Genes for elongation factor EF-1α in the brine shrimp *Artemia*

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A plasmid carrying a cDNA sequence coding for elongation factor EF-1α from *Artemia* was used to probe blots of mRNA and chromosomal DNA from *Artemia*. A messenger length for EF-1α corresponding to 1850 nucleotides was found. Southern blots pointed to a limited number (1–4) of genes, coding for EF-1α. From an *Artemia* gene library a recombinant phage was isolated, which contains genomic sequences of EF-1α. S1-nuclease mapping indicated the presence of intervening sequences within this cloned gene.

Artemia *Elongation factor EF-1α* *Northern blot* *Southern blot* *Gene library*

1. INTRODUCTION

It has been well documented, that the elongation factor EF-1 is composed of more than one component in the case of wheat germ [1], *Artemia* [2], pig liver [3], silk worm gland [4] and chick brain [5]. Especially the isolation and properties of elongation factor EF-1α, the aminoacyl-tRNA binding subunit homologous to EF-Tu in *Escherichia coli* has been well studied and shown to be a major protein in a number of eukaryotic cells [6]. In dormant cysts of the brine shrimp *Artemia*, EF-1 is mainly found in a high M_r form, consisting of a complex of 3 different polypeptide chains, EF-1α, EF-1β and EF-1γ [2]. EF-1α, as such present in nauplii, has an M_r of about 50000 and binds aminoacyl-tRNA, while EF-1$\beta\gamma$, like prokaryotic EF-Ts, stimulates the exchange of guanine nucleotides [7]. Sequence analysis of parts of the EF-1α protein chain of *Artemia* reveals a clear homology to distinct regions in EF-Tu from *E. coli* [8]. EF-Tu is encoded by two nearly identical but unlinked genes (*tufA* and *tufB*), providing a challenging model for studying the regulation of gene expression in *E. coli* [9]. *tufB* in the *rif* region is cotranscribed with 4 upstream tRNA stretches [10]. In analogy to the expression of ribosomal protein gene operons [11] this may explain the autogeneous control of EF-TuB synthesis on a translation level [12]. The *tufA* gene expression is differently regulated [13].

Despite the pivotal role of EF-1 in eukaryotic protein synthesis and its abundance in eukaryotic cells, insight into the number of its genes and the regulation of their expression is lacking.

The present work is directed towards characterization of the gene organization of EF-1α from the brine shrimp *Artemia*. Here we present results concerning the size of the messenger RNA, the number of genes and the presence of intervening sequences. In a separate publication [14], we describe the isolation and sequence analysis of a cDNA clone, containing one-third of the coding sequence of EF-1α.

2. EXPERIMENTAL PROCEDURES

2.1. *Northern hybridization*

RNA from developing *Artemia* cysts was isolated by urea/LiCl precipitation [20] as described [14]. Gel electrophoresis, transfer to Genescreen (NEN) and the subsequent hybridization were performed according to Thomas [15].

2.2. *Southern hybridization*

Free swimming nauplii were homogenized in an Elvejem homogenizer in 10 mM Tris–HCl (pH 8.0), 5 mM $MgCl_2$ at 4°C. After filtration, the in-

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tact nuclei were purified by repeated centrifugation at 4000 × *g* for 5 min through 1.5 M sucrose in the same buffer. After lysing the nuclei in 10 mM Tris–HCl (pH 8.0), 10 mM EDTA and 2% SDS, DNA was purified by phenol extraction, ethanol precipitation, RNase and proteinase K treatment followed by a second phenol extraction and ethanol precipitation. Blotting and hybridization of restriction-digested DNA in 3 × SSC at 65°C was performed as in [16]. After hybridization, blots were washed 3 times for 15 min in 0.2 × SSC at 65°C.

2.3. *Construction of a gene library of* Artemia *DNA in the λ-vector EMBL3A*

Artemia DNA was partially digested with *Mbo*I and fractionated on sucrose gradients as in [16]. After dephosphorylation, DNA fragments were ligated in *Bam*HI sites, located in the polylinkers of the replacement vector EMBL3A [17]. After in vitro packaging, recombinant phages were grown in *E. coli* NM528 host cells. Selection and growing of a phage hybridizing to the cDNA plasmid as well as the purification of phage DNA was done as in [16].

2.4. *S1-nuclease protecting assay*

Protection of λ-clone DNA against S1-nuclease after hybridization with mRNA was accomplished according to Favarolo [18], as described by Maniatis [16]. λ-clone DNA (0.1 μg) was labeled by nick-translation for 45 min at 13.5°C, followed by a 90 min incubation with T4-DNA-ligase at 20°C in order to repair the nicks.

After hybridization at 51°C for 3.5 h the mixture was divided into 3 parts. S1-nuclease was added and the digestion was carried out for 1.75 h at 13.5°C, 20°C and 37°C, respectively. The protected DNA fragments were detected by autoradiography after electrophoresis through denaturing 4% polyacrylamide gels.

2.5. *Miscellaneous*

Enzymes were obtained from NE-Biolabs (England), BRL GmbH (FRG), Boehringer (Mannheim) (Germany), CBL (England) and The Radiochemical Centre (Amersham). ^{32}P-Labeled nucleoside triphosphates were purchased from the Radiochemical Centre (Amersham).

3. RESULTS

3.1. *The size of EF-1α mRNA*

An estimation of the number and size of the EF-1α mRNAs was made by blotting fractionated poly(A)$^+$ RNA and probing the blot with the cloned cDNA. In fig.1 the result of such an experiment is presented. The size markers (left lane, M13mp7 × *Hae*III fragments, labeled at their 5′-ends) were treated in the same way (denaturation by glyoxal prior to electrophoresis and blotting) as was the *Artemia* poly(A)$^+$ RNA (10 μg, right lane). Only hybridization with an RNA fraction of about 1850 nucleotides was observed, large enough to code for a polypeptide chain of approximately 450 residues.

3.2. *EF-1α genes in the* Artemia *genome*

An estimation of the number of EF-1α genes in the *Artemia* genome was made by Southern blot analysis with cloned cDNA as a hybridization probe. DNA from *Artemia* nuclei was digested with various restriction enzymes (fig.2). The completion

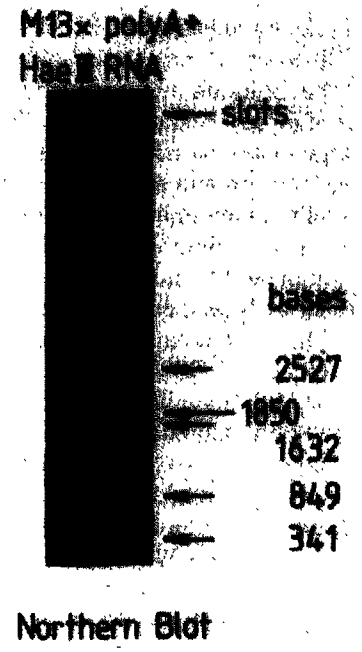

Fig.1. Size determination of EF-1α mRNA of *Artemia*. Total poly(A)$^+$ RNA (10 μg, right lane) from *Artemia* was denatured and analyzed by electrophoresis through a 1.1% agarose gel, adjacent to the size markers (M13mp7 × *Hae*III, left lane) treated in the same way.

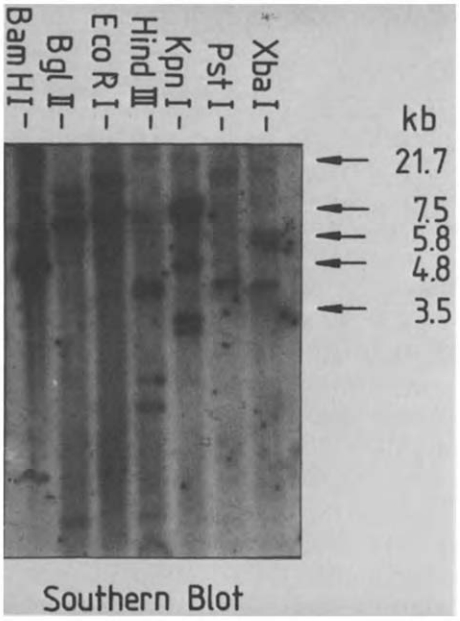

Fig.2. Southern blot analysis of EF-1α coding sequences in *Artemia* DNA: *Artemia* DNA (4–10 μg) was digested to completion with the restriction enzymes indicated. The fragments were fractionated through a 0.7% agarose gel and after blotting hybridized with ^{32}P-labelled cloned EF-1α cDNA. For sizing an *Eco*RI digest of λ-DNA was used.

of the digestions was tested for by incubating aliquots of the reaction mixture with a slight excess of undigested λ-DNA and analysis by electrophoresis through an 0.4% agarose gel. The resulting autoradiogram (fig.2) showed hybridization with the cDNA in all the lanes and the number of bands varied from 1–4 depending on the restriction enzyme used. Unless the genes coding for the elongation factor are reiterated, the number of different genes for EF-1α is maximally 4.

3.3. *Intervening sequences in a cloned EF-1α gene*

After in vitro packaging >140000 recombinant phage plaques were obtained. Assuming an *Artemia* genome size of approximately 1.3×10^9 bp [19] a probability of $\geq$88% can be calculated of having cloned any single-copy sequence [16]. After three successive rounds of screening, one recombinant phage designated λAEI and hybridizing with the EF-1α probe was isolated. On Northern blots DNA from λAEI hybridized with the same mRNA as the cDNA clone in fig.1 (not shown). λAEI was characterized further by Southern blotting after digestion with various restriction enzymes and probed with the cloned cDNA. Prior to these digestions the λAEI DNA was cut by *Sal*I in order to separate the cloned *Artemia* DNA from the arms of its vector. The resulting autoradiogram (fig.3) showed two prominent hybridizing bands in each lane and in addition one and three weaker hybridizing fragments after digestion with *Pst*I and *Hin*dIII, respectively. The 1.5 kb *Hin*dIII fragment was also observed on the blot of chromosomal DNA (fig.2). With respect to the restriction enzymes used, *Kpn*I is the only one that recognizes a site in the nucleotide sequence of the EF-1α cDNA probe [14].

The combined data indicate, that λAEI DNA harbours at least part of an EF-1α gene and that the λAEI DNA sequences homologous to the EF-1α cDNA hybridization probe are separated by DNA carrying restriction sites for *Sal*I, *Pst*I, *Kpn*I and *Hin*dIII. The presence of intervening sequences was tested by determination of the single

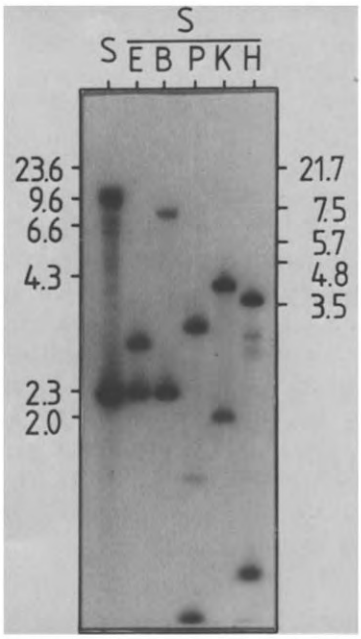

Fig.3. Blot analysis of λAEI DNA. λAEI DNA was digested with *Sal*I. Parts of this *Sal*I digest were digested with *Eco*RI (SE), *Bam*HI (SB), *Pst*I (SP), *Kpn*I (SK) and *Hin*dIII (SH). Digested λAEI DNA (1.5 μg) was applied to each slot. *Eco*RI and *Hin*dIII digests of λ-DNA provided the size markers indicated.

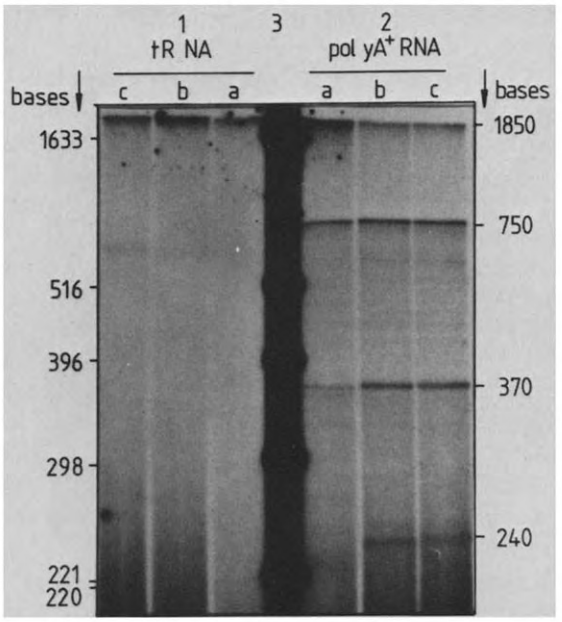

Fig.4. Fragments of λAEI DNA resistant to S_1-nuclease after hybridization with poly(A)$^+$ RNA. ^{32}P-Labeled λAEI DNA (50 ng) was hybridized with 10 μg yeast tRNA [1] and with 30 μg *Artemia* poly(A)$^+$ RNA [2]. Digestion with S_1-nuclease was performed at 13.5°C (c), 20°C (b) and 37°C (a). The samples were analyzed by electrophoresis through a 4% polyacrylamide gel in 7 M urea. pAT 153 DNA × *Hin*fI was used for size determination (lane 3).

stranded λAEI fragments resistant to S_1-nuclease after hybridization with *Artemia* poly(A)$^+$ RNA. As shown in fig.4, four λAEI fragments survived the S_1-nuclease incubation and three of them owed their protection to mRNA hybridization. This indicates the presence of at least two intervening sequences in the cloned gene coding for EF-1α. It is also clear, that the total amount of protected sequences does not cover the entire length of the mature EF-1α transcript estimated to be 1850 nucleotides (fig.1).

4. DISCUSSION

Hybridization of only one class of poly(A)$^+$ RNA indicates the presence of a single-length mRNA species coding for the elongation factor. This experiment does not exclude the possibility, that distinct EF-1α genes give rise to different mature transcripts of approximately the same length. Concerning the estimation of the number of EF-1α genes by Southern blotting (fig.2) the source of the DNA should be taken into consideration. *Artemia* cysts as collected from shores are not 'clonal' as are cultures derived from individual cells. Moreover, *Artemia* DNA could so far only be isolated from free-swimming nauplii, in which differentiation has occurred. For these reasons, restriction enzyme polymorphisms cannot be excluded and therefore Southern blotting may overestimate the actual number of EF-1α genes. The possibility of reiterated EF-1α genes remains, but such repetition has to be perfect with respect to the cleavage sites of the different restriction enzymes to keep the apparent number of genes low.

We propose the presence of only a few distinct genes (1–4) coding for the elongation factor EF-1α per amount of haploid genome. It will be interesting to see, whether the presence of two intervening sequences in an EF-1α gene relates to the occurrence of three domains in the polypeptide chain [8,21].

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