

Primary structure of rat liver elongation factor 2 deduced from the cDNA sequence

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A number of cDNA clones in summary encoding 700 amino acid residues from the N-end of rat liver elongation factor 2 (EF-2) and including 49 nucleotides of the 5'-untranslated mRNA region have been obtained. EF-2 cDNA clones were isolated from gradually constructed small (1000-5000 clones) specific cDNA libraries using the primer extension method for synthesis of the first cDNA chain. The complete primary structure of cDNA and protein EF-2 from rat liver was derived taking into account the primary structure of the 3'-terminal region EF-2 cDNA previously reported [(1986) Proc. Natl. Acad. Sci. USA 83, 4978-4982]. Comparison of this cDNA with hamster cDNA has shown that (i) the base sequences had a 89.7% homology while that of the 5'-untranslated region was 73%; (ii) there are two amino acid replacements in rat liver EF-2 as compared with hamster EF-2.

cDNA cloning; Elongation factor 2; Nucleotide sequence; Amino acid replacement; (Rat liver)

1. INTRODUCTION

Elongation factor 2 catalyzes translocation of a newly synthesized peptidyl-tRNA from the acceptor site to the donor site on the ribosome, thus preparing the protein-synthesizing complex for the next step of polypeptide chain elongation. This protein, consisting of a single polypeptide chain of M_r 90 000-100 000, was isolated from different eukaryotic cells. The primary structure of this protein from hamster embryo cells has been determined recently by cloning and sequencing full-sized EF-2 cDNA [1]. In the same study the primary structure of the C-terminal part (mRNA 3'-terminal region) of rat liver EF-2 was determined. Data have also been reported on cDNA cloning and determination of the primary structure of the C-terminal part of human EF-2 [2]. We have previously reported the

cloning of cDNA coding for the central part of rat liver EF-2 [3]. In this study we have cloned and sequenced DNA complementary to the 5'-terminal region of EF-2 mRNA, and summarize the results on cloning and the primary structure of rat liver EF-2 cDNA. Thus, we have derived the complete structure of cDNA and protein EF-2 taking into account published data on the 3'-terminal region of rat liver EF-2 cDNA [1] and have compared it with the full-sized cDNA and protein EF-2 from hamster [1]. We also discuss the use of specific libraries for rapid cloning and determination of the complete primary structure of mRNA.

2. MATERIALS AND METHODS

2.1. Materials

We used dNTP (Pharmacia), restriction endonucleases, the 5'-end-labelling kit (Boehringer-Mannheim), [3 H]dCTP (32 Ci/mmol), RNasin, *E. coli* DNA polymerase I, ribonuclease H, T₄ DNA polymerase, T₄ DNA ligase, M13 cloning kit, M13 sequencing kit, terminal deoxynucleotidyltransferase (Amersham), and avian myeloblastosis virus reverse transcriptase (Vostok, USSR).

2.2. Synthesis of oligonucleotides

Synthesis was performed on DNA synthesizers: Gene

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The nucleotide sequence presented here has been submitted to the EMBL/GenBank database under the accession no. Y07504

Assembler (Pharmacia; oligo II, III, VI, VII) and System I (Beckman; oligo I, IV, V). The following oligonucleotides were synthesized:

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oligo I   5' TGGATGGCATCAGCATGCAGGGGTCTC 3'
oligo II  5' TCCTTGATCTCATTCAG             3'
oligo III 5' GATCATCTGTAGCAGGGCGTC          3'
oligo IV  5' CCCTCCTTGTCTTGTCC             3'
oligo V   5' ACPTACATQTCNGCPAAQTG          3'
oligo VI  5' TCCATQTTPTTCATCAT             3'
oligo VII 5' TTQTTTCATCATPTCQTC            3'

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where P = G or A, Q = C or T, N = A,T,G,C.

2.3. Synthesis and cloning of specific cDNA

Rat liver RNA was isolated by phenol deproteinization [4]. The poly(A)⁺ mRNA fraction was obtained according to [5]. First-strand synthesis was carried out in 50 µl with 10 µg poly(A)⁺ mRNA as described in [6] except that 140 mM KCl was used and specific oligonucleotides (oligo I, III, V, VI or VII) were chosen as primers instead of oligo(dT). Fast annealing of the primer with the template was carried out as in [7]. Second-strand synthesis was carried out according to [6] and mediated by T₄ DNA polymerase [8]. After the reaction, the enzyme was inactivated by heating to 70°C for 10 min and the double-stranded cDNA (ds cDNA) was precipitated twice with spermine [9] to remove protein, dNTP and short (<100 bp) cDNA fragments. ds cDNA was inserted either into plasmid pBR322 at the *Pst*I site by the homopolymer tailing method [8] (hybrid plasmids series pEN) or into plasmid pUC8 at the *Sma*I site by blunt-end ligation (pUC series of hybrid plasmids). Ligation was carried out in 10 µl with 2.5 units T₄ DNA ligase, 50 ng dephosphorylated vector and 3 ng ds cDNA [8]. After transformation of competent *E. coli* MC 1061 cells, we obtained in both cases about 1000–2000 clones per ng cDNA. The background of non-hybrid clones was 10–15%.

2.4. Isolation of EF-2 cDNA clones

EF-2 cDNA clones were isolated from the screened libraries by hybridization 541 filters with 5'-³²P-labelled oligonucleotides II, IV or VI according to [10] at the appropriate temperature [11]. Hybridization with the *Pst*I-*Eco*RI cDNA fragment from plasmid pUC04-20 N1 was performed as in [10].

2.5. cDNA primary structure determination

The cDNA primary structure was determined by the method of Sanger et al. [12] as modified for the ds plasmid [13] using oligonucleotides II, IV as primers or standard direct and reverse M13 primers (for sequencing pUC plasmids) and after recloning of the cDNA restriction fragments in phage M13 using the Amersham M13 cloning kit. Further, the single-stranded DNA was sequenced with the Amersham sequencing kit.

3. RESULTS

We have previously described in detail the procedure for isolation and sequencing of clone pEN241b and clones of the series pUC01-21 [3]. In brief, their cloning was performed as follows. Oligo I and II were designed according to the published primary structure of the 3'-terminal

region of rat liver EF-2 cDNA [1] (fig.1b). Using oligo I as a primer, we synthesized and then cloned the specific cDNA. Of the 1200 clones of this library, four EF-2 cDNA clones were isolated after hybridization with probe oligo II (fig.1c). The pEN241b insert was sequenced as in [12,13]. Then we synthesized oligo III and IV (fig.1c) used as the primer and probe, respectively. 40 clones of EF-2 cDNA (clones series pUC01-21, fig.1d) were isolated from an 8000 clone library prepared in the same way. From an analysis of the primary structure of cDNA from these clones and homologous hamster EF-2 cDNA [1], we concluded that synthesis of the first strand of cDNA was interrupted at approximately the same point in mRNA, immediately before the GC-rich region (bases 750–716 in [1], fig.1a) and, consequently, that there exists a strong stop signal for reverse transcriptase under the given reaction conditions [3].

Oligo V and VI (fig.1b) were synthesized to clone the 5'-terminal region and to test the possible use of a highly degenerate oligonucleotide as an effective specific primer. The 64-fold degenerate 20-nucleotide-long oligo V designed according to the amino acid sequence region (residues 227–233)

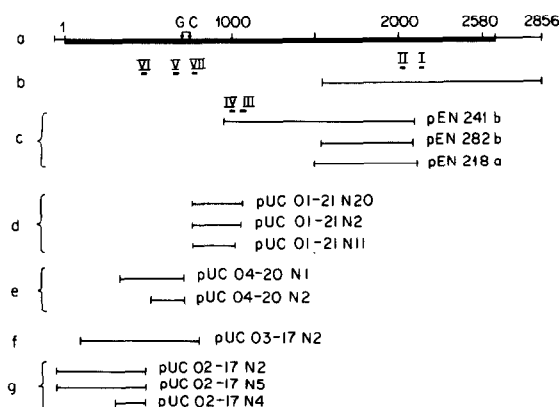


Fig.1. Scheme of cloned EF-2 cDNA. (a) Full-sized EF-2 cDNA from hamster cells (taken from [1]). Bold line shows the coding part, arrows indicate the GC-rich region; (b) rat liver EF-2 cDNA obtained by Kohno et al. [1]; (c) EF-2 cDNA from clones of the pEN series; (d) EF-2 cDNA from clones of the pUC01-21 series. cDNA of three clones of the 40 studied are shown; (e) EF-2 cDNA from the series pUC04-20. cDNA of two clones of the three studied are shown; (f) EF-2 cDNA from clone pUC03-17 N2; (g) EF-2 cDNA from clones series pUC02-17. cDNA of three clones of the four studied are shown. Roman numerals I–VII designate the corresponding oligonucleotides.

of the homologous protein EF-2 from hamster cells [1] was used as a primer. The 4-fold degenerate 17-nucleotide-long oligo VI (synthesized according to the region of amino acid residues 156–161 of hamster EF-2) was used as a probe. After screening the specific library of 4000 clones we isolated one EF-2 cDNA clone (pUC04-20 N1, fig.1e) with a 390 bp insert. Using this insert as a probe we obtained two more EF-2 cDNA clones from the same library with inserts of about 210 bp (fig.1e). All these clones were sequenced by a modified method of Sanger et al. on ds plasmids [13].

Further, the 8-fold degenerate oligo VII synthesized according to amino acid residues 254–259 of hamster EF-2 (synthesized prior to determination of the primary structure of this fragment of rat liver EF-2 cDNA) was used as a primer. Oligo VII is complementary to EF-2 mRNA directly before the stop signal sequence (fig.1b) and we assume that in this case the probability of the enzyme passing this site will be higher. Indeed, screening of the library obtained, comprising 4000 clones, with the labelled *Pst*I-*Eco*RI fragment of plasmid pUC04-20 N1 revealed eight clones of EF-2 cDNA (fig.1f). All contained cDNA inserts of about 750 bp and were sequenced according to Sanger et al. [12].

Oligo VI was also used as a primer, four EF-2 cDNA clones (fig.1g) being isolated from the pUC02-17 library obtained consisting of 8000 clones. One contained a cDNA insert of 200 bp length and the others had inserts of about 530 bp and included a 5'-untranslated region of EF-2 mRNA. These cDNA inserts were sequenced within ds plasmids [13].

4. DISCUSSION

Specific priming of cDNA is sometimes used for cloning of 5'-terminal mRNA regions after partial clones isolated from total cDNA libraries have been obtained and sequenced [14,15]. We believe that this method can be used for rapid cloning of the full-sized cDNA if information on mRNA or the protein in some internal part is available, which is often the case. This method greatly facilitates isolation of DNA clones complementary even to a low-copy template due to enrichment of the cDNA preparation with the appropriate cDNA. Thus, to obtain 1 clone of the rat liver EF-2 cDNA it was

necessary to study 200–1000 clones of the specific library, whereas Kohno et al. [1] employed a total library of 50 000 clones to obtain one clone of the same EF-2 cDNA. This method is very economical due to the small dimensions of libraries (to create a 4000 clone library 1–3 ng cDNA is sufficient). Our results on isolation of clones of the series pUC04-20 indicate that even highly degenerate oligonucleotides can serve as effective primers for synthesis of the specific cDNA to be cloned. The 3'-terminal mRNA region can be cloned in the same way using an oligonucleotide complementary to the non-coding strand as a primer in synthesis of the cDNA second strand after the first strand of the total cDNA has been obtained. In this case, an enriched cDNA preparation (in comparison with the total one) must also result [16].

Since cDNA priming using oligo III located at about 300 bases from the stop signal (fig.1c) yielded no clones containing cDNA beyond the stop signal, we primed cDNA immediately before it (oligo VII, fig.1b). Indeed, it turned out that in this case cDNA synthesis proceeded efficiently enough and extended almost to the 5'-end of mRNA. The reasons for this remain unclear. It may be that in the case of priming with oligo III, the mRNA after denaturation has time to assume a stable secondary structure during synthesis before the stop signal, whereas in priming with oligo VII, the enzyme passes this point before the formation of the stable secondary structure or the already hybridized oligonucleotide itself hinders the formation of such a structure.

Determination of the primary structure of the resulting EF-2 cDNA clones (fig.1) allowed us to represent the complete structure of cDNA coding for rat liver EF-2 (fig.2), taking into account the published primary structure of the 3'-terminal region of rat liver EF-2 cDNA [1], and to deduce the complete primary structure of protein EF-2 (fig.2). Analysis of these structures and those of hamster EF-2 showed that (i) the nucleotide sequences of overlapping parts of rat EF-2 cDNA as reported by Kohno et al. and in this study completely coincide; (ii) the nucleotide sequences of rat and hamster show high homology (89.7%); almost all base replacements are located in the third codon position; (iii) two amino acid replacements have been determined: Glu 441 and Pro 246 in hamster are replaced by Asp and Ala residues in rat, respec-

TCCGCGCAGCGCAGCCATCGTGGGG C C G C T G CCGG T C C T		-49		OGCTTCCCTGTTCAOCTCTGTATTGAGAATCCGACGCGCATCTGCCAACC		-1	
rat							
ATG GTG AAC TTC ACA GTA GAT CAG ATC CGT GCC ATC ATG GAC AAG AAA GCC AAC ATC CCG						60	
Met Val Asn Phe Thr Val Asp Gln Ile Arg Ala Ile Met Asp Lys Lys Ala Asn Ile Arg						20	
AAC ATG TCA GTC ATC GCT CAC GTG GAC CAC GGC AAG TOC ACG TTG ACC GAC TOC CTT GTG						120	
Asn Met Ser Val Ile Ala His Val Asp His Gly Lys Ser Thr Leu Thr Asp Ser Leu Val						40	
TGC AAG GCT GGC ATC ATC GCC TCT GCC CGA GCC GGT GAG ACA CGC TTC ACT GAC ACT CGA						180	
Cys Lys Ala Gly Ile Ile Ala Ser Ala Arg Ala Gly Glu Thr Arg Phe Thr Asp Thr Arg						60	
AAG GAT GAG CAG GAG CGC TGC ATC ACC ATC AAG TOC ACT GCC ATC TOC CTC TTC TAT GAG						240	
Lys Asp Glu Gln Glu Arg Cys Ile Thr Ile Lys Ser Thr Ala Ile Ser Leu Phe Tyr Glu						80	
CTC TOC GAG AAC GAC CTG AAC TTC ATT AAG CAG AGC AAG GAT GGC TCT GGC TTC CTC ATC						300	
Leu Ser Glu Asn Asp Leu Asn Phe Ile Lys Gln Ser Lys Asp Gly Ser Gly Phe Leu Ile						100	
AAC CTC ATT GAC TCT CCA GGT CAT GTG GAC TTC TOC TCA GAG GTG ACC GCT GCT CTG CGT						360	
Asn Leu Ile Asp Ser Pro Gly His Val Asp Phe Ser Ser Glu Val Thr Ala Ala Leu Arg						120	
GTC ACT GAT GGA GCA CTG GTG GTG GTG GAC TGT GTG TCT GGT GTC TGT GTG CAG ACA GAG						420	
Val Thr Asp Gly Ala Leu Val Val Val Asp Cys Val Ser Gly Val Cys Val Gln Thr Glu						140	
ACA GTG CTG CGG CAG GCC ATC GCT GAG CGC ATC AAG CCC GTG CTG ATG ATG AAC AAG ATG						480	
Thr Val Leu Arg Gln Ala Ile Ala Glu Arg Ile Lys Pro Val Leu Met Met Asn Lys Met						160	
GAC CGG GCC CTG CTG GAA CTG CAA CTG GAG CCT GAG GAG CTC TAC CAG ACC TTC CAG CGC						540	
Asp Arg Ala Leu Leu Glu Leu Gln Leu Glu Pro Glu Glu Tyr Phe Gln Thr Arg						180	
ATC GTG GAG AAC GTC AAT GTC ATC ATC TOC ACC TAT GGC GAG GGC GAG AGT GGA CCC ATG						600	
Ile Val Glu Asn Val Asn Val Ile Ile Ser Thr Tyr Gly Glu Gly Ser Gly Pro Met						200	
GGC AAT ATC ATG ATC GAT CCT GTC CTG GGC ACT GTA GGC TTT GGC TCT GGC CTG CAT GGG						660	
Gly Asn Ile Met Ile Asp Pro Val Leu Gly Thr Val Gly Phe Gly Ser Gly Leu His Gly						220	
TGG GGC TTC ACA CTG AAG CAG TTT GCA GAG ATG TAT GTG GGC AAG TTT GCA GGC AAG GGT						720	
Trp Ala Phe Thr Leu Lys Gln Phe Ala Glu Met Tyr Val Ala Lys Phe Ala Ala Lys Gly						240	
GAG GGC CAG CTG GGT GCA GCT GAG CGG GCC AAG AAG GTA GAA GAC ATG ATG AAG AAG CTG						780	
Glu Gly Gln Leu Gly Ala Ala Glu Arg Ala Lys Lys Val GAA Asp Met Met Lys Lys Leu						260	
TGG GGA GAC CGG TAT TTT GAC CGG GCC AAC GGC AAG TTC AGC AAG TCA GGC AAT AGC OCA						840	
Trp Gly Asp Arg Tyr Phe Asp Pro Ala Asn Gly Lys Phe Ser Lys Ser Ala Asn Ser Pro						280	
GAC GGG AAG AAA CTT CCG CGC ACC TTC TGC CAG CTC ATC TTG GAC CCC ATC TTC AAG GTG						900	
Asp Gly Lys Lys Leu Pro Arg Thr Phe Cys Gln Leu Ile Leu Asp Pro Ile Phe Lys Val						300	
TTT GAT GCC ATC ATG AAC TTC AGG AAG GAG GAG ACA GCC AAG CTG ATC GAG AAG CTG GAC						960	
Phe Asp Ala Ile Met Asn Phe Arg Lys Glu Glu Thr Ala Lys Leu Ile Glu Lys Leu Asp						320	
ATC AAG CTG GAC AGT GAG GAC AAG GAC AAG GAG GGC AAA CCA CTG CTG AAG GCT GTG ATG						1020	
Ile Lys Leu Asp Ser Glu Asp Lys Asp Lys Glu Gly Lys Pro Leu Leu Lys Ala Val Met						340	
CGT CGC TGG CTG CCT GCT GGT GAC GCC CTG CTA CAG ATG ATC ACC ATC CAC TTG CCG TOC						1080	
Arg Arg Trp Leu Pro Ala Gly Asp Ala Leu Leu Gln Met Ile Thr Ile His Leu Pro Ser						360	
CCG GTC ACT GCA CAG AAG TAC CGT TGT GAG CTG CTG TAC GAG GGC CCA CCT GAT GAC GAG						1140	
Pro Val Thr Ala Gln Lys Tyr Arg Cys Glu Leu Leu Tyr Glu Gly Pro Pro Asp Asp Glu						380	
GCC GCC ATG GGT ATT AAG AGC TGC GAC CCC AAA GGC CCC CTA ATG ATG TAC ATC TOC AAG						1200	
Ala Ala Met Gly Ile Lys Ser Cys Asp Pro Lys Gly Pro Leu Met Met Tyr Ile Ser Lys						400	
ATG GTG CCA ACA TCT GAC AAA GGC CGC TTC TAT GGC TTC GGT AGA GTG TTC TCT GGG GTG						1260	
Met Val Pro Thr Ser Asp Lys Gly Arg Phe Tyr Ala Phe Gly Arg Val Phe Ser Gly Val						420	
GTG TOC ACA GGT CTG AAG GTC CGG ATC ATG GGC CCC AAC TAT ACA CCT GGG AAG AAG GAG						1320	
Val Ser Thr Gly Leu Lys Val Arg Ile Met Gly Pro Asn Tyr Thr Pro Gly Lys Lys Glu						440	

Fig. 2. Primary structure of rat liver EF-2 cDNA. Bases above the nucleotide sequence of rat EF-2 cDNA show the differences in the base sequence of hamster EF-2 cDNA (according to [1]). **A and A*T denote amino acid replacements in rat EF-2 as compared with hamster EF-2. Dots indicate the nucleotides complementary to the 3'-end of 18 S rRNA. (▼) 5'-end of rat liver EF-2 cDNA from [1].

(▽) 3'-end of cDNA pEN241b. The 3'-untranslated region of rat liver EF-2 cDNA has not been reported in [1].

***A	GAC	CTG	TAC	CTG	AAG	CCT	ATC	CAG	AGG	A	AOC	ATT	CTG	ATG	ATG	GGC	OGC	TAT	GTG	GAA	COO	A	1380
Asp	Leu	Tyr	Leu	Lys	Pro	Ile	Gln	Arg	Thr		Ile	Leu	Met	Met	Gly	Arg	Tyr	Val	Glu	Pro		460	
ATT	GAG	GAT	GTG	COO	TGT	GGC	AAC	ATT	GTG	GGG	TTG	GTC	GGG	GTG	GAC	CAG	TTC	CTT	GTG		1440		
Ile	Glu	Asp	Val	Pro	Cys	Gly	Asn	Ile	Val	Gly	Leu	Val	Gly	Val	Asp	Gln	Phe	Leu	Val		480		
AAG	ACC	GGC	ACC	ATC	ACT	ACC	TTT	GAG	CAC	GCT	CAC	AAC	ATG	CGG	GTG	ATG	AAG	TTC	AGC		1500		
Lys	Thr	Gly	Thr	Ile	Thr	Thr	Phe	Glu	His	Ala	His	Asn	Met	Arg	Val	Met	Lys	Phe	Ser		500		
GTG	AGC	CCT	GTA	GTG	AGG	GTG	GCA	GTG	GAG	GCC	AAG	AAC	CCA	GCT	GAC	CTG	COO	AAG	CTG		1560		
Val	Ser	Pro	Val	Val	Arg	Val	Ala	Val	Glu	Ala	Lys	Asn	Pro	Ala	Asp	Leu	Pro	Lys	Leu		520		
GTG	GAG	GGG	CTG	AAG	OGG	CTG	GCT	AAG	TCT	GAC	OCT	ATG	GTG	CAG	TGC	ATC	ATC	GAG	GAG		1620		
Val	Glu	Gly	Leu	Lys	Arg	Leu	Ala	Lys	Ser	Asp	Pro	Met	Val	Gln	Cys	Ile	Ile	Glu	Glu		540		
TCT	GGG	GAG	CAC	ATC	ATT	GCT	GGT	GCT	GGA	GAG	CTG	CAC	CTG	GAG	ATC	TGC	CTT	AAG	GAC		1680		
Ser	Gly	Glu	His	Ile	Ile	Ala	Gly	Ala	Gly	Glu	Leu	His	Leu	Glu	Ile	Cys	Leu	Lys	Asp		560		
CTG	GAG	GAG	GAC	CAT	GOC	TGC	ATC	CCC	ATC	AAG	AAA	TCT	GAC	OCT	GTG	GTG	TCC	TAC	CGG		1740		
Leu	Glu	Glu	Asp	His	Ala	Cys	Ile	Pro	Ile	Lys	Lys	Ser	Asp	Pro	Val	Val	Ser	Tyr	Arg		580		
GAG	ACA	GTC	AGT	GAG	GAG	TCC	AAC	GTG	CTC	TGT	CTT	TCC	AAG	TCC	CCA	AAC	AAG	CAC	AAC		1800		
Glu	Thr	Val	Ser	Glu	Glu	Ser	Asn	Val	Leu	Cys	Leu	Ser	Lys	Ser	Pro	Asn	Lys	His	Asn		600		
AGG	CTG	TAC	ATG	AAG	GCC	AGG	CCC	TTC	OCT	GAT	GGC	CTA	GCA	GAG	GAC	ATC	GAT	AAG	GGT		1860		
Arg	Leu	Tyr	Met	Lys	Ala	Arg	Pro	Phe	Pro	Asp	Gly	Leu	Ala	Glu	Asp	Ile	Asp	Lys	Gly		620		
GAG	GTA	TCT	GOC	OGC	CAG	GAG	CTC	AAG	GCG	OGT	GOC	OGT	TAC	CTG	GCC	GAA	AAG	TAT	GAG		1920		
Glu	Val	Ser	Ala	Arg	Gln	Glu	Leu	Lys	Ala	Arg	Ala	Arg	Tyr	Leu	Ala	Glu	Lys	Tyr	Glu		640		
TGG	GAT	GTT	GCT	GAA	GOC	OGC	AAG	ATC	TGG	TGC	TTT	GGA	OCT	GAT	GGC	ACT	GGT	CCC	AAC		1980		
Trp	Asp	Val	Ala	Glu	Ala	Arg	Lys	Ile	Trp	Cys	Phe	Gly	Pro	Asp	Gly	Thr	Gly	Pro	Asn		660		
ATT	CTC	ACC	GAC	ATC	ACC	AAG	GGT	GTG	CAG	TAC	CTG	AAT	GAG	ATC	AAG	GAC	AGT	GTG	GTG		2040		
Ile	Leu	Thr	Asp	Ile	Thr	Lys	Gly	Val	Gln	Tyr	Leu	Asn	Glu	Ile	Lys	Asp	Ser	Val	Val		680		
GCT	GGA	TTC	CAG	TGG	GOC	ACT	AAG	GAG	GGT	GCT	CTT	TGT	GAG	GAG	AAC	ATG	OGT	GGT	GTG		2100		
Ala	Gly	Phe	Gln	Trp	Ala	Thr	Lys	Glu	Gly	Ala	Leu	Cys	Glu	Glu	Asn	Met	Arg	Gly	Val		700		
CGA	TTT	GAT	GTC	CAT	GAT	GTG	ACC	CTG	CAT	GCT	GAT	GCC	ATC	CAC	CGA	GGA	GGT	GGT	CAG		2160		
Arg	Phe	Asp	Val	His	Asp	Val	Thr	Leu	His	Ala	Asp	Ala	Ile	His	Arg	Gly	Gly	Gly	Gln		720		
ATC	ATC	CCC	ACA	GGG	OGC	OGC	TGC	CTC	TAT	GCC	AGT	GTG	CTG	ACT	GCA	CAG	CCC	OGC	CTC		2220		
Ile	Ile	Pro	Thr	Ala	Arg	Arg	Cys	Leu	Tyr	Ala	Ser	Val	Leu	Thr	Ala	Gln	Pro	Arg	Leu		740		
ATG	GAG	OCT	ATC	TAC	CTG	GTG	GAG	ATT	CAG	TGT	OCT	GAG	CAA	GTG	GGC	GTG	GGG	ATC	TAT		2280		
Met	Glu	Pro	Ile	Tyr	Leu	Val	Glu	Ile	Gln	Cys	Pro	Glu	Gln	Val	Val	Gly	Gly	Ile	Tyr		760		
GGT	GTG	CTC	AAC	AGG	AAG	OGT	GGC	CAT	GTG	TTT	GAG	GAG	TCC	CAG	GTA	GCT	GGG	ACC	CCC		2340		
Gly	Val	Leu	Asn	Arg	Lys	Arg	Gly	His	Val	Phe	Glu	Glu	Ser	Gln	Val	Ala	Gly	Thr	Pro		780		
ATG	TTC	GTG	GTC	AAG	GOC	TAC	CTG	OCT	GTG	AAT	GAA	TCC	TTT	GGC	TTC	ACT	GCC	GAC	CTT		2400		
Met	Phe	Val	Val	Lys	Ala	Tyr	Leu	Pro	Val	Asn	Glu	Ser	Phe	Gly	Phe	Thr	Ala	Asp	Leu		800		
CGA	TCC	AAC	ACT	GGT	GGC	CAG	GCC	TTC	CCC	CAG	TGT	GTG	TTT	GAC	CAC	TGG	CAG	ATC	CTG		2460		
Arg	Ser	Asn	Thr	Gly	Gly	Gln	Ala	Phe	Pro	Gln	Cys	Val	Phe	Asp	His	Trp	Gln	Ile	Leu		820		
OCT	GGG	GAC	CCC	TTT	GAC	AAC	AGC	AGC	OGC	CCC	AGC	CAA	GTT	GTA	GCT	GAG	ACC	OGC	AAG		2520		
Pro	Gly	Asp	Pro	Phe	Asp	Asn	Ser	Ser	Arg	Pro	Ser	Gln	Val	Val	Ala	Glu	Thr	Arg	Lys		840		
OGC	AAA	GGC	CTG	AAG	GAG	GGC	ATC	CCA	GCG	CTG	GAC	AAC	TTC	CTG	GAC	AAA	CTG	TAG			2577		
Arg	Lys	Gly	Leu	Lys	Glu	Gly	Ile	Pro	Ala	Leu	Asp	Asn	Phe	Leu	Asp	Lys	Leu				858		

tively; (iv) homology in the 5'-untranslated region is considerably lower (73%) due to large differences just at the 5'-end (fig.2). In the 5'-untranslated region of rat liver mRNA, as in hamster EF-2 mRNA, there is a site complemen-

tary to the 3'-end of 18 S rRNA and a eukaryotic consensus (-4) CACC ATG(3) (fig.2).

An interesting feature of pUC02-17 N2, 5 clones was revealed on cloning (fig.1g). It transpired that the growth rate of these clones containing cDNA

from position -49 to position 480 in the sense orientation in site *Sma*I of plasmid pUC8 (but not in-frame with the β -galactosidase gene) is about an order lower than that of clones containing other EF-2 cDNA inserts or without inserts at all (not shown). This cDNA fragment encodes about 160 amino acid residues from the N-end of EF-2, and Kohno et al. [1] refer to this fragment as the GTP-binding region. This effect is being currently studied.

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