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HUMAN ELONGATION FACTOR 1β: cDNA AND DERIVED AMINO ACID SEQUENCE

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Summary: From a cDNA library in λgt11 derived from poly (A+)RNA of human ovarian granulosa cells a cDNA clone λHGP34, containing an EcoRI insert of 829 bp, was identified. After subcloning of the insert into pUC18, the clone pHGP34 was obtained and sequenced. The derived amino acid sequence, corresponding to a protein of 225 amino acids, shows a high degree of homology to elongation factor 1β (EF-1β) of *Artemia salina* (57%) and known peptide sequences of *Xenopus laevis* EF-1β (86%). We therefore assume that the protein coded for by pHGP34 represents human EF-1β. Northern analysis reveals an EF-1β specific mRNA of 900 bp. Southern analysis indicates that EF-1β in the human genome, like EF-1α, appears to be specified by more than one gene. A high degree of sequence homology for EF-1β specific sequences is observed for bovine, rat and mouse species.

Elongation factor 1 (EF-1) is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. EF-1 is a major protein of eukaryotic cells and exists as a heterotrimer consisting of the subunits EF-1α, EF-1β and EF-1γ (1). Recently, the existence of EF-1δ, a homologous form of EF-1β, was demonstrated. Hence, two species of EF-1 with composition EF-1αβγ or EF-1αδγ can be formed (1). EF-1 comprises two different functional domains: a nucleotide binding domain provided by EF-1α and a nucleotide exchange protein complex EF-1βγ (2). A large number of amino acid sequences for EF-1α of various species are known (2, 3). The high degree of homology observed is interpreted as a conservation of active sites as well as protein topological loci that interact with tRNAs and mRNAs. Much less is known about EF-1β; the amino acid sequence for *Artemia salina* by cDNA cloning (4) and limited sequence information by peptide sequencing in the case of *Xenopus laevis* (5) have been described. We report here the characterization of human EF-1β mRNA by cDNA cloning and provide the first amino acid sequence of EF-1β for a mammalian species.

Material and Methods

The human granulosa cells were obtained from follicles of women after ovarian superstimulation by clomiphene; the cells were purified by Percoll gradient centrifugation.

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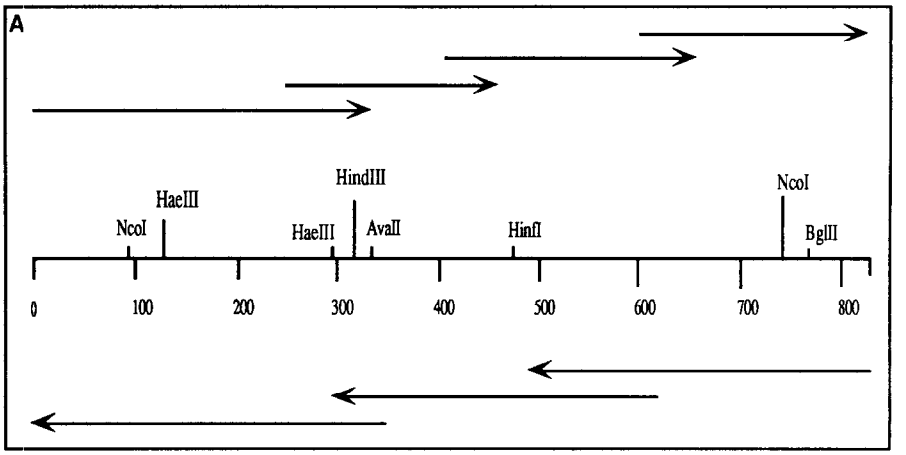
Total RNA and poly (A+)RNA from human granulosa cells were prepared as described (6, 7). cDNA synthesis from poly (A+)RNA followed a modified protocol of Gubler and Hoffmann (8), using a commercial kit (BRL). A cDNA library in λgt11 was prepared employing the λgt11 cloning kit (Amersham). IgGs isolated from an antiserum raised in rabbits against a glycoprotein fraction from human follicular fluid were used to screen 1.5 x 10^5 plaques of the library by standard procedures (9, 10). The EcoRI inserts of positive clones were subcloned into pUC18 and sequences determined by double strand sequencing; internal regions of the insert sequence were obtained employing suitable synthetic primers. For Northern analysis, RNA was electrophoresed in a 1% agarose gel containing formaldehyde (10) and transferred to GeneScreen membranes (NEN). Hybridization of RNA blots followed the protocol of Khandjian (11). Quantification of the results by Northern analysis relative to an internal standard was achieved by densitometry of autoradiographs. For Southern blot analysis genomic DNA was restricted, electrophoresed in 0.7% agarose and transferred to nitrocellulose membranes as described (12). Hybridization was performed at 60°C under stringent conditions following the protocol of Church and Gilbert (13). DNA probes were [^{32}P]-labeled by random priming to a specific activity of 1x10^9 cpm/μg employing a commercial kit (Amersham).
DNA and protein sequence analysis as well as sequence comparisons were performed using the computer program of the University of Wisconsin genetics computer group (14).

Results and Discussion

A cDNA library in λgt11 derived from human ovarian granulosa cells was screened with antibodies against glycoproteins of follicular fluid. A number of positive clones were detected and their respective EcoRI inserts subcloned into pUC18. The subclones were all characterized by partial double strand sequencing. The inserts were also subjected to Northern analysis. The clone pHGP34 appeared to represent a mRNA closely resembling the size of the insert. The strategy of double strand sequencing as well as the nucleotide sequence of pHGP34 are depicted in Fig.1.

The complete nucleotide sequence of the insert of pHGP34 contained an open reading frame (nucleotides 96-770) from which the amino acid sequence of a protein of 225 residues could be deduced (Fig. 1B). The translational start is part of a typical eukaryotic consensus sequence for mRNAs CACCATGG (15, 16, 17). A comparison of the DNA sequence of pHGP34 with sequences of a data base indicated significant homology (65%) to that of the cDNA for EF-1β of *Artemia salina*. The homology of the amino acid sequence deduced from the open reading frame of pHGP34 with that of EF-1β of *Artemia salina* was 57% (Fig. 2A).

Out of 29 amino acid residues of the recently reported EF-1β peptide fragments of *Xenopus laevis* (5) 25 residues (86%) are identical with the amino acid sequence derived from pHGP34 (Fig. 2A). It is of interest that the homology between the C-terminal regions of the sequences for EF-1β of *Artemia salina* and pHGP34 is greater than that obtained by comparison of the total sequences. This is accord with the recent findings of van Damme et al. (1) that the catalytic function of EF-1β resides in the C-terminal, which would therefore be expected to be more highly conserved. As reported by Janssen et al. (18) phosphorylation of EF-1β from *Artemia salina* by an endogenous kinase affects its catalytic nucleotide exchange activity. The target for phosphorylation is residue S(89) of the amino acid sequence located in the sequence region DLFS(89)DEEDEE comprising a sequence motif critical for casein kinase II-type substrate recognition (18). The corresponding region in the pHGP34-derived sequence (see Fig. 2) is remarkably homologous, thus underlining the functional similarity brought about by sequence homology. Limited peptide sequence information on EF-1δ of *Artemia salina*, a



B

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GCCTCGAGGCGGGCGTCTTCGGTCATCTCCGGCGCTTCTAGGGCTGGTTCCCGTCATCTT   60
CGGGAGCCGTGGAGCTCTCGGATACAGCCGACACCATGGGTTTCGGAGACCTGAAAAGCC  120
                                M  G  F  G  D  L  K  S  P

CTGCCGGCCTCCAGGTGCTCAACGATTACCTGGCGGACAAGAGCTACATCGAGGGGTATG  180
  A  G  L  Q  V  L  N  D  Y  L  A  D  K  S  Y  I  E  G  Y  V

TGCCATCACAAGCAGATGTGGCAGTATTTGAAGCCGTGTCCAGCCCACCGCCTGCCGACT  240
  P  S  Q  A  D  V  A  V  F  E  A  V  S  S  P  P  P  A  D  L

TGTGTCATGCCCTACGTTGGTATAATCACATCAAGTCTTACGAAAAGGAAAAGGCCAGCC  300
  C  H  A  L  R  W  Y  N  H  I  K  S  Y  E  K  E  K  A  S  L

TGCCAGGAGTGAAGAAAGCTTTGGGCAAATATGGTCCTGCCGATGTGGAAGACACTACAG  360
  P  G  V  K  K  A  L  G  K  Y  G  P  A  D  V  E  D  T  T  G

GAAGTGGAGCTACAGATAGTAAAGATGATGATGACATTGACCTCTTTGGATCTGATGATG  420
  S  G  A  T  D  S  K  D  D  D  D  I  D  L  F  G  S  D  D  E

AGGAGGAAAGTGAAGAAGCAAAGAGGCTAAGGGAAGAACGTCTTGCACAATATGAATCAA  480
  E  E  S  E  E  A  K  R  L  R  E  E  R  L  A  Q  Y  E  S  K

AGAAAGCCAAAAAACCTGCACTTGTTGCCAAGTCTTCCATCTTACTAGATGTGAAACCTT  540
  K  A  K  K  P  A  L  V  A  K  S  S  I  L  L  D  V  K  P  W

GGGATGATGAGACAGATATGGCGAAATTAGAGGAGTGCGTCAGAAGCATTCAAGCAGACG  600
  D  D  E  T  D  M  A  K  L  E  E  C  V  R  S  I  Q  A  D  G

GCTTAGTCTGGGGCTCATCTAAACTAGTTCCAGTGGGATACGGAATTAAGAAACTTCAAA  660
  L  V  W  G  S  S  K  L  V  P  V  G  Y  G  I  K  K  L  Q  I

TACAGTGTGTAGTTGAAGATGATAAAGTTGGAACAGATATGCTGGAGGAGCAGATCACTG  720
  Q  C  V  V  E  D  D  K  V  G  T  D  M  L  E  E  Q  I  T  A

CTTTTGAGGACTATGTGCAGTCCATGGATGTGGCTGCTTTCAACAAGATCTAAAATCCAT  780
  F  E  D  Y  V  Q  S  M  D  V  A  A  F  N  K  I  *

CCTGGATCATGGCATTTAAATAAAAGATTGAAAGATTAAAAAAAAAAAA  829
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Fig.1. cDNA sequences of pHGP34. A: Sequencing strategy and restriction map of pHGP34. The arrow heads within the map give the extent as well as the 5′-3′orientation of the sequenced fragments. B: Nucleotide sequence of pHGP34 and deduced amino acid sequence of human EF-1β. Consensus sequences are underlined.

homologue of EF-1β was provided by van Damme et al. (1). No homology could be observed between a stretch of 31 N-terminal amino acid residues from EF-1δ of *Artemia salina* and the corresponding region of the sequence specified by pHGP34 (Fig. 2B). The sequence comparisons outlined above identify the amino acid sequence derived from pHGP34 as that of human EF-1β.

Genomic DNA from various sources was restricted with EcoRI and subjected to a comparative Southern analysis employing the insert of pHGP34 as hybridization probe under stringent

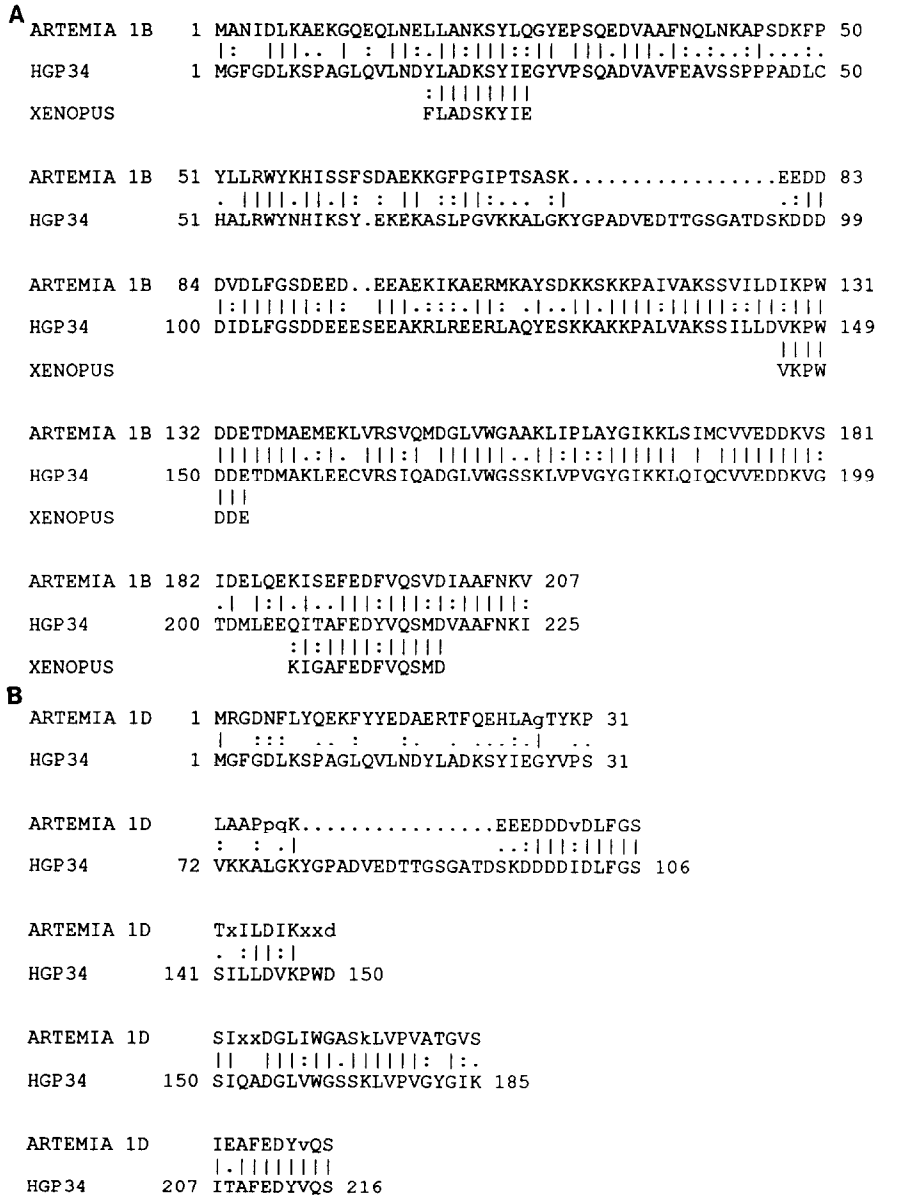

Fig.2. Comparison of amino acid sequences. Alignment based on identity and similarity was done by computer employing the program Bestfit. A) Artemia 1B, EF-1β from *Artemia salina*; HGP34, amino acid sequence derived from pHGP34; Xenopus, EF-1β from *Xenopus laevis*. B) Artemia 1D, EF-1δ from *Artemia salina*; x, unidentified amino acid residue; lower case characters indicate amino acid residues not determined with certainty.

conditions. From the results depicted in Fig. 3 it can be seen that in the human species EF-1β mRNA is apparently not specified by a single copy gene. Furthermore, genomic DNA from rat, mouse and bovine also yielded strong signals indicating a high degree of homology for DNA sequences of EF-1β coding regions. Less pronounced appears to be the homology at the DNA sequence level between human EF-1β and *Xenopus laevis* or chicken. Northern analysis of human granulosa total RNA using the insert of pHGP34 for hybridization identified an mRNA of 900 bp, very close in size to that of the cDNA insert. The Northern Blot depicted in

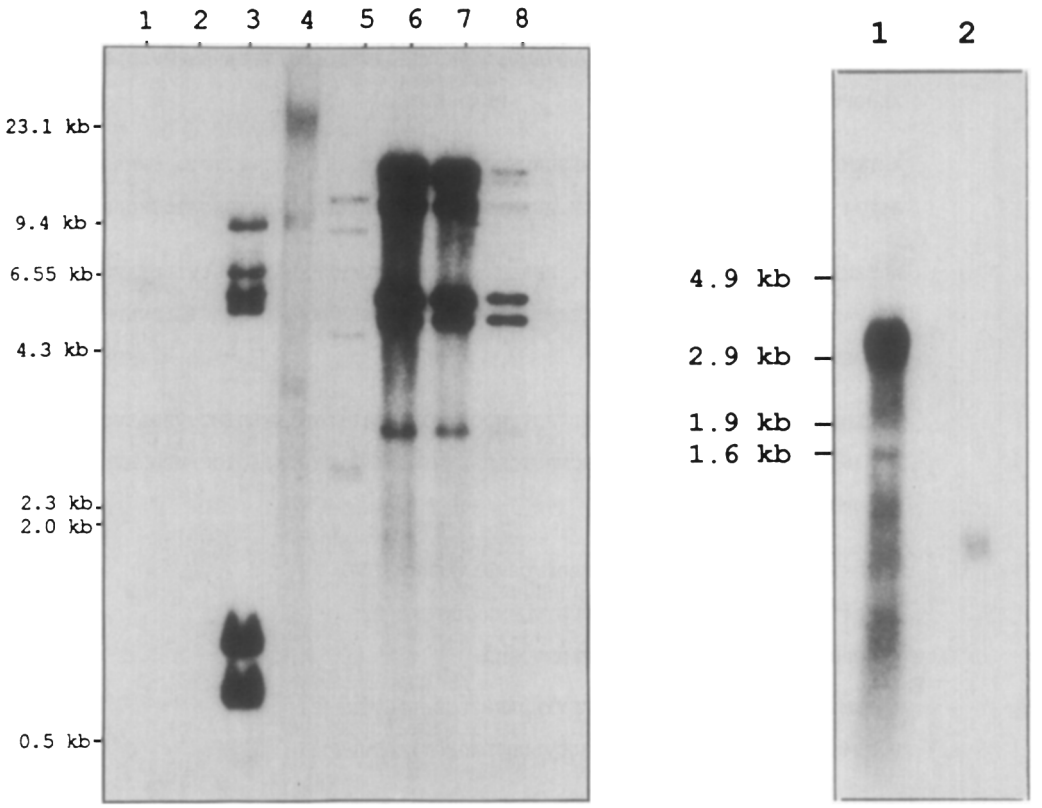

Fig.3. Comparative genomic blot. The genomic DNAs of respective species were restricted with EcoRI. The insert of clone pHGP34 was employed as hybridization probe. Hybridization was performed under stringent conditions. Lane 1, chicken; lane 2, *Xenopus laevis*; lane 3, bovine; lane 4, mouse; lane 5, rat; lane 6, human cell line H2LCL; lane 7, human male; lane 8, human female.

Fig. 4. Northern analysis of total RNA from human ovarian granulosa cells. The lanes contained 10 µg of RNA each. Hybridization was performed with the inserts of clones pHGP34 as well as pHGR81 (EF-2), (Rapp et al., 1989). Lane 1, EF-2; lane 2, EF-1β.

Fig. 4 was quantitated by densitometry. The molar ratio EF-1β mRNA/EF-2 mRNA in human ovarian granulosa cells was determined to approximately 0.15.

cDNA clone pHGP34 comprises the first and complete amino acid sequence of EF-1β for a mammalian species. Now characterization of EF-1β sequences of other species by cDNA cloning should be facilitated.

Acknowledgments

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