 

# Nucleotide sequence of rabbit elongation factor 1α cDNA

Jens Cavallius and William C.Merrick
Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, OH 44106, USA

Submitted February 3, 1992 EMBL accession no. X62245

Elongation factor 1α (EF-1α) binds aminoacyl-tRNAs in a GTP-dependent manner and then positions the bound aminoacyl-tRNA in the A site of the ribosome. After or concomitant with GTP hydrolysis, EF-1α·GDP is released from the ribosome allowing for peptide bond formation with the peptidyl-tRNA in the ribosomal P site. EF-1α is one of four subunits in $EF\text{-}1_H$ (heavy), which is also referred to as EF-1αβγδ (1). EF-1βγ catalyzes the exchange of GDP in the binary complex EF-1α·GDP for free GTP. Besides being involved in the synthesis of every peptide bond, EF-1α is an exceptionally abundant protein comprising 3−10% of the soluble protein in most cells. Beyond its role in protein synthesis, EF-1α has also been of interest as a member of the G-protein family as the only crystal structures known for GTP-binding proteins are for EF-Tu and ras. In addition, there have been reports indicating that EF-1α genes are expressed in a developmentally regulated manner (2) and that the methylation of EF-1α may be regulated in spore germination in fungus (3). Other roles suggested for EF-1α include: being a part of the valyl-tRNA synthetase (4); binding to actin (5); or being associated with the mitotic apparatus (6). While elevated expression of EF-1α in yeast is paralleled by an increase in translational fidelity (7), it is not clear whether it is the increased fidelity or one of the 'other' EF-1α activities that accounts for the longer life span of fruit flies which over express EF-1α (8). Decreased activity of EF-1α has been reported from several organisms, also from normal human fibroblasts undergoing aging *in vitro* (9). Chemical sequencing of EF-Tu and EF-1α has revealed that these proteins are post-translationally modified. To verify that the post-translational addition of glycerylphosphorylethanolamine was to glutamic acid residues at positions 301 and 374, as was found in mouse (10) and human (11) EF-1α cDNA sequences, the cDNA from rabbit was cloned and the EF-1α clones sequenced (X62245). The 59-mer oligonucleotide used for screening was based upon the amino acid sequence of low codon degeneracy (WNGDNMLEPSANMPWFKGWK, corresponding to bases 586 to base 644 in the coding sequence of the human and rabbit EF-1α). The sequence revealed that indeed glutamic acid was encoded at positions 301 and 374. Apart from the very highly conserved coding sequence of EF-1α, striking sequence homologies have been found in the 3′ non-coding region both between rabbit and human and rabbit and mouse mRNA. This suggested two possibilities: first, this stretch may be complementary to the coding region forming some structure which might relate to the stability or translational regulation of the mRNA; second, this sequence by itself may be a highly specific element that is critically important for stability, differential expression or efficiency in translation. If only the highly conserved region (the 60 bases 3′ of the stop codon) between human, mouse and rabbit of the 3′ noncoding end was folded in the Zuker program of the GCG Package (12), an average stability was found (free energy was −7.8 kcal/mol or −0.13 kcal/mol per base). In folding the whole region around the stop codon (from base pair number 1274 to 1502) of the rabbit EF-1α sequence, a very stable secondary structure was observed (−55 kcal/mol or −0.24 kcal/mol per base). This stability occurred in spite of the low homology between the three species found in the most 3′ end of this region (from 68 to 113 bases 3′ of the stop codon). The structure folds a part of the 3′ noncoding region onto the coding region of the EF-1α mRNA. It should be noted that this region is among the most highly conserved in the coding sequence. Similar structures could be found for human and mouse EF-1α mRNAs. The very low free energy obtained in this secondary mRNA structure and the highly conserved sequence in the 3′ noncoding end (100% between rabbit and human and 94% between mouse and human) suggests that this sequence serves some independent function unrelated to the coding region.

## ACKNOWLEDGEMENTS

Supported in part by grants from the National Institutes of Health GM 26796 (W.C.M.) and the Danish Science Academy (J.C.) and American Heart Association, Northeast Ohio Chapter (J.C.).

## REFERENCES

1. Carvalho,M.G., *et al.* (1984) *Arch. Biochem. Biophys.* **234**, 603−611.
2. Peeler,M.T., *et al.* (1990) *Dev. Biol.* **142**, 486−488.
3. Fonzi,W.A., *et al.* (1985) *Mol. Cell. Biol.* **5**, 1100−1103.
4. Bec,G. and Waller,J.P. (1989) *J. Biol. Chem.* **264**, 21138−21143.
5. Yang,F., *et al.* (1990) *Nature* **347**, 494−496.
6. Ohta,K., *et al.* (1990) *J. Biol. Chem.* **265**, 3240−3247.
7. Song,J.M., *et al.* (1989) *Mol. Cell. Biol.* **9**, 4571−4575.
8. Shepherd,J.C.W., *et al.* (1989) *Proc. Natl. Acad. Sci. USA* **86**, 7520−7521.
9. Cavallius,J., *et al.* (1986) *Exp. Gerontol.* **21**, 149−157.
10. Reddy,N.S., *et al.* (1988) *Gene* **70**, 231−243.
11. Brands,J.H.G.M., *et al.* (1986) *Eur. J. Biochem.* **155**, 167−171.
12. Devereux,H. and Smithies,O. (1984) *Nucleic Acids Res.* **12**, 387−395.