Nucleotide sequence of a *Trypanosoma cruzi* cDNA encoding a protein homologous to mammalian EF1γ

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Elongation factor (EF1) participates in protein synthesis by binding aminoacyl-tRNA to 80S ribosomes with the hydrolysis of GTP. EF1 is a major complex in eukaryotic cells. It is composed of subunits α, β and γ of molecular mass 50 kDa, 30 kDa and 48 kDa respectively (1). Following an immunoscreening of a lambda ZAPII *Trypanosoma cruzi* epimastigote cDNA library, using antibodies against three parasite proteins of molecular mass 45, 30 and 25 kDa eluted from glutathione–agarose column (Tc GBP), we could identify two cDNA clones, corresponding to the 30 and 25 kDa proteins, highly homologous in both their nucleic acid and amino acid sequences. Sequence analysis of the clones revealed 40% homology with elongation factor β from both *Artemia Salina* and human (2). Both cloned proteins were expressed by *T.cruzi* developmental stages and each was translated by a different length mRNA. Therefore, the proteins were termed TcEF-1β25 and TcEF-1β30. The nature of the 45 kDa protein comprising TcGBP remains unknown. In order to identify the cDNA clones corresponding to this protein, we used a double immunoscreening strategy that allowed us to isolate a cDNA clone corresponding to the 45 kDa protein.

The cDNA expression library was screened, first using anti-TcGBP immumserum which reacted against three major components of molecular mass 45, 30 (TcEF-1β30) and 25 kDa (TcEF-1β25). We could isolate 17 positive clones which were further reacted with anti-TcEF1-β 25/30 immune sera. One clone which did not react with anti-TcEF-1β25/30 was selected and sequenced by Automated Laser Fluorescent ALF DNA Sequencer (Pharmacia) using Auto Read™ sequencing kit.

Computer analysis detected a single open reading frame of 419 amino acids beginning by the initiation codon ATG at nucleotide 1258. The predicted molecular mass of the 411 amino acids parasite polypeptide is 46.6 kDa.

Screening of GeneBank database for sequences related to that of the clone under study revealed that the gene was not previously identified and that the encoded protein shares significant homology with *Artemia* (3) and human EF-1γ (4). The degree of identity of TcEF-1γ polypeptide to human EF-1γ is 31.3% and 29.3% with *Artemia* EF-1γ. When conservative replacements are included in the calculation, the similarity is 62.4% and 60.2%, respectively. The overall homology is of the same order as that observed between EF-1β proteins of different species and TcEF-1β 25 and 30 (2). The homology in the latter is mainly observed in the C-terminal domain, whereas in EF-1γ, the homology is spread over the sequence.

In some species, EF-1γ is phosphorylated by a cell division-controlled protein kinase, p34cdc2 (5). The recognition sequence for p34cdc2 kinase is a proline residue immediately following a serine or threonine residue (6). Three putatives phosphorylation sites at position 51 (CSPC), at position 90 (RTPL) and at position 265 (PSPF) were found in TcEF-1γ sequence.

The single methionine at position 1 is preceded by nucleotides (ACGCCATG) that conform well to the general translation initiation consensus sequence (CCA/GCCATG) established by Kozak for eukaryotes (7). In yeast, many of the genes encoding transcription and translation factors possess conserved sequences in their 5' untranslated regions (8, 9) which are thought to function as transcriptional promoters and enhancers. Two such consensus sequences derived from ribosomal protein genes have been defined: HOMOLI (AACATCC/TG/ATA/GCA) (10) and RPG (ACCCATACATT/CT/A) (11). Careful examination of the 5' non-coding region of TcEF-1γ indicates the presence of a potential RPG sequence (ATACACA) at position 2 of the nucleic acid sequence. Further biochemical and immunological characterization of TcEF-1γ protein is currently under investigation.

REFERENCES


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