Elongation factor 1 contains two homologous guaninenucleotide exchange proteins as shown from the molecular cloning of beta and delta subunits

P.Cormier, H.B.Osborne¹, J.Morales, T.Bassez¹, O.Minella, R.Poulhe, R.Bellé^{*} and O.Mulner-Lorillon

Physiologie de la Reproduction, UA CNRS 1449, INRA, Université Pierre et Marie Curie, 4 place Jussieu, 75256 Paris Cedex 05 and ¹Biologie et Génétique du Développement, UA CNRS 256, Université Rennes I, 35042 Rennes Cedex, France

Received December 22, 1992; Accepted January 5, 1993

EMBL accession no. X69764

Elongation factor 1 mediates the elongation step of mRNA translation. The transfer of aminoacyl-tRNA to ribosomes under hydrolysis of GTP is catalyzed by a GTP binding protein, EF1 α . A guanine-nucleotide exchange complex now referred as EF1 $\beta\gamma\delta$ replaces GDP by GTP on EF1 α (1). A complex, purified from *Xenopus* oocytes as a substrate for the meiotic and mitotic p34^{cdc2} kinase, was shown to contain the guanine-nucleotide exchange activity (2). The *Xenopus* complex is composed of three main proteins, p30, p36 and p47. Surprisingly, microsequencing of two of its components, p36 and p30 suggested the presence of two related proteins (2). We have previously cloned and sequenced the cDNA encoding for p47 or EF1 γ (3) and p36 or EF1 δ (4), we present here the molecular cloning of p30 or EF1 β . This result allows for the first time, sequence analysis of EF1 β and δ proteins, both present in the same complex.

A λ gt11 Xenopus ovarian library was screened with a polyclonal antibody raised against the EF1 $\beta\gamma\delta$ Xenopus complex. A clone encoding for EF1 β was isolated. The insert (811 bp) was subcloned into pBluescript KS II phagemid and sequenced in both directions using standard techniques (5) (Figure 1A). The encoded protein, of 227 amino acids length, was identified to EF1 β by the presence in the cDNA-deduced sequence of all peptides found by the microsequencing of purified p30 protein (Figure 1B). A putative phosphorylation site for casein kinase II was found at ¹⁰⁸Serine, matching the one identified in the corresponding protein of Artemia (6). Interestingly, sequence identity was high between the C-terminal domain of the EF1 β protein (100-227) and the C-terminal domain of EF-1 δ protein (85%) i.e. in the domain containing the guanine-nucleotide exchange activity (7). The 1-100 N terminal part of EF1 β appears clearly unrelated (13% identity) to that of EF1 δ . The presence of two different guanine-nucleotide exchange proteins in the same complex questions their respective physiological roles, which could be related to their specific N-ter domains.

ACKNOWLEDGEMENTS

We are grateful to Dr deRobertis for the generous gift of the $\lambda g111$ library. We thank E.Ricquier for her contribution.

REFERENCES

- 1. Riis, B., Rattan, S.I.S., Clark, B.F.C. and Merrick, W.C. (1990) Trends Biochem. Sci. 15, 420-424.
- Janssen, G.M.C., Morales, J., Schipper, A., Mulner-Lorillon, O., Bellé, R. and Moller, W. (1991) J. Biol. Chem. 266, 14885-14888.
- Cormier, P., Osborne, H.B., Morales, J., Bassez, T., Pouhle, R., Mazabraud, A., Mulner-Lorillon, O. and Bellé, R. (1991) Nucleic Acids Res. 19, 6644.
- Morales, J., Cormier, P., Mulner-Lorillon, O., Poulhe, R. and Bellé, R. (1992) Nucleic Acids Res. 20, 4091.
- 5. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, N.Y.
- Janssen,G.M.C., Maessen,G.D.F, Amons,R. and Möller,W. (1988) J. Biol. Chem. 263, 11063-11066.
- Van Damme, W.T.F., Amons, R., Karssies, R., Timmers, C.J., Janssen, G.M.C. and Möller, W. (1990) Biochim. Biophys. Acta 1050, 241-247.



Figure 1. A. — Sequencing strategy: Arrows indicate length and direction of sequences. B — cDNA-deduced amino acid sequence: White boxes: peptides obtained by sequencing of the protein.

^{*} To whom correspondence should be addressed