

Apis mellifera cytoplasmic elongation factor 1 α (EF-1 α) is closely related to *Drosophila melanogaster* EF-1 α

Uwe Walldorf² and Bernhard T. Hovemann¹

¹Center for Molecular Biology Heidelberg (Z.M.B.H.), Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG and ²Biocenter of the University of Basel, Klingelbergstraße 70, CH-4056 Basel, Switzerland

Received 18 April 1990; revised version received 18 May 1990

Using low stringency hybridisation with a *Drosophila melanogaster* EF-1 α gene fragment we have isolated a genomic DNA clone encoding elongation factor 1 α (EF-1 α) from *Apis mellifera*. The hybridising *Apis mellifera* sequence could be delineated to two small *Eco*RI fragments that were also revealed by genomic Southern hybridisation. By comparison with the corresponding *Drosophila melanogaster* data the complete translational reading frame has been deduced. It is interrupted by two intervening sequences of 220 and about 790 nucleotides. Comparison with known eucaryotic EF-1 α sequences further confirms that certain amino acid sequences seem to be invariable within the EF-1 α protein family.

Protein synthesis; Elongation factor; Translation; Genomic sequence; *Apis mellifera*; *Drosophila melanogaster*

1. INTRODUCTION

Translation of genetic information into protein is a highly conserved process. This holds true specifically for the chain of interacting events that take place at the ribosome. It is, therefore, not surprising that many of the factors that take part in translation have allowed very little variation of their amino acid sequence during evolution. The elongation of the amino acid chain involves a series of protein components that are known as elongation factor 1 and elongation factor 2 complexes [1]. Elongation factor 1 consists of the three proteins EF-1 α , - β and - γ . EF-1 α catalyses the transport of aminoacyl-tRNA to the 80 S ribosome. A prerequisite for EF-1 α activity is its activation by GTP, a process that is thought to be catalysed by the EF-1 β , γ complex. A growing number of elongation factor 1 α sequences of different origin is now available due to the possibility of screening by crosshybridisation between species. We have recently described two independent genes of *Drosophila melanogaster* that encode two different elongation factors 1 α [2]. They follow an individual pattern of expression during development and were named EF-1 α ,F1 and EF-1 α ,F2.

Here, we describe the cloning and sequence analysis of an EF-1 α gene from *Apis mellifera*. Because of the

evolutionary distance of 250 million years between *Drosophila* and *Apis* the analysed sequence allows an interesting comparison between distantly related insects. Moreover, it completes the knowledge about those amino acid sequences that stay invariable which is indicative of their structural and functional importance. The determined sequence revealed a translational reading frame that is closely related to both *Drosophila* genes, EF-1 α ,F1 and EF-1 α ,F2.

2. MATERIALS AND METHODS

2.1. General methods

Genomic *Apis mellifera* DNA was prepared as described by Walldorf et al. [3] and Blin and Stafford [4]. Restriction endonuclease digestions, gel electrophoresis of DNA fragments, library screening, isolation of phage λ , restriction site mapping and subcloning in plasmid DNA vectors was performed according to Maniatis et al. [5] and references therein.

2.2. Library construction

Genomic *Apis mellifera* DNA was partially digested with the restriction enzyme *Sau*3A. After size selection using NaCl density gradient centrifugation DNA of 15–20 kb in length was cloned in the *Bam*HI site of the EMBL4 phage λ vector [6].

2.3. Screening and hybridisation conditions

1.5×10^5 phages corresponding to 5 genomic equivalents were screened under low stringency hybridisation conditions at 37°C. The hybridisation buffer consisted of 43% deionized formamide, $5 \times$ SSC ($1 \times$ SSC is 0.15 M NaCl, 15 mM Na-citrate), $4 \times$ Denhardt's solution [7], 0.1% SDS, 0.1% Na-pyrophosphate, 20 μ g/ml tRNA and 50 μ g/ml heparin. The filters were washed twice for 30 min each at 50°C in $2 \times$ SSC.

2.4. DNA sequence determination

Phage M13 subcloned restriction fragments were sequenced using the chain termination method of Sanger et al. [8].

Correspondence address: B.T. Hovemann, Center for Molecular Biology Heidelberg, Im Neuenheimer Feld 282, D-6900 Heidelberg, FRG

The nucleotide sequences presented here have been submitted to the EMBL/GenBank database under the accession numbers: X52884 and X52885

3. RESULTS AND DISCUSSION

Intending to clone an EF-1 α gene from *Apis* we first performed genomic Southern hybridisations to obtain information whether a crosshybridisation approach with *Drosophila* EF-1 α sequences is feasible. We used a 0.6 kb *Bam*HI-*Pst*I fragment of the *Drosophila* EF-1 α ,F1 gene that covers the coding sequence from amino acid 52 to 251 and, thus, contains a portion of the most conserved part of EF-1 α [2]. Fig. 1 shows a Southern blot of *Eco*RI-digested *Apis* DNA in parallel with a control lane of *Eco*RI-digested *Drosophila* DNA. Since the *Drosophila* *Bam*HI-*Pst*I probe contains an internal *Eco*RI site, two fragments appear in the *Drosophila* DNA lane after hybridisation. Two positive signals also appear in the *Apis* DNA. We interpret this result as an indication that at least one, possibly two genes are present in the *Apis* genome that seem to be closely related to the *Drosophila* gene because of the strong hybridisation signal. The additionally observed faint band in the high molecular weight range might eventually result from weaker crosshybridisation with sequences that also contain the highly conserved GTP-binding domain which is present in the EF-1 α probe and other translation factors [14]. However, we cannot completely rule out the possibility that this signal indicates an additional elongation factor 1 α of *Apis* which shows only little sequence conservation.

In order to isolate an EF-1 α gene of *Apis* we screened a library of cloned genomic DNA fragments with the same *Drosophila* probe as used for Southern hybridisation. From several positive signals one phage, bef-1, was plaque purified, the DNA was isolated and mapped (Fig. 2). The recombinant contained neighbouring *Eco*RI fragments of 1.0 kb and 1.1 kb, the same size that had been observed in the genomic Southern analysis (Fig. 1), favoring the conclusion that a single copy of the *Drosophila* EF-1 α homologue is present in *Apis*. These two fragments were subsequently cloned in pUC18, mapped and smaller subfragments were then cloned in phage M13 and used for sequence determination according to Sanger et al. [8]. The DNA sequence between the *Cla*I and *Hind*III sites (see expanded map region in Fig. 2) revealed 77% sequence conservation when compared to the EF-1 α ,F1 *Drosophila* reading frame. The determined sequence is shown in Fig. 3. Surprisingly, a nucleotide sequence conservation of 77% is also revealed when compared to the second *Drosophila* elongation factor gene, EF-1 α ,F2 that by itself shares 78% homology with the EF-1 α ,F1 gene. Because of this high degree of sequence conservation we were able to assign the translation start and stop signal within the *Apis* sequence to nucleotide 365 and 2121, respectively. The interruption of the reading frame by two introns, like in the *Drosophila* EF-1 α ,F2 gene, is also corroborated from sequence comparison.

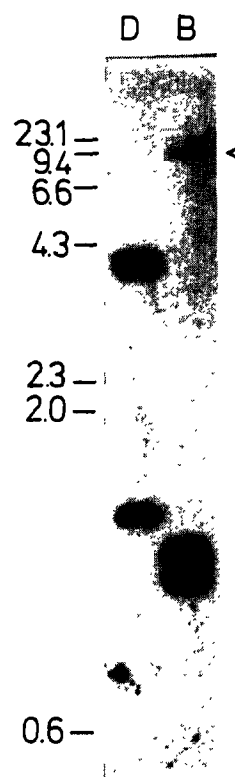


Fig. 1. Genomic Southern hybridisation with a *Drosophila melanogaster* EF-1 α gene fragment as probe. (Left lane) *Drosophila melanogaster* strain CantonS DNA (D). (Right lane) *Apis mellifera* DNA (B), both digested with *Eco*RI. The size marker is deduced from *Hind*III digested phage λ DNA that has been run in parallel. The open triangle indicates the position of weak hybridisation.

Within the *Apis* intron sequence – intron two has only partly been sequenced – no reasonable homology with the *Drosophila* genes is found. We conclude from sequence comparison with the *Drosophila* genes and several other EF-1 α sequences [9–17] (Fig. 4) that the identified *Apis* reading frame comprises a complete EF-1 α protein sequence. It consists of 461 amino acids and has a calculated molecular mass of 50.5 kDa. Since our interest was mainly focussed on the amino acid sequence of EF-1 α we made no further attempts to obtain additional nucleic acid sequence information.

We observe 49 and 44 amino acid differences between the *Apis* elongation factor and *Drosophila* EF-1 α ,F1 and EF-1 α ,F2, respectively. Nineteen of the exchanges between *Apis* and either one of the *Drosophila* sequences occur at identical positions. On the other hand, 19 *Drosophila* EF-1 α ,F1 sequence changes are present at positions where EF-1 α ,F2 and *Apis* are unchanged. Conversely, 14 times the EF-1 α ,F2 amino acid sequence changed leaving EF-1 α ,F1 and *Apis* identical. In 11 positions all 3 sequences are different (Fig. 4). In summary, about the same number of changes happened comparing each of the amino acid or nucleotide sequences with the two remaining ones.

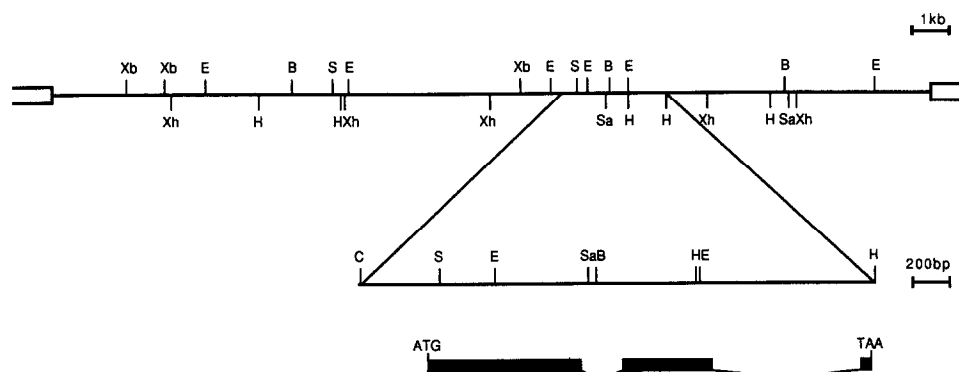


Fig. 2. Structure of the *Apis mellifera* EF-1 α gene locus as contained in phage bef-1. Restriction enzyme abbreviations are: B, *Bam*HI; C, *Clal*; E, *Eco*RI; H, *Hind*III; S, *Sal*I; Sa, *Sac*I; Xb, *Xba*I; Xh, *Xho*I. The EF-1 α gene region is expanded 5-fold between the *Clal* and the *Hind*III site. The additional *Clal* sites are not shown. The putative gene structure of the region that gives rise to the amino acid reading frame as revealed by sequence comparison with the *Drosophila melanogaster* EF-1 α genes is depicted. Filled boxes confine the open reading frame, lines represent intron sequences.

Two introns interrupt the reading frame in the genomic *Apis* sequence as well as in the *Drosophila* EF-1 α ,F2 sequence. However, only the position of the first intron is identical in *Apis* and *Drosophila* EF-1 α ,F2. In contrast, the EF-1 α ,F1 coding sequence is free of introns. Taken together, these observations lead us to conclude that *Drosophila* EF-1 α ,F2 and the *Apis* gene evolved from a common ancestor. The addition of the second intron at different positions in the *Apis* and the *Drosophila* EF-1 α gene may then be the result of a more recent insertion event.

Whether *Apis* also contains a second *cytoplasmic* elongation factor 1 α gene copy is still an open question. We consider this as less likely because of the insignificance of the hybridization to the high molecular weight fragment (Fig. 1). However, *nuclear* encoded mitochondrial elongation factors that are more related to the procaryotic gene type exhibit a low degree of sequence conservation to cytoplasmic EF-1 α genes and this might result in a weak crosshybridisation signal [13].

Our aim was to obtain additional information on the

```

1 ATCGATTAAA ACATATATGT GCATCTGGCC TTTATCTTGG GCGGAGCTTA TTTATATCGT GTCAAAAGAA AGTTGTAATC GAGAGTAAAA ATCTTAAAG
101 TTTAGATAAA AGGTGTATTA AAAAAAAAAA AAGCTATTTT TATACGGATA AATATATGTT AAGTTTCTTT TCTCTCTCTC TTCTCTCTC
201 TCTCTCTTTT CTCTTCTCTC CATATCAAA AGTAATTAAC CACATTGTGA AAGAATATC GCGGCGGACG CETAATCTGG TTTCACGAG GCGAGGACG
301 AATCGATAGC GAAAGCGGAG TATTCACGTA GGTGTGTAAT TTTCACGTA GCTAGAGCGT CGAC ATG GGT AAG GAG AAG ATT CAT ATA AAC
392 ATC GTG GTG ATC GGC CAC GTT GAT TCG GGC AAG TCG AGC ACT ACC GGT CAT CTG ATT TAC AAA TGC GGC GGC ATC GAG AAC
473 CGT ACC ATC GAA AAG TTC GAG AAG GAG GGT CAA GAA ATG GGC AAG GGC TCG TTC AAG TAC GCC TGG GTG TTG GAG AAC CTG
554 AAG GGT GAA CCG GAG GGT GGT ATC ACC ATC GAT ATC GCC CTG TGG AAG TTC GAG ACT GCG AAA TAT TAC TGC ACC ATC ATA
635 GAC GCG CCG GCG CAT CCG GAT TTC ATC AAA AAT ATG ATC ACC GCG ACG AGT CAG GCG GAT TGT GCG GTG TTA ATA GTC GCG
716 GGT GGT ATA GGT GAA TTC GAA GGT GGT ATC TCG AAG AAC GGA CAG ACC CCG GAA CAC GGT TTG CTG GCG TTC ACC GTT GCG
797 GTC AAG CAG TTG ATC GTT GGA GTG AAG AAC ATG GAT ATG ACC GAT CCG CCG TAT TCG GAG GCG GGT TTC GAG GAG ATC AAG
878 AAA GAG GTG TCA TCG TAC ATA AAA AAG ATC GGT TAC AAC ACA GCC TCG GCG TTC GTA CCG ATC TCC GGA TGG CAC GCG
959 GAC AAC ATG TTG GAA CCG CCG GAG AAC CCG TCG TAC AAG GGA TGG AAA GTG GAG CCG AAA GAT GGT AAC GGT GAC GGA
1040 AAG ACC CTG ATC GAA GCG CTC GAC GGT ATA TTA CCG CCG TCG AGA CCG ACC GAT AAG GGT CTT GGT TTA CCG CTT CAG GAC
1121 GTT TAT AAG ATC GGT GGT ATC GGT ACT GTA CCG GTC GGT CCG GTG GAG ACC GGT ATT TTG AAA CCA G GTATGGATCG ATATTAGAA
1208 CGGTGTTAAG CGTGGAGCTC CAGAGATCG AGGAATCTTT CGTTCTGTCG ATCTCTAAAA TTTATGAAT TTTAATGCTC CGTCCGACCG ATGTTTCCCT
1308 TCAGAAATATC ATCTGGAAGA AATTTCTTAG TTTTCTTTCT TAAAGTTTA AACTTTCATA TCAGATATCG CGCATTAATG TTGAATGTTG GACGACAG
1407 GT ATC CTG GTA ATC TTC GGT CCA GCA GCG CTC ACC ACC GAA GTG AAA TCG GTC GAG ATG CAT CAC GAG GGA TTG ACG GAG
1487 GCG TTA CCG GCG GAC AAC GTT GGC TTC AAC GTG AAG AAT TGT GTG AAA GAG TTG ACG GGT GGT TAC GTG GCG GGA GAT
1568 TCG AAA AAT CAA CCG CCA CCG GCG GCA GCG GAT TTC ACA GCG CAG GTG ATC GTC CTC AAT CAT CTT GCG GAG ATA ACG AAC
1649 GGT TAT ACA CCG GTG CTC GAT TGT CAC ACC GGT CAT ATC GGT TGC AAA TTT GCG GAG ATC AAG GAG AAA TGC GAC CCG CCG
1730 ACC GCG AAG ACC ACC GAG AAT CCG AAG ACC ATC AAG ACC GGA GAC GCG GCG ATC GTG ATG TTG CAA CCG ACT AAG CCG
1811 ATG TCG GTC GAT TCG GAG AAA TTC CCG CCG CTT GGT CCG TTC GCG GTT CCG GAG ATC CCG CAG ACT GTC GGT GTC GCG
1892 GTC ATT AAG GTACGCTTCT CTCTATTCCA TCTTTTTTTC TTAACTTCCA ACCTAATCAC GCATGAT... ATCGATATTC GACGATATAA
1991 AATATACGTA ACCTAATAAT TTTCGGAAAA AATATTAACA TTAAAAATTT CTTATGTTTC ATAG AGT GTC ACC TTT AAA GAT ACC CAG GCG
2082 AAG GTC ACA AAG GCG GCG GAG AAA GCG CAG AAG AAA AAG TAA CCATCAAGGC T
K V T K A A E K A Q K K K ***

```

Fig. 3. Sequence of the *Apis mellifera* EF-1 α gene. The amino acid reading frame as delineated by the putative translation start and stop codons is denoted by asterisks. Within the reading frame area intron sequences appear above the gaps of the deduced amino acid sequence. About 700 nucleotides of intron 2 were not determined.

10 20 30 40 50 60 70 80 90 100

1 MGKEKSHINVVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAWVLDKLAERERGITIDIALWKFETPKYQVTVIDAPGHRDFIK yeast I
1 MGKEKSHINVVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAWVLDKLAERERGITIDIALWKFETPKYQVTVIDAPGHRDFIK yeast II
1 MGKEKTHVNVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEFEKEAAELGKGSFKYAWVLDKLAERERGITIDIALWKFETPKYNVTVIDAPGHRDFIK Mucor racemosus I
1 MGKEKTHVNVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAWVLDKLAERERGITIDIALWKFETPKYNVTVIDAPGHRDFIK Mucor racemosus II
1 MGKEKTHVNVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAELGKGSFKYAWVLDKLAERERGITIDIALWKFETPKYNVTVIDAPGHRDFIK Mucor racemosus III
1 MGKEKIHINIVVIGHVDSGKSTTTGHLIYKCGSIDKRTIEKFEKEAQMKGSKFYAWVLDKLAERERGITIDIALWKFETAKYVTVIIDAPGHRDFIK Artemia salina
1 MGKEKIHINIVVIGHVDSGKSTTTGHLIYKCGSIDKRTIEKFEKEAQMKGSKFYAWVLDKLAERERGITIDIALWKFETAKYVTVIIDAPGHRDFIK D. melanogaster F1
1 MGKEKIHINIVVIGHVDSGKSTTTGHLIYKCGSIDKRTIEKFEKEAQMKGSKFYAWVLDKLAERERGITIDIALWKFETSKYVTVIIDAPGHRDFIK D. melanogaster F2
1 MGKEKIHINIVVIGHVDSGKSTTTGHLIYKCGSIDKRTIEKFEKEAQMKGSKFYAWVLDKLAERERGITIDIALWKFETAKYVTVIIDAPGHRDFIK Apis mellifera
1 MGKEKTHIKIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMKGSKFYAWVLDKLAERERGITIDISLWKFETSKYVTVIIDAPGHRDFIK Xenopus laevis
1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMKGSKFYAWVLDKLAERERGITIDISQKFKETSKYVTVIESPGHRDFIK mouse
1 MGKEKTHINIVVIGHVDSGKSTTTGHLIYKCGGIDKRTIEKFEKEAAEMKGSKFYAWVLDKLAERERGITIDISLWKFETSKYVTVIIDAPGHRDFIK human

101 NMITGTSQADCAILIIAGGVGEFEAGISKDQGTREHALLAFTLGVRQLIVAVNKMDSVK--WDSRFQEVKETSNIKKVGNPKTVFPVPSGWNQDN yeast I
101 NMITGTSQADCAILIIAGGVGEFEAGISKDQGTREHALLAFTLGVRQLIVAVNKMDSVK--WDSRFQEVKETSNIKKVGNPKTVFPVPSGWNQDN yeast II
101 NMITGTSQADCAILIIAGGTGEFEAGISKDQGTREHALLAFTLGFRQLIVAINKMDTTK--WSQDRYNEIVKEVSGFIKKIGFNPKSVPFVPSGWNQDN Mucor racemosus I
101 NMITGTSQADCAILIIAGGTGEFEAGISKDQGTREHALLAFTLGFRQLIVAINKMDTTK--WSQDRYNEIVKEVSGFIKKIGFNPKSVPFVPSGWNQDN Mucor racemosus II
101 NMITGTSQADCAILIIAGGTGEFEAGISKDQGTREHALLAFTLGVRQLIVAINKMDTTK--WSQDRYNEIVKEVSGFIKKIGFNPKSVPFVPSGWNQDN Mucor racemosus III
101 NMITGTSQADCAVLIIAAGVGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN Artemia salina
101 NMITGTSQADCAVQIDAAGTGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN D. melanogaster F1
101 NMITGTSQADCAVLIIAAGTGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN D. melanogaster F2
101 NMITGTSQADCAVLIIAAGTGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN Apis mellifera
101 NMITGTSQADCAVLIIAAGVGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN Xenopus laevis
101 NMITGTSQADCAVLIIAAGVGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN mouse
101 NMITGTSQADCAVLIIAAGVGEFEAGISKNQGTREHALLAYTLGVKQLIVGVNKMDSTEPFSEARFEEIKKEVSAYIKKIDYNPAAVFVPSGWNQDN human

199 MIEATTNAPWYKWEKETKAGVVGKTLLEAIDATEQPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVTFAPAGVTEVKSVMHHHEQLE yeast I
199 MIEATTNAPWYKWEKETKAGVVGKTLLEAIDATEQPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMVVTFAPAGVTEVKSVMHHHEQLE yeast II
199 MLDSTNMPWFKGNKWKETKAGSKTGKTLLEAIDATEPPVRPSDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKAGMVVTFAPAAVTEVKSVMHHHETLT Mucor racemosus I
199 MLDSTNMPWFKGNKWKETKAGSKTGKTLLEAIDATEPPVRPSDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKAGMVVTFAPAAVTEVKSVMHHHETLT Mucor racemosus II
199 MLDSTNMPWFKGNKWKETKAGSKTGKTLLEAIDATEPPVRPSDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKAGMVVTFAPAAVTEVKSVMHHHETLT Mucor racemosus III
201 MLEASDRLPWYKGNWIERKEGKADGKTLDAIDAILPPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVIKPGMIVTFAPANITTEVKSVMHHHEQLE Artemia salina
201 MLEPSTNMPWFKGMEVRKEGNADGKTLDAIDAILPPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGTVVVFAPANITTEVKSVMHHHEQLE D. melanogaster F1
201 MLEPSEKMPWFKGMSVERKEGKAGKCLIDALDAILPPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGMVVNFAPVNLTEVKSVMHHHEQLE D. melanogaster F2
201 MLEPSPKTPWYKGNWIERKGNADGKTLLEAIDAILPPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGMVTFAPAAVTEVKSVMHHHEQLE Apis mellifera
201 MLEPSPNMPWFKGKWKIRKEGSGGTLLEALDAILPPSRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGMVTFAPVNVTEVKSVMHHHEQLE Xenopus laevis
201 MLEPSANMPWFKGKWKIRKDSAVAPTLEALDAILPP--RPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGMVTFAPVNVTEVKSVMHHHEQLE mouse
201 MLEPSANMPWFKGKWKIRKDSAGTTLLEALDAILPPTRPTDKPLRLPLQDVYKIGGIGTVPVGRVETGVLPKGMVTFAPVNVTEVKSVMHHHEQLE human

299 QGVPDGNVGFNVKNVSVKEIRRGNVCGDAKNPPKGCASFNATVIVLNHPGQISAGYSPVLDCHTAHIAACRFDELLEKNDRSGKKLEDHPKFLKSGDAA yeast I
299 QGVPDGNVGFNVKNVSVKEIRRGNVCGDAKNPPKGCASFNATVIVLNHPGQISAGYSPVLDCHTAHIAACRFDELLEKNDRSGKKLEDHPKFLKSGDAA yeast II
299 EGLPGDGNVGFNVKNVSVKDIRRGNVCGDAKNPPKGCASFNATVIVLNHPGQISAGYAPVLDCHTAHIAACKFSELIEKIDRRSGKKMEDSPKFVKSQDAA Mucor racemosus I
299 EGLPGDGNVGFNVKNVSVKDIRRGNVCGDAKNPPKGCASFNATVIVLNHPGQISAGYAPVLDCHTAHIAACKFSELIEKIDRRSGKKMEDSPKFVKSQDAA Mucor racemosus II
299 EGLPGDGNVGFNVKNVSVKDIRRGNVCGDAKNPPKGCASFNATVIVLNHPGQISAGYAPVLDCHTAHIAACKFSELIEKIDRRSGKKMEDSPKFVKSQDAA Mucor racemosus III
301 QASPGDGNVGFNVKNVSVKELRRGYVAGDSKNPPARGSDFFAQVIVLNHPGQISNGYTPVLDCHTAHIAACKFAEIEKCDRRTGKTTEAPKFIKSGDAA Artemia salina
301 EAVPGDGNVGFNVKNVSVKELRRGYVAGDSKANPPKGAADFTAQVIVLNHPGQISNGYTPVLDCHTAHIAACKFAEIEKCDRRTGKTTEENPKFIKSGDAA D. melanogaster F1
301 EAMPDGNVGFNVKNVSVKELRRGYVAGDSKNPPKGAADFTAQVIVLNHPGQISNGYTPVLDCHTAHIAACKFAEIEKCDRRTGKTTEENPKFIKSGDAA D. melanogaster F2
300 EALPGDGNVGFNVKNVSVKELRRGYVAGDSKNPPKGAADFTAQVIVLNHPGQISNGYTPVLDCHTAHIAACKFAEIEKCDRRTGKTTEENPKFIKSGDAA Apis mellifera
301 EAVPGDGNVGFNVKNVSVKDVRRGNVAGDSKNPPMEAGSFTAQVIVLNHPGQISAGYAPVLDCHTAHIAACKFAELKEKIDRRSGKKLEDNPKFLKSGDAA Xenopus laevis
300 EALPGDGNVGFNVKNVSVKDVRRGNVAGDSKNPPMEAGSFTAQVIVLNHPGQISAGYAPVLDCHTAHIAACKFAELKEKIDRRSGKKLEDNPKFLKSGDAA mouse
301 EALPGDGNVGFNVKNVSVKDVRRGNVAGDSKNPPMEAGSFTAQVIVLNHPGQISAGYAPVLDCHTAHIAACKFAELKEKIDRRSGKKLEDNPKFLKSGDAA human

399 LVKFVPSKPMCEAFSEYPLGRFAVRDMRQTVAVGVKISVDKTEK-AAKVTKAAQKAAK-K* yeast I
399 LVKFVPSKPMCEAFSEYPLGRFAVRDMRQTVAVGVKISVDKTEK-AAKVTKAAQKAAK-K* yeast II
399 IVKMVPSKPMCEAYTDYPLGRFAVRDMRQTVAVGVKAVEKVDK-AGKVTKAAKAAK-K* Mucor racemosus I
399 IVKMVPSKPMCEAYTDYPLGRFAVRDMRQTVAVGVKAVEKVDK-AGKVTKAAKAAK-K* Mucor racemosus II

399	IVKMVPSKPMCV EAYTDY PPLGRFAVRDMRQTVA VGV I KAVEKVDK-AGKVT KAAAKASK-K*	<i>Mucor racemosus</i> 111
401	MITLVPSKPLC V EAFSDFPPLGRFAVRDMRQTVA VGV I KSVNFKDPTAGKVT KAAEKAGK-KK*	<i>Artemia salina</i>
401	IVNLVPSKPLC V EAFQEFPLGRFAVRDMRQTVA VGV I KAVNFKDASGGKVT KAAEKATKGKK*	<i>D. melanogaster</i> F1
401	IIVLVPSKPLC V EAFQEFPLGRFAVRDMRQTVA VGV I KSVNFKETTSGKVT KAAEKAGK-KK*	<i>D. melanogaster</i> F2
397	IVMLQPTKPMCV EAFQEFPLGRFAVRDMRQTVA VGV I KSVTFKDT-QGKVT KAAEKAGK-KK*	<i>Apis mellifera</i>
401	IVDMIPGKPMCV EFSFSDY PPLGRFAVRDMRQTVA VGV I KAVEKKAAGSGKVT KSAQKAATK*	<i>Xenopus laevis</i>
400	IVDMVPGKPMCV EFSFSDY PPLGRFAVRDMRQTVA VGV I KAVDKKAAGAGKVT KSAQKAQKAK*	mouse
401	IVDMVPGKPMCV EFSFSDY PPLGRFAVRDMRQTVA VGV I KAVDKKAAGAGKVT KSAQKAQKAK*	human
	* * * * *	

Fig. 4. Comparison of the amino acid sequences as deduced from published eucaryotic EF-1 α gene sequences [9–17].

degree and position of sequence conservation in EF-1 α . Since our previous comparison [2] a complete mouse and *Xenopus laevis* sequence have been published [16,17]. With the *Apis mellifera* sequence now determined by us we can compare sequences derived from a wide spectrum of the evolutionary tree that ranges from yeasts to mammals. This new comparison further reveals an extreme sequence conservation. Even by including 12 elongation factor sequences of 8 different species in our comparisons, the *Apis mellifera* sequence differences are, with two exceptions in amino acid 186 and 315, restricted to positions that had already found to be different between the as yet examined species.

Acknowledgements: We are grateful to W. Gehring for support and I. Sures for helpful criticism in the preparation of this manuscript. This work was supported by the Deutsche Forschungsgemeinschaft (Wa 556/1-1 and 1-2 to U.W. and SFB 229/C3 to B.H.).

REFERENCES

- [1] Kazi, Y. (1978) *Biochim. Biophys. Acta* 505, 95–127.
- [2] Hovemann, B., Richter, S., Walldorf, U. and Cziepluch, C. (1988) *Nucleic Acids Res.* 16, 3175–3194.
- [3] Walldorf, U., Richter, S., Ryseck, R.-P., Steller, H., Edström, J.E., Bautz, E.K.F. and Hovemann, B. (1984) *EMBO J.* 3, 2499–2504.
- [4] Blin, N. and Stafford, D.W. (1976) *Nucleic Acids Res.* 3, 2303–2308.
- [5] Maniatis, T., Fritsch, E.F. and Sambrook, J. (1982) *Molecular Cloning: A Laboratory Handbook*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- [6] Frischauf, A.-M., Lehrach, H., Poustka, A. and Murray, N. (1983) *J. Mol. Biol.* 170, 827–842.
- [7] Denhardt, D.T. (1966) *Biochem. Biophys. Res. Commun.* 23, 641–646.
- [8] Sanger, F., Nicklen, S. and Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* 74, 5463–5467.
- [9] Nagata, S., Nagashima, K., Tsunetsugu-Yokota, Y., Fujimura, K., Miyazaki, M. and Kazi, Y. (1984) *EMBO J.* 3, 1825–1830.
- [10] Schirmaier, F. and Philippsen, P. (1984) *EMBO J.* 3, 3311–3315.
- [11] Cottrelle, P., Thiele, D., Price, V.L., Memet, S., Micouin, J.-Y., Marck, C., Buhler, J.-M., Sentenac, A. and Fromageot, P. (1985) *J. Biol. Chem.* 260, 3090–3096.
- [12] Sundstrom, P., Lira, L.M., Choi, D., Linz, J.E. and Sypherd, P. (1987) *Nucleic Acids Res.* 15, 9997–10006.
- [13] Van Hemert, F.J., Amons, R., Pluijms, W.J.M., Van Ormondt, H. and Möller, W. (1984) *EMBO J.* 3, 1109–1113.
- [14] Brands, J.H.G.M., Maassen, J.A., Van Hemert, F.J., Amons, R. and Möller, W. (1986) *Eur. J. Biochem.* 155, 167–171.
- [15] Roth, W.W., Bragg, P.W., Corrias, M.V., Reddy, N.S., Dholakia, J.N. and Wahba, A.J. (1987) *Mol. Cell. Biol.* 7, 3929–3936.
- [16] Lu, X. and Werner, D. (1989) *Nucleic Acids Res.* 17, 442.
- [17] Krieg, P.A., Varnum, S.M., Wormington, W.M. and Melton, D.A. (1989) *Dev. Biol.* 133, 93–100.