Volume 248, number 1,2, 131-136 FEB 07127 May 1989

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

A.V. Oleinikov, G.G. Jokhadze and Yu.B. Alakhov

Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

Received 16 March 1989

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.

Correspondence address: A.V. Oleinikov, Institute of Protein Research, Academy of Sciences of the USSR, 142292 Pushchino, Moscow Region, USSR

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

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, T_4 DNA polymerase, T_4 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

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

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

oligo I	5′ TGGATGGCATCAGCATGCAGGGGTCTC 3′
oligo II	5′ TCCTTGATCTCATTCAG 3′
oligo III	5′ GATCATCTGTAGCAGGGCGTC 3′
oligo IV	5′ CCCTCCTTGTCCTTGTCC 3′
oligo V	5′ ACPTACATQTCNGCPAAQTG 3′
oligo VI	5′ TCCATQTTPTTCATCAT 3′
oligo VII	5′ TTQTTCATCATPTCQTC 3′

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 T_4 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 T_4 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′-^{32}P-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)

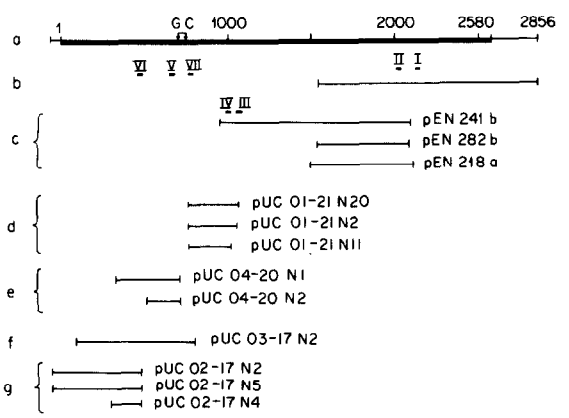


Fig.1. Scheme of cloned EF-2 cDNA. (a) Full-sized EF-2 cDNA from hamster cells (taken from [1]). Bold line shows the coding part, arrows indicate the GC-rich region; (b) rat liver EF-2 cDNA obtained by Kohno et al. [1]; (c) EF-2 cDNA from clones of the pEN series; (d) EF-2 cDNA from clones of the pUC01-21 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 50000 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-

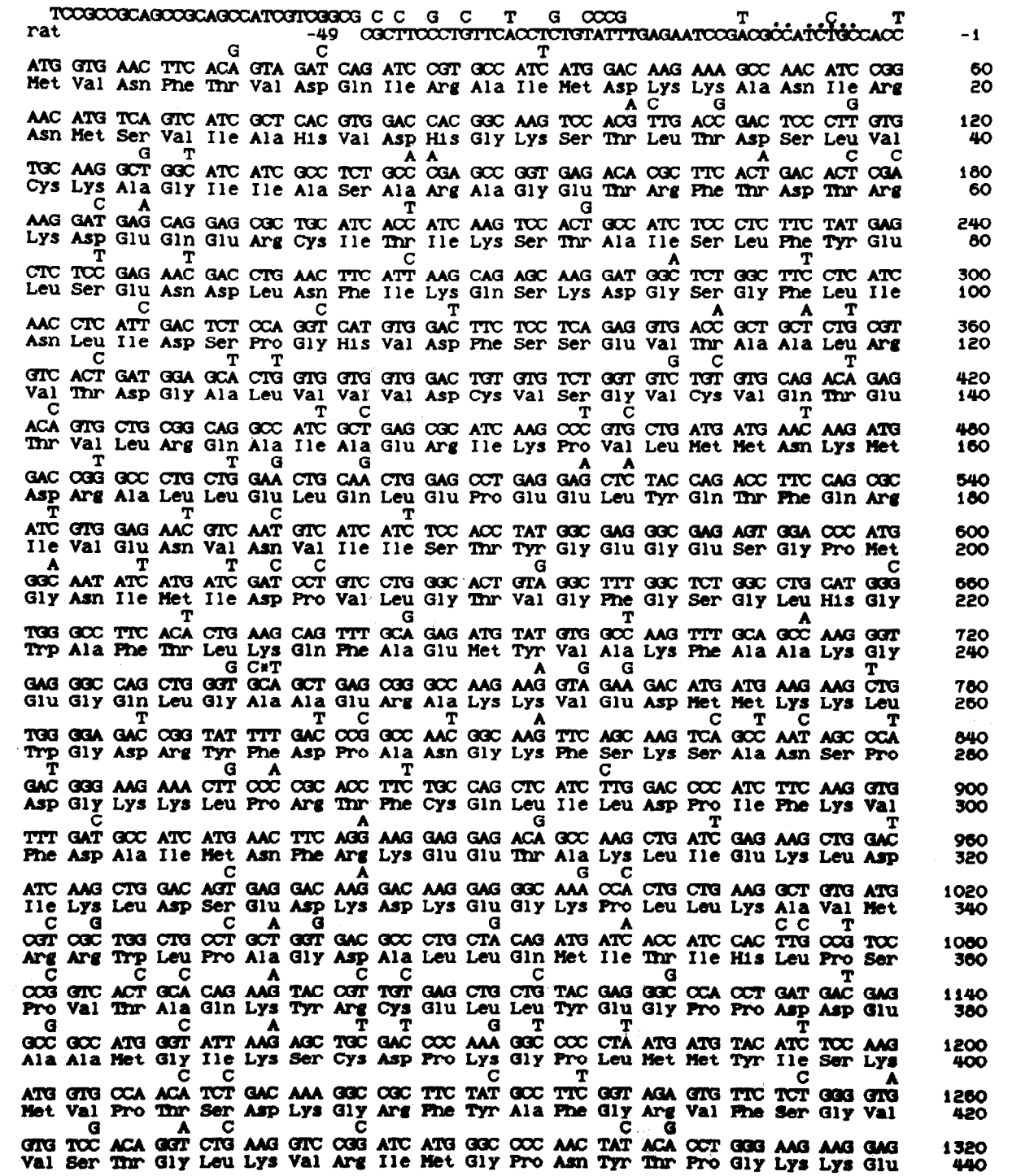
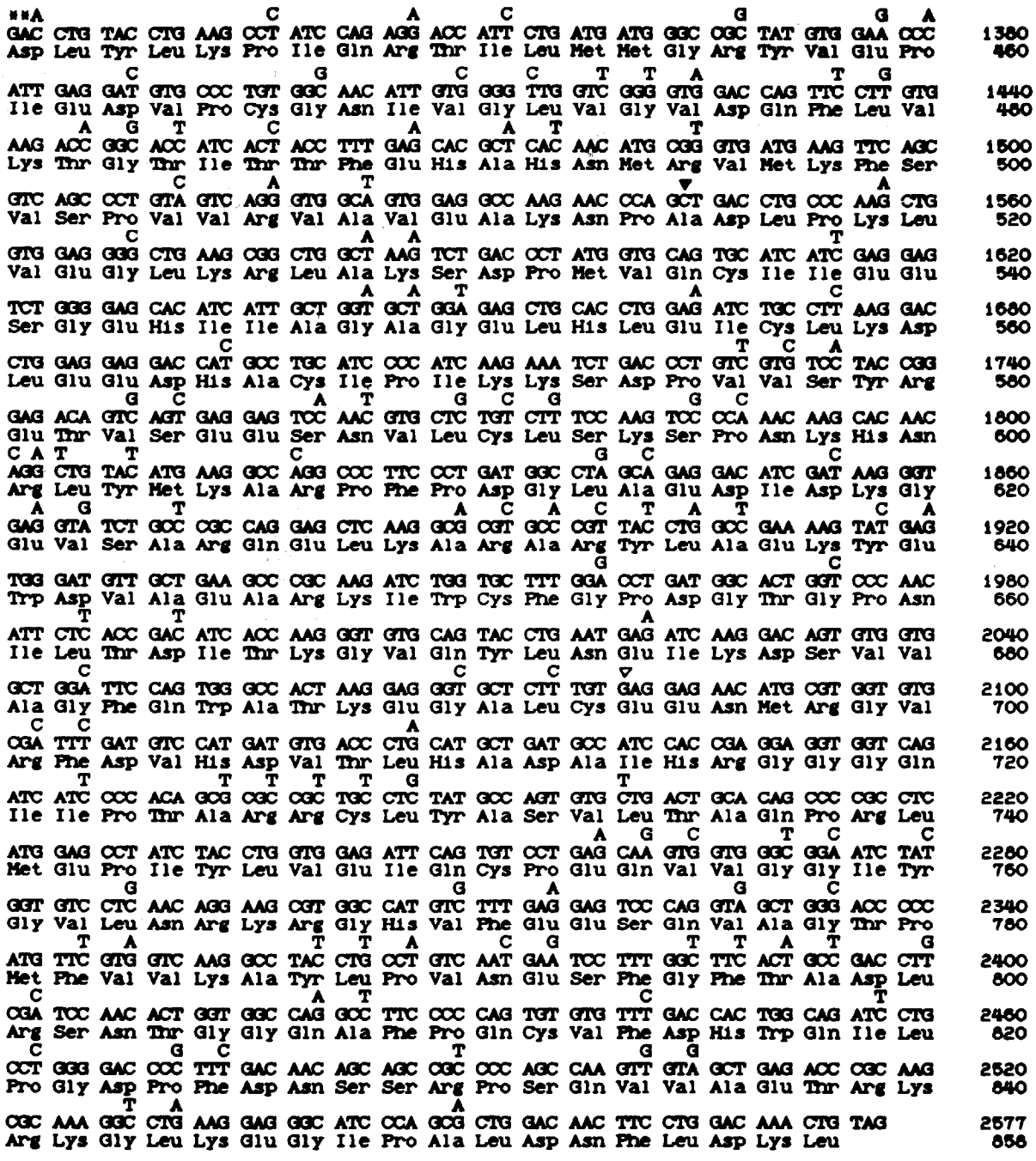


Fig.2. Primary structure of rat liver EF-2 cDNA. Bases above the nucleotide sequence of rat EF-2 cDNA show the differences in the base sequence of hamster EF-2 cDNA (according to [1]). **A and A*T denote amino acid replacements in rat EF-2 as compared with hamster EF-2. Dots indicate the nucleotides 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].



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 complementary 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 *SmaI* 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.

REFERENCES

[1] Kohno, K., Uchida, T., Ohkubo, H., Nakanishi, S., Nakanishi, T., Fukui, T., Ohtsuka, E., Ikehara, M. and Okada, Y. (1986) Proc. Natl. Acad. Sci. USA 83, 4978–4982.

[2] Rapp, G., Mucha, I., Einspanier, R., Luck, M. and Scheit (1988) Biol. Chem. Hoppe-Seyler 369, 247–250.

[3] Oleinikov, A.V., Jokhadze, G.G. and Alakhov, Yu.B. (1989) Bioorg. Khim., in press.

[4] Shore, G.C. and Tata, J.R. (1977) J. Cell Biol. 72, 726–735.

[5] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 69, 1408–1414.

[6] Gubler, U. and Hoffman, B.J. (1983) Gene 25, 263–269.

[7] Merrit, S.C., Tschudi, C., Konigsberg, W.H. and Richards, F.F. (1983) Proc. Natl. Acad. Sci. USA 11, 1536–1540.

[8] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

[9] Hoopes, B.C. and McClure, W.R. (1981) Nucleic Acids Res. 9, 5493–5404.

[10] Gergen, J.P., Stern, R.H. and Wensink, P.C. (1979) Nucleic Acids Res. 7, 2115–2136.

[11] Meinhoth, J. and Wahl, G. (1984) Anal. Biochem. 138, 267–284.

[12] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463–5467.

[13] Hattori, M. and Sakaki, Y. (1986) Anal. Biochem. 152, 232–238.

[14] Noda, M., Furutani, Y., Takahashi, H., Toyosato, M., Hirose, T., Fnayama, S., Nakanishi, S. and Numa, S. (1982) Nature 295, 202–206.

[15] Nishimura, Y., Rosenfeld, M.G., Kreibich, G., Gubler, U., Sabatini, D.D., Adesnik, M. and Andy, R. (1986) Proc. Natl. Acad. Sci. USA 83, 7292–7296.

[16] Houghton, M., Stewart, A.G., Doel, S.M., Emtage, J.S., Eaton, M.A.W., Smith, J.C., Patel, T.P., Lewis, H.M., Porter, A.G., Birch, J.R., Cartwright, T. and Carey, N.H. (1980) Nucleic Acids Res. 8, 1913–1931.