FEBS Letters 464 (1999) 148–152 FEBS 23122

Molecular cloning and characterization of the *Arabidopsis thaliana* α-subunit of elongation factor 1B

François Héricourt, Isabelle Jupin*

Laboratoire de Virologie Moléculaire, Institut Jacques Monod, UMR 7592, CNRS-Universités Paris 6 & Paris 7, 2 place Jussieu, 75251 Paris Cedex 05, France

Received 27 September 1999; received in revised form 25 November 1999

Edited by Marc Van Montagu

Abstract Using a PCR-based approach, we have isolated two *Arabidopsis thaliana* cDNA clones (α1 and α2) encoding the α-subunit of translation elongation factor 1B (eEF1Bα). They encode open reading frames of 228 and 224 amino acids respectively, with extensive homology to eEF1Bα subunits from different organisms, particularly in the C-terminal half of the protein. They both lack a conserved phosphorylation site that has been implicated in regulating nucleotide exchange activity. Using a plasmid shuffling experiment, we demonstrated that both α1 and α2 clones are able to complement a mutant yeast strain deficient for the eEF1Bα subunit. This provides evidence that *Arabidopsis* encodes at least two functional isoforms of this subunit, termed eEF1Bα1 and eEF1Bα2. A third cDNA clone was isolated that appeared to result from an alternative splicing event of the eEF1Bα1 gene.

Key words: Elongation factor; α-Subunit of translation elongation factor 1B; cDNA; Functional complementation; *Arabidopsis thaliana*

1. Introduction

Elongation factor 1 (eEF1, the old designation being EF-1) plays a central role in the elongation step of protein biosynthesis (reviewed in [1]). It catalyzes the GTP-dependent binding of aminoacyl-tRNA to the aminoacyl site on ribosomes concomitant with the hydrolysis of GTP. Elongation factors from a wide variety of eukaryotic cells are very similar to each other in structure and function [2]. eEF1 from various eukaryotes such as wheat and *Artemia salina* is composed of four different subunits, eEF1A, eEF1Bα, eEF1Bβ and eEF1Bγ [3]. eEF1A reacts with GTP and aminoacyl-tRNA to form a ternary complex, while eEF1Bαβγ catalyzes the exchange of GDP bound to eEF1A with exogenous GTP and stimulates the eEF1A-dependent aminoacyl-tRNA binding to ribosomes.

Compared with eEF1A (the old designation being EF-1α), there is a paucity of knowledge concerning the eEF1B subunits which are required for recycling eEF1A-GDP [1]. The subunits eEF1Bα (previously designated EF-1β' in plants and EF-1β in animals) and eEF1Bβ (previously designated EF-1β in plants and EF-1∂ in animals) consist of two similar, but non-identical proteins with indistinguishable guanine nucleotide exchange activities [3] that differ in their ability to interact with eEF1Bγ (previously designated EF-1γ) [4]. The latter subunit is suggested to 'anchor' the eEF1 complex in the endoplasmic reticulum [5].

While sequences for eEF1A cDNAs have been reported in many plant species including *Arabidopsis thaliana* (reviewed in [6]), less information is available regarding the genes encoding the eEF1B subunits. Amino acid sequences of plant cDNAs for the eEF1Bα subunit have been described only in rice and wheat [7,8], those corresponding to eEF1Bβ subunit have been reported in rice and *Arabidopsis* [9,10], while only the rice eEF1Bγ cDNA sequence is available so far [11]. Although a number of expressed sequence tags from various plant species displaying homologies with these sequences can be found in sequence databases, evidence for their functionality is still lacking.

In order to enlarge our knowledge about plant eEF1B subunits, we have cloned and analyzed cDNAs encoding *A. thaliana* eEF1Bα. We show that there exist at least two genes encoding this subunit and that both are able to complement the corresponding yeast mutant, demonstrating that two isoforms of this subunit are present and functional in *A. thaliana*.

2. Materials and methods

2.1. Database search and primer design

Using the rice and wheat eEF1Bα amino acid sequences [7,8], a search was performed within the *A. thaliana* database (genome-www.stanford.edu/Arabidopsis) using the TBLASTX program. A 80 kb genomic clone named MXC9 and located on chromosome 5 (accession number AB007727) was retrieved, whose deduced protein sequence displayed high homology with wheat and rice eEF1Bα subunits (e numbers ranging from $6.3\times e^{-36}$ to $6.2\times e^{-37}$). Because the N- and C-termini of the protein sequences were highly conserved, specific primers were designed to PCR amplify the corresponding *Arabidopsis* open reading frame within a cDNA library. Primer 1 CCGGGATCCCAATGGCGGTTACATTTTCTGAT and primer 2 CTGAATTCTGTCTAAATCTTGTTAAAAGCGAC correspond respectively to nucleotides 22 985–23 007 (top strand) and 24 239–24 216 (bottom strand) of the genomic clone MXC9 and contain *Bam*HI and *Eco*RI sites, respectively (underlined).

2.2. PCR and cloning

The *A. thaliana* Matchmaker cDNA library (Clontech), containing 3×10^6 independent clones, was used as a template. The PCR reaction was performed in a total volume of 100 μl containing 200 ng of template, 0.75 μM of each primer, 0.2 mM dNTP and 5 U of Pfu polymerase (Stratagene). After a 5 min denaturation step at 94°C, 25 cycles were performed, consisting of denaturation at 94°C for 30 s, annealing at 56°C for 1 min and elongation at 72°C for 2 min. The amplified fragments were separated on a 1% agarose gel, purified, digested with *Bam*HI and *Eco*RI restriction enzymes and subcloned into the similarly restricted pACT2 vector (Clontech).

2.3. Sequence analysis and sequence comparisons

DNA sequencing was performed on an ABI PRISM 377 DNA

*Corresponding author. Fax: (33)-1-44 27 35 80.
E-mail: jupin@ccr.jussieu.fr

PII: S0014-5793(99)01694-4

sequencer (Applied Biosystem) using a BigDye Terminator Sequencing kit (Applied Biosystem).

Protein alignments were performed with the CLUSTAL W program of the expasy package (www.expasy.ch/tools) and viewed with the BOXSHADE program (franklin.burnham-inst.org/box).

2.4. Functional complementation assay by plasmid shuffling

The LEU2 expression vector pACT2Δ was constructed by digesting the pACT2 plasmid (Clontech) with *Hin*dIII followed by religation, in order to eliminate a 758 bp fragment encoding the Gal4 activation domain. Cloning of the *A. thaliana* eEF1Bα cDNA clones in pACT2Δ, between the yeast ADH1 promoter and terminator, was performed by homologous recombination in yeast [12]. Primers 3 TCAAGCTATACCAAGCATACAATCAACTCCAAGCTTATGGCGGTTACATTTTCTGATCT and 4 ACTTGACCAAACCTCTGGCGAAGAAGTCCAAAGCTTCTAAATCTTGTTAAAAGCGACAAT were used to PCR amplify the *Arabidopsis* α1, α2 and α3 cDNA clones. These primers contain 24 nucleotides identical to the 5′ and 3′ ends of the *A. thaliana* eEF1Bα cDNA clones, respectively, and 36 nucleotides homologous to pACT2Δ (underlined) to promote integration in the vector. 500 ng of PCR products was then transformed together with 500 ng of *Hin*dIII-linearized pACT2Δ vector into the JWY4200 yeast strain (MATa ura3-52 trp1-Δ101 lys2-801 leu2-Δ1 his3-Δ200 tef5::TRP1 [pJWB2937 TEF5 URA3 CEN4] [13]). Transformants were selected on a medium lacking leucine and uracil (−LU) and the presence of the expected plasmid was confirmed by PCR amplification using pACT2Δ-specific primers followed by a digest with restriction enzymes (*Hae*III and *Sma*I) that discriminate between α1 and α2 cDNA clones. One colony of each yeast transformant was then streaked onto a medium containing 1 g/l of 5-fluoroorotic acid (5FOA) and was grown at 30°C for 3 days to promote the loss of the URA3 plasmid pJWB2937 expressing yeast TEF5.

3. Results and discussion

3.1. PCR-based isolation of A. thaliana eEF1Bα cDNAs

Database searches with the amino acid sequence of rice and wheat eEF1Bα [7,8] led to the identification of a fragment of *A. thaliana* chromosome 5, named MXC9, that putatively contained the coding sequence for the *Arabidopsis* homologue of this subunit. Based on this homology, specific primers were designed and PCR amplifications were performed using an *Arabidopsis* cDNA library as template. Two PCR products

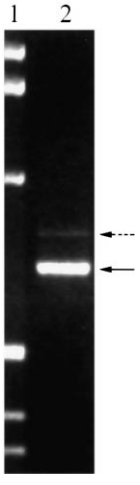

Fig. 1. PCR amplification of the *A. thaliana* cDNA library using EF1Bα-specific primers. Lane 1: DNA molecular weight markers (1 kb ladder, Life Technologies); lane 2: DNA products obtained after PCR amplification of the *Arabidopsis* cDNA library with primers 1 and 2. The fragments of ca. 0.7 kb and 0.8 kb are indicated by a full arrow and a dashed arrow, respectively.

```
A.
  1 caatggcggttacattttctgatctccacacagagcgaggtctcaaaaccctcgaggaa
      M  A  V  T  F  S  D  L  H  T  E  R  G  L  K  T  L  E  E
 61 catctcgccggtaaaacgtacatctccggcgatcagctttcggttgatgatgtgaaggt
     H  L  A  G  K  T  Y  I  S  G  D  Q  L  S  V  D  D  V  K  V
121 ttacgctgccgtattggagaatccaggtgatggttttcccaatgctagcaagtggtacg
      Y  A  A  V  L  E  N  P  G  D  G  F  P  N  A  S  K  W  Y  D
181 attctgttgcttctcacctagctaaaagttttcccgggaaagcagacggagtgagagtt
      S  V  A  S  H  L  A  K  S  F  P  G  K  A  D  G  V  R  V
241 ggtggtggtgttgctcctccatctgaagctcatccacatactgaggaacctgctgctga
     G  G  G  V  A  P  P  S  E  A  H  P  H  T  E  E  P  A  A  D
301 tggtgatggtgatgatgatgatattgatcttttcgctgatgagactgaagatgaga
      G  D  G  D  D  D  D  D  I  D  L  F  A  D  E  T  E  D  E  K
361 aaaaagctgctgaagagagagaagctgctaagaaagatactaagaagactaaagagagt
      K  A  A  E  E  R  E  A  A  K  K  D  T  K  K  T  K  E  S
421 ggaaaatcttctgtgctactggaagtaaaaccatgggatgatgaaaccgacatgaagaa
     G  K  S  S  V  L  L  E  V  K  P  W  D  D  E  T  D  M  K  K
481 gttagaagaagctgttcgtagtgttcagatgccaggtcttacatggggagcttcgaaat
      L  E  E  A  V  R  S  V  Q  M  P  G  L  T  W  G  A  S  K  L
541 tggtacctgttggttacgggataaagaaactcacaatcatgatgacaattgttgatgac
      V  P  V  G  Y  G  I  K  K  L  T  I  M  M  T  I  V  D  D
601 cttgtgtctgtggacaacctcattgaagaccatctcacctcagagcctaacaacgagta
     L  V  S  V  D  N  L  I  E  D  H  L  T  S  E  P  N  N  E  Y
661 catccaaagtgtcgatattgtcgcttttaacaagatttagaca
      I  Q  S  V  D  I  V  A  F  N  K  I  *

B.
  1 caatggcggttacattttctgatctacacacagaggagggtgtcaaatccgtggaggag
      M  A  V  T  F  S  D  L  H  T  E  E  G  V  K  S  V  E  E
 61 cacctcgccggaaaaacctacatctccggagatcaattgtctgtggatgatgtcaaggt
     H  L  A  G  K  T  Y  I  S  G  D  Q  L  S  V  D  D  V  K  V
121 ttacgctgccgttccagtgaaacccagcgatgccttccccaatgctagcaagtggtacg
      Y  A  A  V  P  V  K  P  S  D  A  F  P  N  A  S  K  W  Y  E
181 agtccgtggcttcccaacttgctaaaagtttccctggaaaggccgttggagtacaattc
      S  V  A  S  Q  L  A  K  S  F  P  G  K  A  V  G  V  Q  F
241 ggtggctctgctgctgctccagctgttgaagctgaggcacctgcagctgcagctga
     G  G  S  A  A  A  A  P  A  V  E  A  E  A  P  A  A  A  A  D
301 tgatgatgatgacatggatctctttggtgacgagaccgaagaagaaaagaaagctgcag
      D  D  D  D  M  D  L  F  G  D  E  T  E  E  E  K  K  A  A  E
361 aggagagggaggctgctaagaaggacaccaagaagcctaaagagagtggaaagtcttct
      E  R  E  A  A  K  K  D  T  K  K  P  K  E  S  G  K  S  S
421 gtgctcatggatgttaagccatgggatgatgaaaccgacatgaagaaactggaagaggc
     V  L  M  D  V  K  P  W  D  D  E  T  D  M  K  K  L  E  E  A
481 tgttcgtggtgttgagatgcctggtcttttctggggagcctcaaaacttgtaccagttg
      V  R  G  V  E  M  P  G  L  F  W  G  A  S  K  L  V  P  V  G
541 gttatgggatcaagaaactcacaattatgttcacgattgttgatgacctcgtgtccccg
      Y  G  I  K  K  L  T  I  M  F  T  I  V  D  D  L  V  S  P
601 gacaacctcattgaagacttcctcacctcagagcctaacaacgagtacatccagagttg
     D  N  L  I  E  D  F  L  T  S  E  P  N  N  E  Y  I  Q  S  C
661 tgacattgtcgcttttaacaagatttagaca
      D  I  V  A  F  N  K  I  *
```

Fig. 2. Nucleotide and deduced amino acid sequences of α1 (A) and α2 (B) cDNA clones. The nucleotide sequence of the primers used to PCR amplify the cDNAs is boxed. The stop codons are indicated by asterisks. The EMBL accession numbers for α1 and α2 cDNAs are AJ249596 and AJ249597, respectively.

of ca. 700 and 800 bp were obtained, the former being the most abundant (Fig. 1). After subcloning these products into the pACT2 vector, the sequence analysis of two independent clones corresponding to the 700 bp PCR product revealed the existence of two different types of cDNA molecules, that we designated α1 and α2. Although these molecules are highly homologous, they can be differentiated by their restriction pattern using *Eco*RI and *Sma*I restriction enzymes. Based on this restriction polymorphism, 12 additional clones were analyzed and were found to correspond to α1 and α2 cDNAs in equal proportion (data not shown). Cloning and sequencing of the 800 bp fragment revealed the existence of a third type of cDNA molecule, that we designated α3.

The nucleotide and deduced amino acid sequences of α1 and α2 cDNAs are presented in Fig. 2. By sequence comparison, we were able to assign α1 cDNA to the genomic sequence previously retrieved from the *Arabidopsis* database.

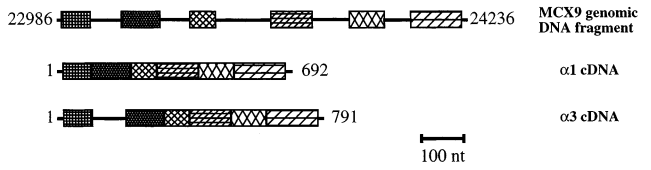

Fig. 3. Comparison of MXC9 genomic DNA fragment with α1 and α3 cDNA clones. Exons are represented by shaded boxes. The numbers refer to the nucleotide positions in the MXC9 genomic DNA fragment or in the cloned cDNAs. The EMBL accession number for α3 cDNA is AJ249598.

Five introns were identified (Fig. 3), whose splice sites are in good match with the *Arabidopsis* intron splice site consensus sequence [14], and whose length, ranging from 85 to 169 nucleotides, is in good agreement with the standard length of *Arabidopsis* introns (ca. 100 nucleotides) [14]. Because the PCR-amplified fragment was limited to the coding sequence of eEF1Bα, the 5′ and 3′ non-coding regions of the different cDNAs are therefore not available.

The protein encoded by α1 is 228 amino acids long with a calculated molecular weight of 24 788 Da, while α2 encodes a slightly shorter protein product (224 amino acids) with a calculated molecular weight of 24 201 Da. Both proteins are extremely similar to each other (93% similarity and 81% identity) and present higher homology to wheat and rice eEF1Bα subunits (ranging from 83% to 88%) than to the *Arabidopsis* eEF1Bβ subunit (81%) (Table 1). This extensive homology strongly suggests that these two cDNA clones encode the eEF1Bα subunit of *A. thaliana*. The cloning of these two cDNAs therefore provides evidence that at least two genes

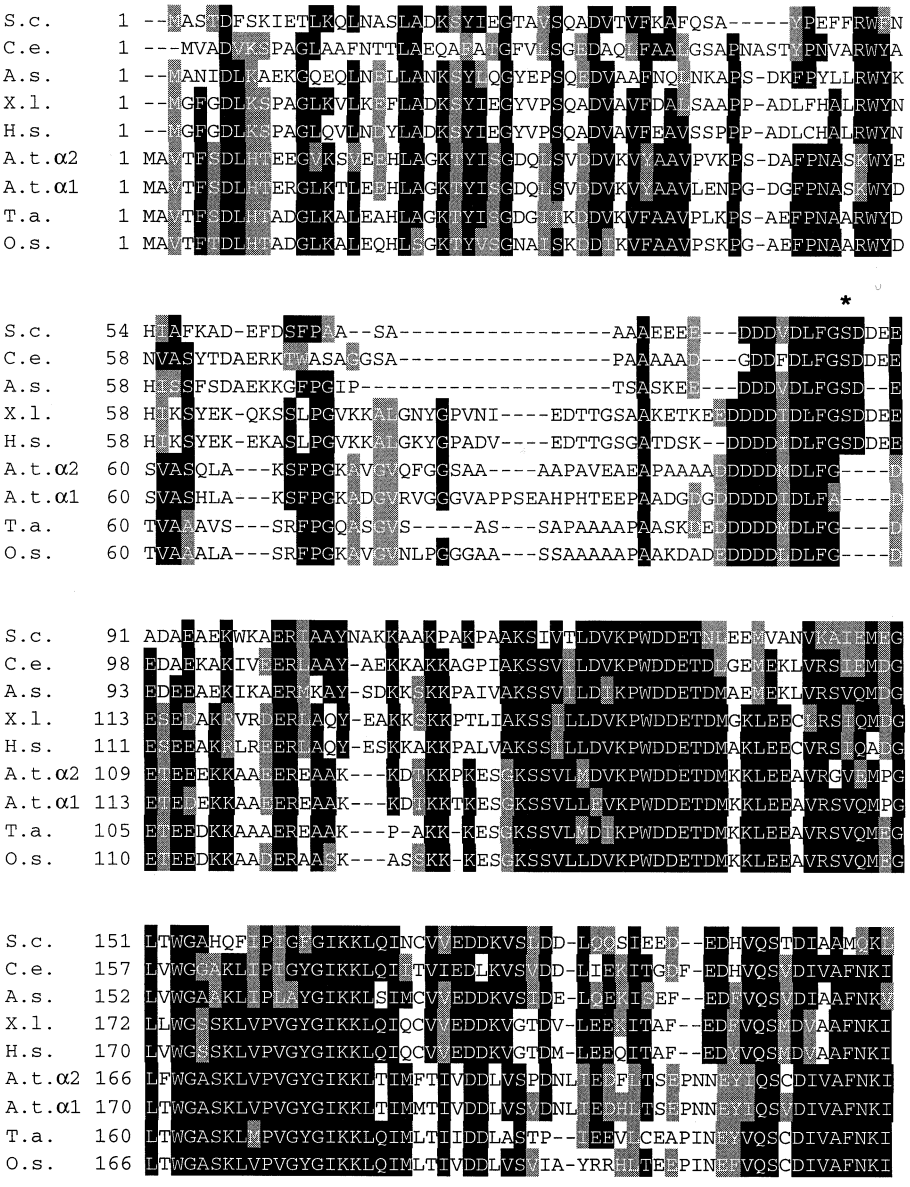

Fig. 4. Amino acid sequence comparison of eEF1Bα subunits from different organisms. Sequence alignment was performed with CLUSTAL W and viewed with BOXSHADE. Regions of identity (black-shaded) and similarity (gray-shaded) are indicated. The serine residue that is phosphorylated in *A. salina* eEF1Bα [15] is indicated by an asterisk. Sequences used in the alignment are from *S. cerevisiae* (S.c.), *C. elegans* (C.e.), *A. salina* (A.s.), *X. laevis* (X.l.), *H. sapiens* (H.s.), *A. thaliana* (A.t.α1 and A.t.α2), *T. aestivum* (T.a.) and *O. sativa* (O.s.). The accession numbers are identical to those described in Table 1.

are present in this plant. We named the corresponding proteins eEF1Bα1 and eEF1Bα2.

α3 cDNA, which is present in lower amounts in the cDNA library (Fig. 1), appeared to derive from the same genomic sequence as α1. However, it is likely to result from a partial maturation process of the α1 mRNA or from an alternative splicing event since it contains 99 additional nucleotides that are identical to the first intron of the corresponding genomic sequence MXC9 (Fig. 3). Because the presence of this unspliced intron generates a stop codon prematurely in the coding sequence, α3 cDNA encodes a putative truncated protein of 29 amino acids.

3.2. Comparison of amino acid sequences from different organisms

Table 1 presents the overall homology and identity levels between the eEF1Bα subunits of *Arabidopsis* and the eEF1Bα and eEF1Bβ subunits from other organisms. Although the highest homology was observed between *Arabidopsis* eEF1Bα1, eEF1Bα2 and wheat and rice eEF1Bα subunits, extensive homology (between 66% and 71%) was also observed upon comparison with eEF1Bα subunits from other organisms. The extent of homology with eEF1Bβ subunits from different organisms was also significant although weaker than the corresponding eEF1Bα subunit.

As shown in Fig. 4, the C-terminal half of the protein (residues 138–228 of *Arabidopsis* eEF1Bα1) corresponds to the most conserved region. Because this domain of *A. salina* eEF1Bα has been shown to retain the guanine nucleotide exchange activity [3], it is likely that both *Arabidopsis* eEF1Bα1 and eEF1Bα2 subunits possess a similar function in the GDP/GTP exchange reaction.

It has been demonstrated previously that phosphorylation of *A. salina* eEF1Bα by casein kinase II on the Ser-89 residue affects the GDP/GTP exchange rate on eEF1A [15]. Interestingly, this target serine residue is conspicuously missing in *Arabidopsis* eEF1Bα1 and eEF1Bα2 (Fig. 4), as observed previously in wheat and rice eEF1Bα subunits [7,8]. This observation suggests that, similarly to the wheat eEF1Bα [16,17], the *Arabidopsis* eEF1Bα subunits are probably not phosphorylated by casein kinase II. Whether plant eEF1 relies on other different regulatory mechanisms remains to be determined.

Table 1
Percentage of homology (and percentage of identity) between the eEF1Bα subunits of *Arabidopsis* and eEF1Bα and eEF1Bβ of different organisms

eEF1Bα	*Arabidopsis* eEF1Bα1	*Arabidopsis* eEF1Bα2
Wheat	83.3 (67.1)	85.7 (69.6)
Rice	86.4 (69.3)	88.0 (68.9)
Yeast	69.4 (36.7)	66.7 (37.7)
Nematode	70.8 (42.9)	71.4 (43.7)
Brine shrimp	69.8 (42.2)	67.1 (41.7)
Xenopus	70.5 (44.9)	70.0 (43.8)
Human	71.3 (45.7)	70.0 (43.5)
eEF1Bβ		
Arabidopsis	81.5 (56.5)	81.5 (57.3)
Rice β1	84.3 (59.4)	84.7 (60.7)
Rice β2	84.7 (58.5)	86.3 (59.3)
Brine shrimp	64.2 (37.0)	66.0 (38.4)
Xenopus	65.3 (35.0)	62.3 (36.5)
Human	61.6 (35.2)	56.0 (32.7)

Sequences (Swiss-Prot accession numbers) used in this comparison are from *Triticum aestivum* eEF1Bα (P29546), *Oryza sativa* eEF1Bα and eEF1Bβ (P29545 and Q40680), *Saccharomyces cerevisiae* eEF1Bα (P32471), *Caenorhabditis elegans* eEF1Bα (P34460), *A. salina* eEF1Bα and eEF1Bβ (P12262 and P32192), *Xenopus laevis* eEF1Bα and eEF1Bβ (P30151 and P29693), *Homo sapiens* eEF1Bα and eEF1Bβ (P24534 and P29692) and *A. thaliana* eEF1Bβ (P48006).

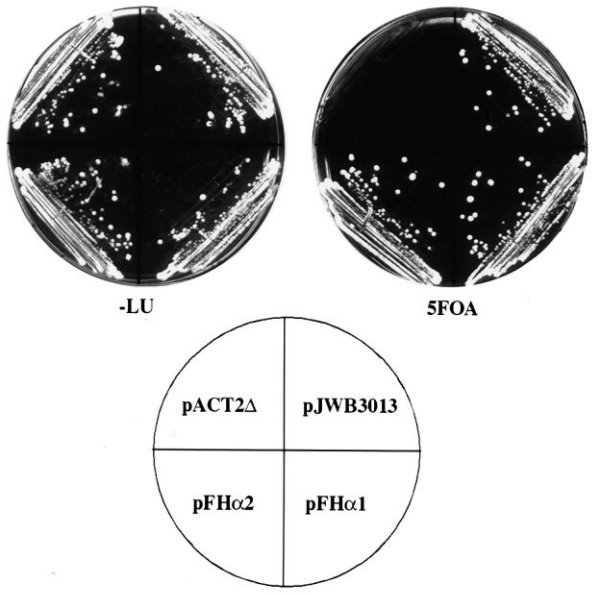

Fig. 5. Complementation assay of the yeast tef5 mutant strain. The yeast tef5 mutant strain JWY4200 complemented with the URA3 vector pJWB2937 expressing the yeast TEF5 gene was transformed with LEU2 vectors pFHα1 and pFHα2 expressing α1 and α2 cDNA clones of *Arabidopsis*, respectively. Control experiments were performed using the empty vector pACT2Δ, or the LEU2 vector pJWB3013 that expresses the yeast TEF5 gene. One colony of each transformant was streaked onto −LU and 5FOA plates and incubated for 3 days at 30°C.

3.3. Functional complementation of a yeast mutant

To gain a further insight into the role of these different *Arabidopsis* eEF1Bα subunits, we investigated whether they were functional in yeast, by assaying their ability to complement a mutant yeast strain deficient for this subunit. We made use of the yeast strain JWY4200 [13] that is disrupted in the TEF5 gene encoding the yeast eEF1Bα subunit. Because disruption of this gene is lethal, the mutant strain is rescued by the TEF5 gene carried on an autonomous URA3 vector pJWB2937. We therefore tested whether expression of *Arabidopsis* α1 and α2 cDNA clones was able to rescue the strain JWY4200, after promoting the loss of the vector pJWB2937. This technique, known as plasmid shuffling [18], is based on the counter-selection of yeast transformed with a vector harboring the URA3 marker gene, by plating them on a medium containing 5FOA.

For this purpose, the expression vectors pFHα1 and pFHα2 carrying the *Arabidopsis* α1 and α2 cDNA clones, respectively, under the control of the yeast ADH1 promoter, were constructed. They derive from the pACT2Δ vector and possess the LEU2 marker gene. Each expression vector was transformed into the tef5 mutant yeast strain containing the pJWB2937 plasmid and transformants were selected by plating the cells on a medium lacking leucine and uracil (−LU). Control experiments were performed using the empty vector

pACT2Δ or the plasmid pJWB3013 that expresses the yeast TEF5 gene and the LEU2 marker gene. Loss of the vector pJWB2937 was then promoted by streaking independent colonies of each transformant on 5FOA-containing medium, and the survival of the cells was observed after 3 days at 30°C.

We observed that the plasmids pFHα1 and pFHα2, expressing the *Arabidopsis* eEFIBα1 and eEFIBα2 subunits, respectively, were able to complement the yeast tef5 mutant strain, as did the plasmid pJWB3013 that expresses the yeast TEF5 gene (Fig. 5). No growth was observed when the yeast mutant strain was transformed with the empty vector pACT2Δ.

These results demonstrate that the *Arabidopsis* eEF1Bα1 and eEF1Bα2 subunits not only display sequence similarity to other eEF1Bα subunits but also represent their functional homologues. Interestingly, the existence of different isoforms of elongation factors has already been reported in various organisms including plants. *Drosophila* encodes two eEF1A subunits which are differently regulated during development [19] and four genes encoding this subunit have been described in *Arabidopsis* and rice [20,21]. eEF1Bβ subunit is encoded by four different genes in human [22] and by two genes in rice, whose expression displays a tissue-specific pattern [23]. The expression pattern and the role of each isoform of *Arabidopsis* eEF1Bα has yet to be determined, but their molecular cloning should now facilitate molecular and genetic approaches to define the regulatory mechanisms of plant eEF1 complex.

Acknowledgements: We would like to thank Dr. Terry Kinzy for providing the plasmid pJWB3013 and the strain JWY4200. We are indebted to Oliver Clay for his kind help in computer analysis. F.H. was the recipient of a Fellowship from the Ministère de l'Education Nationale de la Recherche et de la Technologie (MENRT) and from the Association pour la Recherche sur le Cancer (ARC). This work was supported in part by grants from MENRT and CNRS.

References

[1] Proud, C.G. (1994) Mol. Biol. Rep. 19, 161–170.
[2] Ejiri, S. (1986) Methods Enzymol. 118, 140–153.
[3] van Damme, H.T., Amons, R., Karssies, R., Timmers, C.J., Janssen, G.M. and Moller, W. (1990) Biochim. Biophys. Acta 1050, 241–247.
[4] Ejiri, S., Kawamura, R. and Katsumata, T. (1994) Biochim. Biophys. Acta 1217, 266–272.
[5] Janssen, G.M., van Damme, H.T., Kriek, J., Amons, R. and Moller, W. (1994) J. Biol. Chem. 269, 31410–31417.
[6] Browning, K.S. (1996) Plant Mol. Biol. 32, 107–144.
[7] Matsumoto, S., Oizumi, N., Taira, H. and Ejiri, S. (1992) FEBS Lett. 311, 46–48.
[8] Oizumi, N., Matsumoto, S., Taira, H. and Ejiri, S. (1992) Nucleic Acids Res. 20, 5225.
[9] Matsumoto, S., Terui, Y., Xi, S., Taira, H. and Ejiri, S. (1994) FEBS Lett. 338, 103–106.
[10] Gidekel, M., Jimenez, B. and Herrera-Estrella, L. (1996) Gene 170, 201–206.
[11] Kidou, S., Tsukamoto, S., Kobayashi, S. and Ejiri, S. (1998) FEBS Lett. 434, 382–386.
[12] Manivasakam, P., Weber, S.C., McElver, J. and Schiestl, R.H. (1995) Nucleic Acids Res. 23, 2799–2800.
[13] Kinzy, T.G. and Woolford Jr., J.L. (1995) Genetics 141, 481–489.
[14] Simpson, G.G. and Filipowicz, W. (1996) Plant Mol. Biol. 32, 1–41.
[15] Janssen, G.M., Maessen, G.D., Amons, R. and Moller, W. (1988) J. Biol. Chem. 263, 11063–11066.
[16] Ejiri, S. and Honda, H. (1985) Biochem. Biophys. Res. Commun. 128, 53–60.
[17] Matsumoto, S., Mizoguchi, T., Oizumi, N., Tsuruga, M., Shinozaki, K., Taira, H. and Ejiri, S. (1993) Biosci. Biotechnol. Biochem. 57, 1740–1742.
[18] Boeke, J.D., Trueheart, J., Natsoulis, G. and Fink, G.R. (1987) Methods Enzymol 154, 164–175.
[19] Hovemann, B., Richter, S., Walldorf, U. and Cziepluch, C. (1988) Nucleic Acids Res. 16, 3175–3194.
[20] Axelos, M., Bardet, C., Liboz, T., Le Van Thai, A., Curie, C. and Lescure, B. (1989) Mol. Gen. Genet. 219, 106–112.
[21] Kidou, S. and Ejiri, S. (1998) Plant Mol. Biol. 36, 137–148.
[22] Pizzuti, A., Gennarelli, M., Novelli, G., Colosimo, A., Lo Cicero, S., Caskey, C.T. and Dallapiccola, B. (1993) Biochem. Biophys. Res. Commun. 197, 154–162.
[23] Terui, Y., Tsutsumi, K., Kidou, S., Sawazaki, T., Kuroiwa, Y., Yamaki, M. and Ejiri, S. (1998) Biochim. Biophys. Acta 1442, 369–372.