Molecular cloning of a new guanine nucleotide-exchange protein, EF1δ

J.Morales, P.Cormier, O.Mulner-Lorillon, R.Poulhe and R.Bellé*
Laboratoire de Physiologie de la Reproduction, CNRS (URA1449)/INRA, Université Pierre et Marie Curie, 4 Place Jussieu, 75256 Paris Cedex 05, France

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Elongation factor 1 mediates the elongation step of mRNA translation by transferring aminoacyl-tRNA to ribosomes under hydrolysis of GTP. EF1 is composed of two different factors with complementary functions: EF1α, a GTP binding protein and EF1βγ, a nucleotide exchange complex. The exchange activity was shown to reside in EF1β whereas the role of EF1γ is not well established (1). EF1β and γ are associated in *Xenopus* oocytes with a third protein of 36 kDa (2). On the basis of peptide microsequencing, p36 is different but related to EF1β, it is now refered to EF1δ (3). We present here the first sequence of EF1δ subunit.

A polyclonal antibody raised against the EF1βγδ *Xenopus* complex (4) was used to screen a λgt11 *Xenopus* ovarian library. Twelve positive clones were obtained. Among them, a clone encoding for EF1δ was identified on two criteria. First, λgt11 produced-protein was not reactive with affinity purified EF1γ and EF1β antibodies, and secondly, affinity purified antibodies obtained from this clone were specific for p36 of the *Xenopus* complex.

The insert (986 bp) was subcloned into pBluescript KS II phagemid and both strands were sequenced using standard techniques (5) as indicated (Figure; A). The encoded protein (Figure; B) is 265 amino acids length. All peptides found by the microsequencing of purified p36 subunit matched with the sequence. Searches in the data banks indicate, as expected, significant homology with EF1β. Interestingly, the homology was high between the 130−265 C-terminal region of the protein and the 100-end C-terminal region of all EF-1β proteins analysed, respectively 66, 80 and 79% with EF-1β from Artemia, *Xenopus* and Human. Since, in EF1β, the catalytic activity resides in the carboxyterminal half of the protein chain (6), we suggest that the 130−165 domain of EF1δ contains the guanine nucleotide exchange activity. The 1−130 N terminal part of EF1δ shows no significant homology with any other protein. Interestingly, this domain contains a leucine zipper motif (L58 to L93) involving six leucine residues. The N terminal domain could therefore be involved either in dimerisation of EF1 or in interaction with other cell components. Leucine zipper motifs have been reported in aminoacyl-tRNA synthetases (7). Since Valyl-tRNA synthetase is able to associate with EF1 (8), the (1−130) N terminal domain of EF1δ could be responsible for this interaction. Putative phosphorylation sites for $p34^{cdc2}$ and casein kinase II are found, respectively at T^{122} (TPAAK) and at S^{146} (SDNEE). Biochemical analysis of these sites are under investigation.

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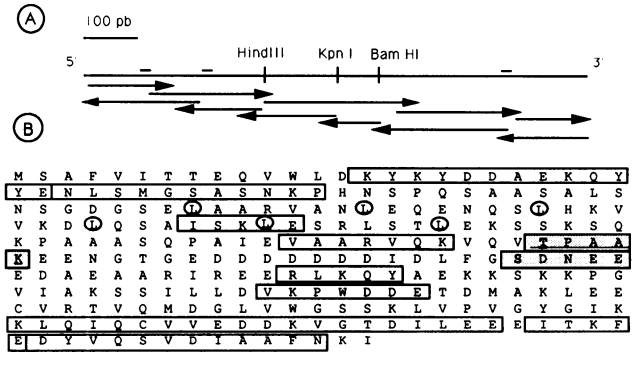

Figure. A—Sequencing strategy : Arrows indicate length and direction of sequences; dashes show position of oligonucleotides used as primers. B—cDNA-deduced amino acid sequence: White boxes, peptides obtained by sequencing of the protein. Grey boxes, putative phosphorylation site for $p34^{cdc2}$ (underlined) and CKII. Circled, Leucine residues involved in the leucine zipper motif.

* To whom correspondence should be addressed