A bacterial clone carrying sequences coding for elongation factor EF-1α from *Artemia*

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A bacterial cDNA clone was identified carrying one third of the nucleotides coding for elongation factor EF-1α from the brine shrimp *Artemia*. The sequence of codons corresponds with the known sequence of amino acids of EF-1α in the region involved.

Artemia *Elongation factor EF-1α* *Copy DNA cloning and sequencing*

1. INTRODUCTION

The aminoacyl-tRNA binding enzymes of prokaryotes and eukaryotes are major cellular proteins [1,2], which possess related primary structures in both cell types [3–6]. Much less is known about the structure and function of these proteins in eukaryotes than in prokaryotes.

Like EF-Tu in *Escherichia coli*, EF-1α plays the same pivotal role in protein synthesis, but it aggregates often with other proteins, the complex being called EF-1 [7]. In dormant cysts of the brine shrimp *Artemia* the high M_r form consists of a complex of 3 different polypeptide chains, EF-1α, EF-1β and EF-1γ [8]. In nauplii the aminoacyl-tRNA binding activity is mainly found in the free EF-1α protein chain of about 50 kDa and with an isoelectric point of 8.5, while the EF-1$\beta\gamma$ complex stimulates guanine nucleotide exchange [9].

To study the regulation of the elongation cycle of protein synthesis in eukaryotic cells, we have started an investigation of EF-1α in *Artemia*. Here, we describe the molecular cloning in *E. coli* of an EF-1α encoding mRNA fraction [10] and the sequence analysis of an EF-1α cDNA clone, carrying a 542 bp insert.

2. MATERIALS AND METHODS

2.1. *Isolation of RNA from developing* Artemia *cysts*

For extraction of RNA from cysts the urea/LiCl procedure [11] was adapted. Dried cysts were washed and developed as in [10]. The cysts were ground in a mortar at 4°C in the presence of 6 M urea, 3 M LiCl and homogenized in a Sorvall omnimixer. After filtration the suspension was diluted with urea, 3 M LiCl and homogenized in a Sorvall omnimixer. After filtration the suspension was diluted with urea/LiCl and stored overnight at −20°C. Following centrifugation (20 min at 20000 × *g*) the pellet was dissolved in 10 mM Tris–HCl (pH 8.8), 5 mM EDTA, 1% SDS and the solution extracted 2 or 3 times with phenol, chloroform, isoamylalcohol (50/50/1, by vol.). After precipitation with ethanol, poly(A)$^+$ RNA was selected and subsequently fractionated on sucrose gradients containing CH_3HgOH [10].

2.2. *cDNA synthesis and molecular cloning*

Most of the methods employed are slightly modified versions of the procedures referred to. The assay of mRNA and immunoprecipitation of

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the translation product were performed as in [10]. Synthesis of cDNA, digestion with S_1-nuclease, DNA gel electrophoresis and the sources of the various enzymes and chemicals were as in [12]. Transformation of competent *E. coli* K12 RR1 cells, the plating, growing of colonies, and the isolation of plasmid DNA have been published [13–15].

2.3. *Homopolymer tailing*

Double-stranded, S_1-nuclease treated cDNA was extended with dC tails essentially as in [16]. *Pst*I restricted pAT 153 DNA; dG-tailed in the same way was used as a cloning vehicle. To assay the rate of addition of dC residues, fractions were diluted with an equal volume of water, inactivated by heating for 10 min at 65°C and restriction digested with *Hin*fI. Agarose gel-electrophoretic analysis clearly showed a 'growing fragment' (fig.1). The optimum length of the dG tails was determined by transformation of competent *E. coli* cells after annealing with dC-tailed plasmid DNA.

2.4. *Hybridization-released translation*

Plasmid DNA was linearized with *Eco*RI, denatured and spotted on nitrocellulose filters (Genescreen, NEN). For testing a large number of clones a Hybridot spotting device (BRL) has shown to be convenient. The filters were baked and prehybridized in 0.1 M Tris–HCl (pH 7.6), 0.75 M NaCl, 2 mM EDTA, 0.5% SDS, 50% formamide, 60 μg/ml yeast tRNA, and 70 μg/ml poly(A). After 4 h at 37°C, 0.5 mg poly(A)$^+$ RNA from *Artemia* was added and the hybridization was continued for 20 h at 37°C. The filters were washed at 20°C with 10 mM Tris–HCl (pH 7.6), 1 mM EDTA, 100 mM NaCl (TEN), 0.2% SDS, with TEN and with TE, about 10-times each. Subsequently, the hybridized RNA was eluted by incubating each filter for 5 min at 80°C in 2 mM EDTA (pH 7.0) in an Eppendorff tube. The elution step was repeated and after addition of yeast tRNA as a carrier, the nucleic acids were precipitated twice with ethanol and analyzed as above.

2.5. *Sequencing methods*

For DNA sequence determination the cDNA clone was digested with *Pst*I and the insert was eluted by means of electrophoresis from a preparative agarose gel. The insert was ligated in the *Pst*I site of pUC8 and recloned in *E. coli* JM 101 [17]. Besides the *Pst*I side the polylinker of this vector provides a *Sal*I site suitable for 5′ (T4 polynucleotide kinase) as well as 3′ (Klenow DNA polymerase) labeling and *Eco*RI or *Bam*HI sites for the generation of single-end labeled fragments. The chemical modification method of Maxam and Gilbert [18] was employed for DNA sequencing.

3. RESULTS AND DISCUSSION

3.1. *Molecular cloning and clone identification*

For extraction of RNA from *Artemia* cysts the urea/LiCl procedure applied turned out to be faster and less expensive than sedimentation through CsCl gradients used in [10]. Template activity does not show marked differences between the two methods.

Using about 1.5 μg poly(A)$^+$ RNA fraction enriched in sequences coding for EF-1α, we encountered no special difficulties up to the stage of tailing and annealing. These were optimized using pAT 153 DNA as a model in two separate experiments. Concerning the dC tailing, the gel-electrophoretic assay (fig.1) shows that the addition of dC residues proceeds linearly with time at about 3 residues/min. With respect to the dG tailing, pAT 153 DNA was linearized by digestion with *Pst*I and divided in two portions, which were tailed with dG and dC residues, respectively. After annealing and transformation of competent *E. coli* cells the ampicillin-sensitive fraction of the tetracycline-resistant transformants was determined. We concluded that 4–5 min of dC tailing and 7–15 min of dG tailing provided satisfactory results.

After annealing the dC-tailed cDNA with the dG-tailed vector a sample was used to transform Ca^{2+}-treated *E. coli* RR1 cells. The efficiency of transformation was about 8×10^5 colonies/μg pAT 153 DNA and about 50% of the obtained transformants were found to be sensitive to ampicillin. Clones carrying inserts of more than 400 bp were used for hybrid-releasing translation. One of them (clone 13) showed a faint band of 50 kDa on an SDS gel after immunoprecipitation with EF-1α antibodies. Plasmid DNA from the clones 13 and 21 was purified by CsCl gradient centrifugation and the hybrid-released translation

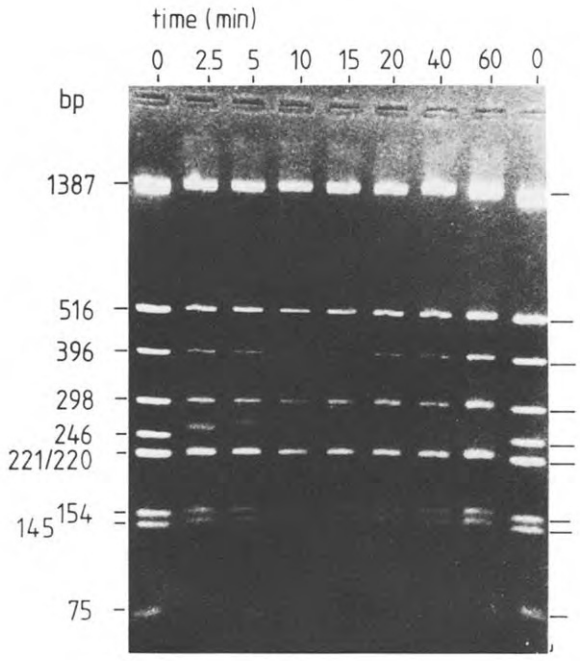

Fig.1. Homopolymer tailing. *Pst*I-restricted pAT 153 DNA was tailed with dC residues by means of terminal transferase (EC 2.7.7.31). At the times indicated samples were inactivated, digested with *Hin*fI and analyzed on a 1.5% agarose gel in the presence of ethidium bromide. The 1393 and 246 bp fragments are carrying the dC tails at one of their 3′-ends.

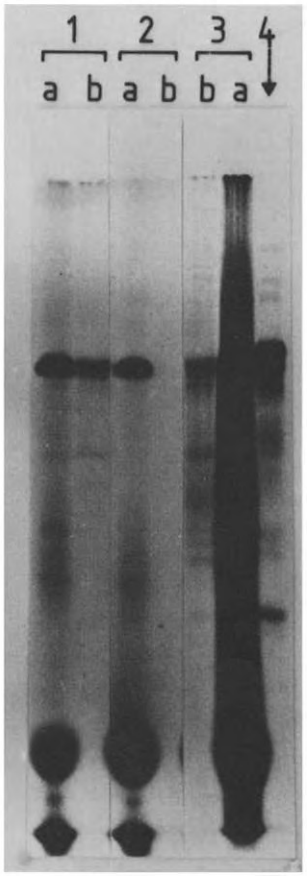

Fig.2. Hybrid-released translation. Plasmid DNA was used to select specific mRNAs. After translation in vitro of the selected mRNAs the products were analyzed on a 12% SDS–polyacrylamide gel: (a) products synthesized in vitro (1 μl); (b) immunoprecipitation of 5 μl assay with anti EF-1α IgG; (1) clone 13 selected mRNA; (2) clone 21 selected mRNA; (3) *Artemia* poly(A)$^+$ RNA (after selection); (4) ^{3}H-labelled EF-1α. The 50 kDa band, almost comigrating with EF-1α and present in all translation assays, is thought to be a product of residual mRNA activity in the lysate.

procedure was repeated (fig.2). Only clone 13 shows a 50 kDa polypeptide in the protein synthesizing assay after subsequent immunoprecipitation with specific anti EF-1α IgG. The nucleotide sequence analysis presented below provides definite proof for the identity of the cDNA clone.

3.2. *Nucleotide sequence of the cDNA insert*

The strategy for the sequence analysis of the 542 bp insert is presented in fig.3a. Some DNA sequences were obtained after isolation of the *Eco*RI–*Hin*dIII pUC8 fragment containing the cDNA insert in the *Pst*I sites. Fig.3b shows the entire nucleotide sequence of the cDNA insert and the derived amino acid sequence, which is in agreement with the partial sequence of the protein chain [6]. Both sides of the cDNA show the presence of oligo(dC) tails, but no poly(A) tract was found at the 3′-end of the coding sequence.

The cDNA sequence codes for the protein se-

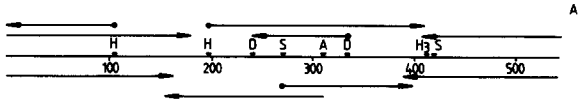

Fig.3.(a) Strategy for the sequence analysis of the cloned EF-1α cDNA, after recloning in pUC8. Positions of relevant restriction sites: A, *Alu*I; H, *Hin*fI; H3, *Hae*III; S, *Sau*3AI.

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   P  V  G  R  V  E  T  G  I  I  K  P  G  M  I  V  T  F  A  P
GACCTGTGGGTCGGGTTGAAACTGGTATCATTAAGCCAGGTATGATAGTCACATTTGCCC
         10         20         30         40         50         60
                                                                  B

                                                            E
                                                            *
   A  N  I  T  T  E  V  K  S  V  E  M  H  H  E  S  L  E  Q  A
CAGCCAACATAACCACTGAAGTCAAATCCGTCGAAATGCACCACGAATCCCTTGAACAGG
         70         80         90        100        110        120

                                                   *
   S  P  G  D  N  V  G  F  N  V  K  N  V  S  V  K  E  L  R  R
CATCTCCAGGTGATAACGTTGGTTTCAATGTCAAAAACGTTTCAGTCAAAGAACTTCGTC
        130        140        150        160        170        180

                                                      Q  E
                                                      *  *
   G  Y  V  A  S  D  S  K  N  N  P  A  R  G  S  Q  D  F  F  A
GTGGCTACGTCGCCTCAGATTCAAAGAACAATCCAGCCAGAGGCTCTCAAGATTTCTTTG
        190        200        210        220        230        240

   E                  G         E
   *                  *         *
   Q  V  I  V  L  N  H  P  G  Q  I  S  N  G  Y  T  P  V  L  D
CTCAGGTTATTGTCTTGAACCACCCTGGTCAGATCTCAAACGGTTACACTCCTGTCTTGG
        250        260        270        280        290        300

                                                !
   C  H  T  A  H  I  A  C  K  F  A  *  I  K  E  K  C  D  R  R
ACTGCCACACAGCTCACATTGCTTGCAAGTTTGCTTAGATTAAAGAGAAGTGTGACAGAC
        310        320        330        340        350        360

   T  G  K  T  T  E  A  E  P  K  F  I  K  S  G  D  A  A  M  I
GTACTGGCAAAACAACTGAAGCTGAGCCAAAATTTATCAAGTCAGGTGATGCGGCCATGA
        370        380        390        400        410        420

   T  L  V  P  S  K  P  L  C  V  E  A  F  S  D  F  P  P  L  G
TCACTTTGGTACCTTCCAAGCCGTTGTGTGTTGAAGCCTTTTCCGACTTCCCACCTCTTG
        430        440        450        460        470        480

   R  F  A  V  R  D  M  R  Q  T  V  A  V  G  V  I  K  S  V  N
GTCGATTTGCTGTCCGTGACATGAGACAAACAGTCGCTGTCGGAGTTATCAAGTCCGTCA
        490        500        510        520        530        540

AC
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Fig.3.(b) Complete nucleotide sequence of the cloned EF-1α cDNA and the derived sequence of amino acids. The positions where the deduced and the published [6] amino acid sequence differ, are indicated by an asterisk and by the residue in the determined sequence of amino acids (see section 4). The lysine residue at position 169 (*) is an ϵ-trimethyllysine in the protein. The amino acid at the position of the stopcodon (337(!), see the text) has not yet been identified.

quence as determined for a C-terminal part of the protein [6]. Points of difference are that the Glu residue in position 99 corresponds to the serine codon TCC and a histidine triplet is found instead of a glycine at position 262. In this connection one has to keep in mind, that EF-Tu in *E. coli* is coded for by two unlinked genes (*tufA* and *tufB*, respectively) [19], which differ at 13 positions in their coding sequences. The corresponding EF-Tu gene products are identical except for the C-terminal residue. The Gln residues identified by us as CAG and GAA codons in the cDNA were given as Glu using CNBr peptides where deamination of the protein during the cleavage step occurs [6].

The presence of a stopcodon at position 337 in the coding sequence is not as expected. The most likely explanation is a copying error of one of the enzymes used for reverse transcription or second strand synthesis [20]. Sequence analysis of more EF-1α cDNA clones will clarify this point. The EF-1α cDNA clone characterized in this report, has been used successfully for the determination of the size of the mRNA, a rough estimation of the number of genes, the isolation of a clone in an *Artemia* genomic library and the presence of intervening sequences in this cloned gene [21].

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REFERENCES

[1] Furano, A.V. (1976) Eur. J. Biochem. 64, 597–606.
[2] Slobin, L.I. (1980) Eur. J. Biochem. 110, 555–563.
[3] Jones, M.D., Petersen, T.E., Nielsen, K.M., Magnussen, S., Sottrup-Jensen, L., Gausing, K. and Clark, B.F.C. (1980) Eur. J. Biochem. 108, 507–526.
[4] Arai, K.-I., Clark, B.F.C., Duffy, L., Jones, M.D., Kaziro, Y., Laursen, R.A., L'Italien, J., Miller, D.L., Nagarkatti, S., Nakamura, S., Nielsen, K.M., Petersen, T.E., Takahishi, K. and Wade, M. (1980) Proc. Natl. Acad. Sci. USA 77, 1326–1330.
[5] Slobin, L.I. (1981) Biochemistry 20, 5761–5767.
[6] Amons, R., Pluijms, W., Roobol, K. and Möller, W. (1983) FEBS Lett. 153, 37–42.
[7] Clark, B.F.C. (1980) Trends Biochem. Sci. 5, 207–210.
[8] Slobin, L.I. and Möller, W. (1976) Eur. J. Biochem. 69, 351–375.
[9] Slobin, L.I. and Möller, W. (1978) Eur. J. Biochem. 84, 66–77.
[10] Van Hemert, F.J., Roobol, C. and Möller, W. (1981) Eur. J. Biochem. 120, 137–142.
[11] Auffray, C. and Rougeon, F. (1980) Eur. J. Biochem. 107, 303–314.
[12] Dijkema, R., Dekker, B.M.M., Van Ormondt, H., De Waard, A., Maat, J. and Boyer, H.W. (1980) Gene 12, 287–299.
[13] Dagert, M. and Ehrlich, S.D. (1979) Gene 6, 23–28.
[14] Peacock, S.L., McIver, C.M. and Mohanan, J.J. (1981) Biochim. Biophys. Acta 655, 243–250.
[15] Birnboim, H.C. and Doly, J. (1979) Nucleic Acids Res. 7, 1513–1523.
[16] Hoeymakers, J.H.J., Borst, P., Van den Burg, J., Weissmann, C. and Cross, G.A.M. (1980) Gene 8, 391–417.
[17] Vieira, J. and Messing, J. (1982) Gene 19, 259–268.
[18] Maxam, A.M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564.
[19] Jaskunas, S.R., Lindahl, L., Nomura, M. and Burgess, R.R. (1975) Nature 257, 458–462.
[20] Loeb, L.A., Weymouth, L.A., Kunkel, T.A., Gopinathan, K.P., Beckman, R.A. and Duke, D.K. (1979) Cold Spring Harbor Symp. Quant. Biol. 43, 921–927.
[21] Van Hemert, F.J., Lenstra, J.A. and Möller, W. (1983) FEBS Lett. 157, 295–300.
[22] Blackburn, P. (1979) J. Biol. Chem. 254, 12484–12487.