Molecular cloning of *Xenopus* elongation factor 1γ, major M-phase promoting factor substrate

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Elongation factor-1 (EF-1), participates in mRNA translation by binding aminoacyl-tRNAs to 80S ribosomes under hydrolysis of GTP (reviewed in 1). During meiotic cell division of *Xenopus* oocytes, the γ subunit of EF-1 βγδ is phosphorylated concomitantly with a change in the specificity and efficiency of mRNAs translation (2, 3). The γ subunit of EF-1 is phosphorylated by the M-phase promoting factor (MPF), a universal regulator of the G2 to M transition of the cell cycle, which consists of a complex of two proteins. One, p34, posssesses H1 kinase activity and the other is member of a class of proteins designed as cyclins (reviewed in 4). An antibody raised against *Xenopus* EF1βγδ, was used to screen a λgt11 *Xenopus* oocyte cDNA library. A number of positive clones were isolated and the cDNA inserts were subcloned in the Bluescript KS phagemid by standard techniques (5). Both strands were sequenced completely (6) after subcloning of the appropriate restriction fragments. An insert was identified as encoding for EF-1 γ since peptides obtained from the purified protein as described in (7) were found in the deduced protein sequence of the clone. This cDNA was further used as a probe to screen a λgt10 *Xenopus* cDNA library. Two positive clones were purified and used to complete the sequence of the coding region (1308 nucleotides) of *Xenopus* EF1 mRNA. These cDNAs and the initial cDNA isolated from the λgt11 library had identical sequences in their overlapping portions. The encoded protein (Figure) consists of 436 amino acids with a putative molecular weight of 49,000 and a pHi of 7.9 which correspond to determinations from the purified protein (2, 3). All the peptides found by microsequencing of the purified protein could be matched in the sequence with only 2 differences on 56 amino acids (Figure). Comparison between *Xenopus* EF-1γ and *Artemia* EF-1γ (8) showed 58% similitude. Putative phosphorylation sites (KTPE and TPKK) were found respectively at positions 46 and 230. Neither of these phosphorylation sites are present in the sequence of *Artemia* which is compatible with the lack of phosphorylation of this protein by the kinase activity of MPF (3). Biochemical determination of the phosphorylation site(s) can now be performed using the cDNA-deduced protein sequence and is currently under investigation.

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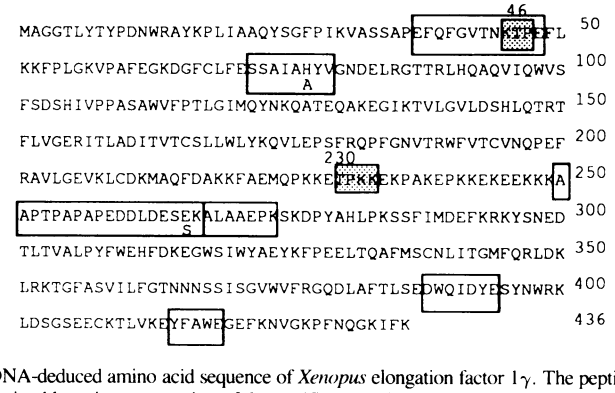

cDNA-deduced amino acid sequence of *Xenopus* elongation factor 1γ. The peptides obtained by microsequencing of the purified protein are indicated in white boxes, peptide 73 was SSAIAAYV, peptide 250 was AAPTPAPAPEDDLDSK, the others were exactly identical. Putative MPF phosphorylation sites are indicated in grey boxes at positions 46 and 230.

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