Vol. 197, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1993 Pages 154-162

HUMAN ELONGATION FACTOR EF-1β: CLONING AND CHARACTERIZATION OF THE EF1β5a GENE AND ASSIGNMENT OF EF-1β ISOFORMS TO CHROMOSOMES 2,5,15 AND X

Antonio Pizzuti[1§], Massimo Gennarelli[2], Giuseppe Novelli[3*],
Alessia Colosimo[2], Stefania Lo Cicero[2],
C.Thomas Caskey[1], and Bruno Dallapiccola[2,4]

[1]Institute for Molecular Genetics and Howard Hughes Medical Institute,
Baylor College of Medicine, Houston, Texas 77030

[2]Department of Public Health and Cell
Biology, Tor Vergata University of Rome, Rome, Italy

[3]Chair of Human Genetics, Catholic University
of Rome, Rome, Italy

[4]CSS Hospital IRCCS, San Giovanni Rotondo, Italy

Received October 4, 1993

Summary: We report here the isolation and characterization of a novel human elongation factor-1 β (EF-1β) gene by cDNA selection from YAC mapping on chromosome 5q12-q14. This gene is specifically transcribed in fetal brain and in skeletal muscle and is characterized by a complete sequence homology with previously described EF-1β cDNAs. We also assigned the loci for three other EF-1β isoforms, to human chromosomes 2, 15 and X. The multiple chromosomal assignments of EF-1β loci demontrates the genetic heterogeneity of human EF-1β peptides.

Introduction: Elongation factor-1(EF-1) is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome (1). EF-1 is a multimer of three different peptides, EF-1α (MIM*130590), EF-1β (MIM*130591), and EF-1γ (MIM*130593) (1). The α subunit of EF-1 corresponds to the nucleotide binding domain, and it is equivalent to the bacterial EF-Tu (1). The nucleotide exchange complex,functionally related to the procaryotic EF-Ts

§Present address: Institute of Neurology, University of Milan, Italy.

*To whom correspondence should be addressed at: C.R.I., Laboratorio Centrale, Via Ramazzini 15, 00151 ROMA, Italy. FAX: + 39/6/65741183 or 65741213.

0006-291X/93 $4.00

factor, is composed by the γ/β dimer (1). The EF-1α gene is present in multiple copies in the human genome, with different chromosomal localizations (2). No chromosomal assignment has been reported for the EF-1β coding subunit. Two highly homologous cDNAs coding for this subunit, and differing only in the 5' untranslated region have been cloned (3,4). Although sequences difference may be attributed to an alternatively spliced 5' exon from a unique gene, Southern blot experiments using EF-1β cDNA as a probe, suggest the presence of multiple homologous sequences in the genome (3). We report the isolation of a new EF-1β gene, highly homologous to the known EF-1β cDNAs, by cDNA selection on a human chromosome 5 YAC clone. RT-PCR experiments indicate that the new gene (EF-1β5a) transcribes a specific cDNA with some tissue specificity. We demonstrate that in addition to EF-1β5a, at least three other EF-1β genes are present in the human genome on chromosomes 2 ,15 and X.

Materials and Methods

cDNA direct selection from total YAC DNA: Whole YAC DNA (100 ng) was separated from the yeast chromosomes by pulsed field gel electrophoresis (PFGE) and digested to completation with MboI. The YAC DNA fragments were ligated to adapter A (Table 1) and amplified by PCR using a biotin-conjugated-oligonucleotide complementary to the adapter A sequence as a polymerization primer. Poly-A+ RNA from 5 different human tissues (brain, skeletal and cardiac muscle, placenta, liver) was isolated and ds-cDNA synthesized. The cDNAs were ligated to adapter B (Table 1) and amplified by PCR using an oligonucleotide complementary to the adapter B sequence as a polymerization primer. cDNA (500 ng) and YAC fragments (30 ng) were denatured and hybridized for 24 hours at 65°C. The hybridization was carried out in 5 X SSPE, 5 X Denhard, 0.1% SDS. 10 μg of human genomic DNA and 5 μg of yeast DNA were used to compete for repetitive sequences. The hybridization mix was completed by adding of 50 μl of streptavidin-conjugated magnetic beads (DYNABEADS, Dynal,Inc., USA). The beads-biotinilated YAC fragments-cDNAs complexes were separated magnetically. After washing in 0.5 X SSC at 65°C for 15 min, the DNA was recovered by boiling (5 min at 100°C). The released cDNAs were amplified by PCR using the oligonucleotide complementary to the adapter B sequence as a polymerization primer. PCR products (500 ng) were used for a new selection step. The resulting cDNAs were digested using EcoRI restriction enzyme and cloned into EcoRI digested pBluescript (Stratagene,Inc. USA).

Polymerase chain reaction (PCR) and chromosomal mapping panel: Oligonucleotide primers complementary to published sequences of EF-1 β (3,4) were synthesized and used to prime PCR reactions. EF-1β5a specific amplimers were prepared from the nucleotide sequence (Fig.1). The complete set of oligonucleotide primers used in our experiments are reported in Table 1 and Figure 1. PCR was performed according to Saiki et al.(5) in 50 μl of a mixture containing 50 ng genomic DNA, 20 pmol each primer, 20 mM Tris-HCl pH 8.7, 200 μM each dNTP, 50 mM NaCl, 1.5 mM $MgCl_2$, and 2 units Taq DNA polymerase (Perkin-Elmer-Cetus, USA). The cycling conditions were 1 min of denaturation at 94 °C, 50

Table 1: Oligonucleotides and primers used for cDNA selection and PCR experiments

Primer code	Position	Primer sequences
EF5A1A	8→30	5' -TCGGAGACCTGAAAAGGTCCCGCG- 3'
EFZ2A	675→654	5' -GATCTTGTTGAAAGCAGCCACA- 3'
EF684	268→290	5' -GTGGAGCTACAGATAGTAAAGAT- 3'
EF274	699→675	5' -TGCCATGATCCAGGATGGATTTTAG-3'
EFNEW1A	-188→-167	5' -GAGAAGTGGGAATGCAAAAGTA- 3'
EFNEW3	28→8	5' -CAGGGCTTTTCAGGTCTCCGA- 3'
EF273	-50→-27	5' -CTGGTTCCCGTCATCTTCGGGAGC- 3'
EF685	115→92	5' -CAAATACTGCCACATCTGCTTGTG- 3'
Adapter A	-	5' CTGGCTACGAATTCGAGCTCAGATG 3'
		5' GATCCATCTGAGCTCGAATTCGTCGACCAG 3'
Adapter B	-	5' GGCCGCAAGCATGCGAATTCAGGATCC 3'
		5' GGATCCTGAATTCGCATGCTTGCGGCC 3'

s of annealing at 57°C and 1 min of extension at 72°C. The cycle were repeated 30 times (Thermocycler, Perkin-Elmer-Cetus, USA) followed by 8 min of a final extension at 72°C. Amplification products were analysed on ethidium bromide stained agarose gels (2 per cent w/v). Amplified DNA was purified from low melting point agarose (FMC BioProducts, USA), reamplified by asymmetric PCR (6) and sequenced using a Sequenase kit (US Biochemical Corp., USA). Sequencing products were analysed on 8M urea-8 percent polyacrylamide gels. In some cases, PCR products were transferred to Hybond-N filters (Amersham) and subjected to Southern blot analysis. The BIOS Somatic Cell Hybrid PCRable DNAs (New Haven, USA) was used for chromosome localization of specific PCR amplified fragments.

cDNA synthesis and expression studies: Multiple tissue poly(A)$^+$ RNAs were purchased from Clontech Laboratories, Inc. (USA). Reverse transcription of mRNAs into single-stranded cDNA was essentially carried out according to the method of Kawasaki (7) and by using the cDNA synthesis kit supplied by Clontech Laboratories, Inc. (USA). The reaction was performed with avian myeloblastosis virus reverse transcriptase, oligo $(dT)_{15}$ primers, RNasin (Amersham International, U.K.) and 0.5 μg of mRNAs in a final volume of 25 μl. Aliquots of 10 μl were used in the PCR experiments. Human β-actin (Clontech Laboratories, Inc., USA) was used as an internal standard.

Fluorescent *in situ* hybridization: Metaphase chromosomes were prepared from human peripheral blood lymphocytes as described (8). Purified EF-1β5a cosmid DNA was labelled with biotinylated-dCTP and dUTP by nick translation and hybridized to chromosome spreads according to routine procedures (8). Chromosomes were counterstained with propidium iodide and visualized as described (8).

Results

Isolation and characterization of the EF-1β5a gene: The CEPH human genomic YAC library was screened by PCR for the presence of the marker D5S125 $(CA)_n$ dinucleotide repeat (9). This marker, is tighly linked to the spinal muscular atrophy (SMA, MIM* 253300) locus on human chromosome 5 (9). A single YAC, 311H5 was isolated. This YAC, is about 350 Kb long, and was used for cDNA selection. 100 selected cDNA clones were analysed for the presence of transcripts mapping in the YAC. Fourteen clones were isolated, specifically hybridizing to the YAC DNA. They were derived from 5 different single copy genes. Five cDNAs (average length 250 bp) showed complete homology to the 5' portion of the human EF-1β cDNA described by von der Kammer et (3). In order to isolate the complete EF-1β gene, a total yeast DNA cosmid library (supercos-Stratagene, USA) from clone 311H5 was prepared. The library was screened using the EF-1β cDNAs isolated from cDNA selection. A 35 Kb cosmid clone (BcELF-1) was isolated and a 4.4Kb BglII fragment, containing most of the EF-1β gene was subcloned in pBluescript (Stratagene, Inc. USA) and sequenced using oligonucleotides designed based on known sequences of EF-1β cDNAs. As shown in Fig.1, the coding sequence of the EF-1β5 gene is highly homologous (96%), but not identical, to the known EF-1β cDNAs. Regions of high homology include the whole coding region, the 3' untranslated, and the last 83 bp of the 5' untranslated region. The most striking difference is absence of a TT sequence at base 559 after the starting ATG that creates a STOP codon thereafter, truncating 31 codons from the translated sequence. EF-1β5a is colinear to the cDNAs in the whole 3' untranslated and coding regions indicating absence of introns after the starting ATG. The two known cDNAs are completely identical to each other up to bp -22 from the starting ATG, where their sequences diverge. The EF-1β5a sequence follows the structure of one of these cDNAs in the 5' untranslated up to bp -83, where the two sequences also diverge (Fig.1). The region at the diverging point conforms to an exon-intron junction sequence, suggesting the presence of an intron at 5' to the divergent point. This assumption is confirmed by RT-PCR experiments using oligonucleotides designed on the "intronic" and "exonic" side of the EF-1β5a sequence. "Exonic" primers coupled to EF-1β cDNA internal oligonucleotide give products of the expected length, while experiments using "intronic" oligonucleotides are always negative (data not shown). In order to look for the presence of non-homologous sequences belonging to the very 5' end of the known cDNAs in the EF-1β5a gene, oligonucleotides were designed on their sequences and used for hybridizing the YAC and the cosmid BcELF-1 DNAs. No signal was detected. The presence of specific transcripts of EF-1β5a, was investigated by designing a specific oligonucleotide on its sequence (EF5a1A). EFZ2A was used as co-amplimer for detecting by cDNA-PCR a fragment of 668 bp in fetal brain and muscle. A barely visible signal was also detected in placenta and no products were observed when liver cDNA was analysed (Fig.2).

Chromosome confirmation of the EF-1β5a and assignment of the other EF-1β genes: Two oligomers (EF684 and EF274) were designed on the EF-1β cDNA sequence in order to amplify a very short region of 430 bp in the coding region (Table 2).They were used as PCR primer on a hamster-human DNA panel obtained from BIOS Laboratories, Inc, (USA). The

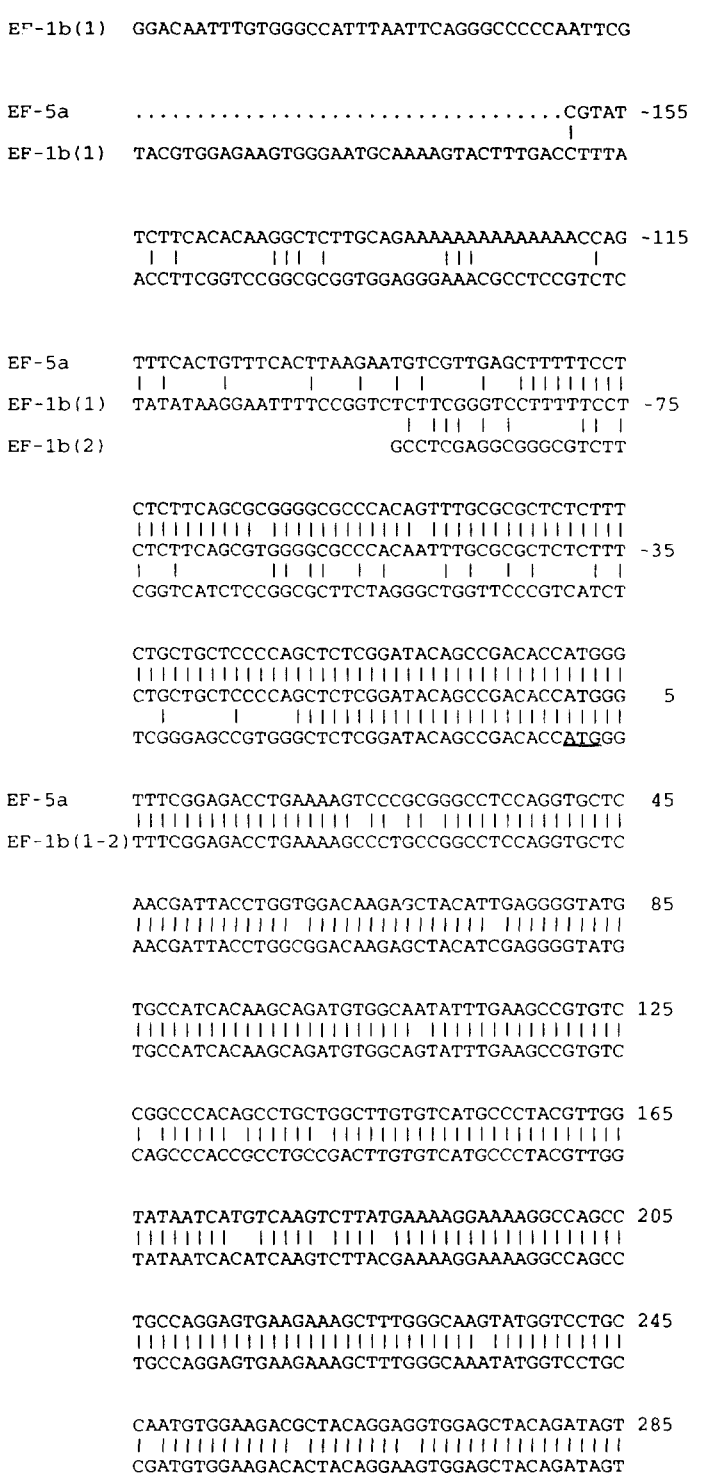

Fig. 1. Sequence comparison between EF-1β5a and the EF-1β cDNA described by von der Kammer et al. (3) and Sanders et al. (4). After the start codon ATG the sequence of EF-1 (3) and EF-1β (4) are identical and are indicated as EF-1β (1-2). The STOP codons (TAA for EF-1β (1-2) and TGA for EF-1β5a are also underlined.

```
AAAGATGATGATGACATTGACCTCTTTGGATCTGATGATG 325
AAAGATGATGATGACATTGACCTCTTTGGATCTGATGATG

AGGAGGAAAGTGAAGAAGCAAAGAGGCTAAGGGAAGAAAG 365
AGGAGGAAAGTGAAGAAGCAAAGAGGCTAAGGGAAGAACG

TCTTGCACAATATGAATCAAACTTAGCCAAAAACCCTGCA 405
TCTTGCACAATATGAATCAAAGAAAGCCAAAAAACCTGCA

CTTGTTGCCAAGTCTTCCATCTTACTAAATGTGAAACCTT 445
CTTGTTGCCAAGTCTTCCATCTTACTAGATGTGAAACCTT

GGGATAAGGAGGCAGATCTGGCGAAATTAGAGGAGTGCGT 485
GGGATGATGAGGCAGATATGGCGAAATTAGAGGAGTGCGT

CAGAAGCATTCAAGCAGACGGCTTAGTCTGGGGCTCATCT 525
CAGAAGCATTCAAGCAGACGGCTTAGTCTGGGGCTCATCT

AAACTAGTTCCAGTGGGATACAGAATTAAGAAAC--CAAA 565
AAACTAGTTCCAGTGGGATACGGAATTAAGAAACTTCAAA

TACAGTGTGTAGTTGAAGATGATAAAGTTGGAACAGATAT 605
TACAGTGTGTAGTTGAAGATGATAAAGTTGGAACAGATAT

TCTGGAGGAGCAGATCACTGCTTTTGAGGACTATGTGCAG 645
GCTGGAGGAGCAGATCACTGCTTTTGAGGACTATGTGCAG

TCCATGGATGTGGCTGCTTTCAACAAGATCTAAAATCCAT 685
TCCATGGATGTGGCTGCTTTCAACAAGATCTAAAATCCAT

CCTGGATCATGGC 698
CCTGGATCATGGC
```

Fig. 1 - Continued

fragment was amplified from hybrid containing human chromosomes 2,5,15 and X, indicating the presence of EF-1β sequences in these chromosomes (Table 2). Specific oligomers for the EF1β5a sequence (EF5A1A - EFZ2A) and for the EF-1β cDNA isolated by Sanders et al. (4) (EFNEW1A - EFNEW3) were then synthesized for the amplification of these EF-1β related genes. These amplimers specifically showed PCR products in hybrids containing respectively chromosomes 5 and 2 (Table 2). Similarly, the von Kammer cDNA (3) was mapped to chromosome 15 using the oligomers EF273 - E F685. EF-1β5a gene was sublocalized on chromosome 5q12-q14 by FISH using BcELF-1 cosmid as a probe (Table 2).

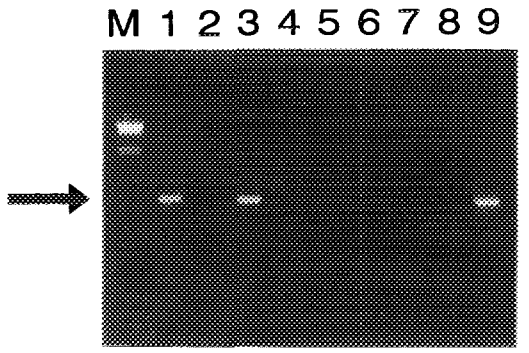

Fig.2. Detection of EF-1β5a transcript. Ethidium bromide visualization of DNA fragment amplified after RT-PCR from skeletal muscle (lane 1), fetal brain (lane 3), human placenta (lane 5), liver (lane 7); lanes 2, 4, 6 and 8 are reverse transcriptase free negative controls; lane 9 genomic DNA positive control.

Discussion

We have reported the isolation and characterization of a novel human EF-1β gene by direct cDNA selection using a YAC. This clone, designed as EF-1β5a, maps on chromosome 5 and shows high homology with the EF-1β cDNAs isolated by von der Kramer et al.(3) and Sanders et al.(4). However, many divergences in nucleotide sequences are found at the 5' region, suggesting that this isoform is the product of a different gene.Using selective PCR for the differential region of each of the EF-1β isoforms, the three genes were assigned to chromosomes 2, 5, and 15. Furthermore, an additional homologous sequence was scored in somatic cell hybrids containing the sole human X chromosome when the PCR was performed using the 3' common coding region of EF-1βgene. These results demonstrate genetic heterogeneity of the human EF-1β gene, similarly to the EF-1α gene which is encoded by a multigene family, distributed on chromosomes 1,2,4,5,6,7, and 15 (2). The existence of multiple copies of the EF-1 genes could reflect a functional role of their products. In fact, eukaryotic protein elongation factors, are involved in a wide-range of cellular functions including cell division, differentiation, ageing and transformation (1,10,11,12). Using sequence comparative analysis and PCR-mapping experiments between

Table 2: Chromosome assignment of EF1-β cDNAs

EF-1β cDNA	Reference	Chromosome	Method
EF-1β5a	Present study	5q12-q14	PCR - FISH
EF-1β	von der Krammer et al.(3)	15	PCR
EF-1β	Sanders et al. (4)	2	PCR
EF-1β 3' common coding region	Present study; von der Krammer et al (3); Sanders et al. (4)	2, 5, 15, X	PCR

the isoforms of the EF-1β genes, we found that the 3' coding region is common to all copies of genes as well as to possibly processed pseudogens present in the eukaryote genomes. This is confirmed by zoo-PCR analysis using DNA from different vertebrates (data not shown) which revealed specific EF-1β 3' region amplification products in chicken, mouse, rabbit, pig, chimpanzee and old world monkeys. However, only in these two latter species PCR experiments were positive when the EF-1β 5' region was amplified. These data suggested that the 5' coding region of EF-1β genes is distinctive of each isoform and provide important insights into the evolutionary relationship between the EF-1β peptides. We suggest that the 5' untranslated region contains regulatory elements for EF-1β expression. In fact, we have recognized intron-sequences only at 5' region of the EF1-β5a gene and demonstrated a restricted pattern of expression of this isoform using 5' region-derived oligomers. RT-PCR experiments suggest that the EF-1β isoforms are tissue-specific, with a precise but still unknown function. Complete genomic characterization of all the EF-1β genes could elucidate in future the origin of this new multigene family. The chromosomal localization and the selective expression of the EF-1β5a mRNA in brain and muscle, the major target sites of neurodegenerative diseases, indicate that EF-1β5a couls be a candidate gene for the SMA locus. The biochemical defect of this disorder is unknown, but linkage studies have mapped the three forms of SMA to chromosme 5q12 - q13.1 (9,13), a location concordant with the EF-1β5a locus.

Acknowledgments

We thank A. Baldini for FISH experiments, M. Magnani and J. Reichardt for review of this manuscript and useful discussions. This research was supported by the Italian Telethon, MURST (fondi 60%) and ASM, Milan. S.L.C. is a fellow of A.I.R.H. (Italy).

References

1. Riis, B., Rattan, S.I.S., Clark, B.F.C. and Merrick, W.C. (1990) Trends Biochem. Sci. (review)15, 420-424.
2. Opdenakker, G., Cabeza-Arvelaiz, Y., Fiten, P., Dijkmans, R., Van Damme, J., Volckaert, G., Billiau, A., Van Elsen, A., Van der Schueren, B., Vanden Berghe, H. and Cassiman, J.J. (1987) Hum. Genet. 75, 339-344.
3. von der Kammer, H., Klaudiny, J., Zimmer, M. and Scheit, K.H. (1991) Biochem. Biophys. Res. Commun. 177, 312-317.
4. Sanders, J., Maassen, I.A., Amons, R. and Möller,W. (1991) Nucleic Acids Res. 19, 4551.
5. Mullis, K.B. and Faloona, F.A. (1987) Methods Enzymol. 155, 335-350.

6. Gyllensten, U.B. and Erlich, H.A. (1988) Proc. Natl. Acad. Sci. U.S.A.. 85, 7652-7656.

7. Kawasaki, E.S. (1991) In: PCR Protocols, a guide to Methods and Applications, pp 21-27, Innis et al., eds., Academic Press, Inc., San Diego, CA.

8. Baldini, A., and Word, D.C. (1991) Genomics, 9, 770-774.

9. Daniels, R.,J., Suthers,G.K., Morrison, K.E., Thomas, N.H., Francis, M.G., Mathew, C.G., Loughlin, S., Heiberg, H.E., Wood, D., Dubowitz, V.and Davies, K.E. (1992) J. Med. Genet. 29, 165-170.

10. Hiraga, K,. Suzuki, K., Tsuchiya, E. and Miyakawa, T. (1993) FEBS Lett. 316, 165-169.

11. Sonnemann, J., Bauerle, A., Winchler, T. and Mutzel, R. (1991) J. Biol. Chem. 266, 23091-23096.

12. Tatsuka, M., Mitsui, H., Wada, A., Nojima, H. and Okayama, H. (1992) Nature. 359, 333.

13. Melki, J., Abdelhak, S., Sheth, P., Bachelot, M.F., Burlet, P., Marcadet, A., Aicardi, J., Barois, A., Carriere, J.P., Fardeau, M., Fontan, D., Ponsot, G., Billette, T., Angelini, C., Barbosa, C., Ferriere, G., Lanzi, G., Ottolini, A., Babron, M.C., Cohen, D., Hanauer, A., Clerget-Darpoux, F., Lathrop, M. and Munnich, A. (1990) Nature. 344, 767-768.