTCACCGACACGGCCAAGCAGATCA
353   K  M  S  A  S  D  P  N  S  S  I  F  L  T  D  T  A  K  Q  I
1261  AGACCAAGGTCAACAAGCACGCATTTTCTGGGGGCAGAGACACCATCGAGGAGCACCGGC
373   K  T  K  V  N  K  H  A  F  S  G  G  R  D  T  I  E  E  H  R
1321  AGTTTGGGGGCAACTGTGACGTGGACGTCTCCTTCATGTACCTGACCTTCTTCCTGGAGG
393   Q  F  G  G  N  C  D  V  D  V  S  F  M  Y  L  T  F  F  L  E
1381  ACGACGACAAGCTGGAGCAGATCAGAAAGGATTACAGCAGCGGCGCCATGCTCACCGGCG
413   D  D  D  K  L  E  Q  I  R  K  D  Y  S  S  G  A  M  L  T  G
1441  AGCTGAAGAAGGAGCTGATAGACGTCCTGCAGCCGCTGGTCGCCGAGCACCAGGCCCGGC
433   E  L  K  K  E  L  I  D  V  L  Q  P  L  V  A  E  H  Q  A  R
1501  GCAAGGAGGTCACGGACGAGATGGTGAAGGAGTTCATGACGCCCCGGCAGCTGTGCTTCC
453   R  K  E  V  T  D  E  M  V  K  E  F  M  T  P  R  Q  L  C  F
1561  ACTATCAGTAACACGGGGTGGGAGTTACCCATAACCCAGCCCAGTGGGGTCGCCGCAGCC
473   H  Y  Q  STOP
1621  CGTGGGCTCTGTCGCACGGTACTGCTGGGCCTGGTGTCGTGAGCCCTGTGTGTACCGACC
1681  CTGTTTCTTCATCGTCTGTCTTGGCCAGAACACTGCGGGTGATTGGGTGCTGGCTAAGGT
1741  GGCGTGGTCGCATAGCAGCGCCTGCTGTGAGTCTCACCCACAAAGCAGCCAGGGTGCCGC
1801  GCCTCTGCCTCGGAGGGCTGCCCTGGAAAACAGCCCAGGGTTCCAAATGTACCCTTAGAA
1861  TCTGCTCTTAAAATCTGCACTCTGTGGACACATACATGGAGCCAGAGTGTCGCCTTGTGA
1921  AACCTAAGGCTTGACAGGCTGACCTGGGCAGTTTAATGGGCACCTAGTACACTCCAGCCC
1981  TCGCCAGGAAAGAAGCAGTGAGTGGCCCAAAGCTTTGCCCGACGTGGAAGTGACACAGCA
2041  CCACTTTCGAGCTGTTTGCACGGGGCTGTGGGCAGAGGTGGGCAGGCTCACTGAGCTGGG
2101  TCCTGTGGGTGAGACTTGCGGACTGCGCTTCCTAACCCAGACCATGCATGTATCCCCGTT
2161  CTCTTCCCCGACTACCAATGTGGGCTCCACCGAGCCATCCCAGTGGTGTCCGGTGGCCTG
2221  GCCGCGCTGCCTTTGCAGGGCTTCTCTCTGTCACCCGACCGCAGTGGGCACACAGAGCAG
2281  TGCACTGGCCAAGCGGTGGACGCCGCCTGGGGGACCCTCCGCGGTTTCTCCGTGTACCGT
2341  GTGTGGTCCTGGGAAAACAGAAGCCACATCTCAGGGAAAGGCTCTGGCTGGAGTCCGCCC
2401  TGCCCCTTGTCACTCGCTTGGGTATAAATAAACTTTGCTCCCACCCCC
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FIG. 3. The complete nucleotide and predicted amino acid sequence of the rabbit eRF cDNA. The predicted initiator methionine codon begins at nucleotide position 144; the numbers on the left indicate the nucleotide and amino acid positions. The polyadenylylation signal AATAAA is underlined and the poly(A) sequence has been omitted.

FIG. 4. Sequence comparison of the predicted rabbit eRF protein and the bacterial and yeast mitochondrial tryptophanyl-tRNA synthetases. Top line, rabbit eRF (R. eRF); the second, third, and fourth lines, tryptophanyl-tRNA synthetases from yeast mitochondria (Y. mit), *E. coli* (E. col), and *B. stearothermophilus* (B. ste). The amino acid sequences were aligned by the program GENALIGN (36) and visual inspection. Gaps are represented by dashes and only those residues found to be conserved with eRF are boxed.

A comparison of the predicted eRF protein sequence to the *E. coli* RF1 and RF2 protein sequences failed to demonstrate any significant amino acid similarity. This is not surprising since mammalian and bacterial RFs, although demonstrating functional similarities when each is tested on its own ribosomes, do not crossreact functionally when tested on the other's ribosomes (4, 21). A computer-aided search of sequences in the National Biomedical Research Foundation protein data bank (Release 59) identified a short region of significant sequence similarity to tryptophanyl-tRNA synthetase peptide sequences from yeast mitochondria, *E. coli*, and *Bacillus stearothermophilus* (25–27). Fig. 4 shows one possible alignment of these proteins; there is sequence similarity throughout the entire eRF protein and especially between amino acid positions 280 and 375, where 20% of the amino acids are identical in all four proteins. In one particular region between amino acids 352 and 375, almost 70% of the eRF residues are identical with those of one or more of the tryptophanyl-tRNA synthetases. There is also a short region of sequence similarity between the carboxyl terminus of eRF and that of yeast cytosolic methionyl-tRNA synthetase (data not shown).

The eRF region exhibiting the highest degree of similarity to the tryptophanyl-tRNA synthetases (eRF amino acids 353–360) is also the most highly conserved region among the different tRNA synthetases (28). Table 2 compares this region with the corresponding region of a number of tRNA synthetases. Based upon biochemical and crystallographic data (28, 32, 33), this region is thought to contribute to the active site of the synthetases. Crosslinking studies of the methionyl- and tyrosyl-tRNA synthetases place the lysine residue of the consensus Met-Ser-Lys sequence in close proximity to the 3′ acceptor stem of their respective tRNAs (32, 33). The recently determined crystal structure of the glutamyl-tRNA synthetase bound to its cognate tRNA and ATP demonstrates an interaction between the same lysine and ATP, further implicating this region in the catalytic function of the synthetase (34). Since RFs do not have an associated ATPase activity and the lysine in this region of eRF has been replaced with an alanine, it is unknown what role these residues may play in eRF function; however, the high degree of sequence conservation within this region clearly suggests that it is important.

DISCUSSION

We have identified three cDNA clones encoding the rabbit RF. The clones were isolated by screening a λZAP expres-

Table 2. Alignment of eRF residues with the highly conserved putative active-site region of the tRNA synthetases

eRF/tRNA synthetase	Residues	Sequence	Ref.
		* **** ****	
eRF (rabbit)	350–360	AQTKMSASDPN	—
Met (*E. coli*)	329–339	NGAKMSKSRGT	28
Met (yeast cyt.)	522–532	ENGKFSKSRGV	28
Ile (*E. coli*)	599–609	QGRKMSKSIGN	28
Trp (*E. coli*)	192–202	PTKKMSKSDPN	27
Trp (*B. ste.*)	189–199	PTKKMSKSDPN	25
Trp (yeast mit.)	241–251	PEKKMSKSDPN	26
Leu (yeast mit.)	657–667	SYEKMSKSKYN	29
Gly (*E. coli*)	86–96	AEGKPSKAPEG	30
Glu (*E. coli*)	234–244	DGKKLSKRHEA	31
Tyr (*E. coli*)	223–233	TVPLITKADGT	28
Tyr (*B. ste.*)	219–229	TIPLVTKADGT	28
Gln (*E. coli*)	264–274	EYTVMSKRKLN	28
Phe (*E. coli*)	60–70	DKLRVTKVNVG	28
Ala (*E. coli*)	813–823	LIAGVSKDVTD	28

The aligned tRNA synthetases are as follows: methionyl (Met), isoleucyl (Ile), tryptophanyl (Trp), leucyl (Leu), glycyl (Gly), glutamyl (Glu), tyrosyl (Tyr), glutaminyl (Gln), phenylalanyl (Phe), and ananyl (Ala). The yeast mitochondrial (mit.) and cytoplasmic (cyt.) sequences are from *Saccharomyces cerevisiae*. *B. ste.*, *B. stearothermophilus*. Asterisks indicate positions of identical or chemically similar amino acids in at least three synthetases and eRF.

sion library with RF-specific monoclonal antibodies. Functional studies of the protein encoded by this cDNA demonstrated that it has *in vitro* activities characteristic of a mammalian RF. Comparison of the predicted eRF amino acid sequence failed to demonstrate any homology to the *E. coli* RF1 or RF2 proteins but did reveal amino acid sequence similarities to several regions of the tryptophanyl-tRNA synthetases of yeast mitochondria, *E. coli*, and *B. stearothermophilus*. It is of interest that the region of highest homology between eRF and tryptophanyl-tRNA synthetases is also highly conserved in a group of other tRNA synthetases.

What could account for the similarity to the tryptophanyl-tRNA synthetases? Several possibilities can be envisioned. One is that the conserved amino acids provide eRF with a structural conformation required for peptidyl-tRNA binding during peptide chain termination. We speculate that in the absence of an occupied aminoacyl (A) site, eRF binds the peptidyl-tRNA occupying the peptidyl (P) site. Since the eRF "active site" lacks the lysine required for ATP binding, it does not form an ester bond but rather alters the peptidyl-tRNA conformation, thereby allowing the peptidyltransferase center access to the peptidyl-tRNA ester linkage, where water then acts as a nucleophile to hydrolyze the peptidyl-tRNA ester bond.

Another possibility is that the primordial eRF used a tRNA for decoding specificity and hence bound a tRNA similar to $tRNA^{Trp}$. One argument against the use of stop-codon-specific tRNAs is that in *Tetrahymena thermophila* the two independent sense tRNAs specific for UAA and UAG are proposed to have arisen from the existing $tRNA^{Gln}$ by a gene duplication event rather than by the independent evolution of separate tRNAs (35). Alternatively, since a mechanism was required for decoding stop codons, a primitive RF may have "borrowed" features from the primitive UGA-decoder tryptophanyl-tRNA synthetase, leading to the present-day sequence similarities with the evolutionarily more primitive tryptophanyl-tRNA synthetases.

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