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Cloning and characterization of the cDNA encoding rice elongation factor 1β

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Abstract

We have cloned and sequenced a cDNA coding for rice elongation factor 1β (EF-1β). The clone was 1420 bp long and contained an open reading frame coding for 229 amino acids. The overall identity between rice EF-1β and rice EF-1β' [Matsumoto, S., Oizumi, N., Taira, H. and Ejiri, S. (1992) FEBS Lett. 311, 46–48] is 60% at the amino acid sequence level; a higher percent identical residues (81%) were especially observed in the C-terminal region. Rice EF-1β has no conserved phosphorylation site for casein kinase II and no leucine zipper motif, although these motifs are well conserved in EF-1δ (= β in plants) subunits of animal EF-1.

Key words: cDNA cloning; Elongation factor 1β; Rice; Translation

1. Introduction

Eukaryotic elongation factor 1 (EF-1) is composed of four non-identical subunits, EF-1α, β, β' and γ. EF-1α, corresponding in function to prokaryotic EF-Tu, reacts with GTP and aminoacyl-tRNA to form a ternary complex, and catalyzes the binding of aminoacyl-tRNA to the A site of ribosome concomitant with the hydrolysis of GTP. EF-1$\beta\beta'\gamma$, corresponding in function to prokaryotic EF-Ts, catalyzes the exchange of GDP bound to EF-1α with exogenous GTP, and stimulates the EF-1α-dependent aminoacyl-tRNA binding to ribosomes. Interestingly, both EF-1β and β' have GDP/GTP exchange activity. They were named simply from the order of their molecular weights [1,2]; although confusing, in animals, EF-1β and EF-1β' are termed EF-1δ and EF-1β, respectively [3].

In *Artemia salina*, it was demonstrated that the activity of EF-1β (β' in plants) was regulated by phosphorylation of the serine residue at position 89 by endogenous casein kinase II (CK II) [4]. The consensus sequence for phosphorylation was well conserved in EF-1δ and EF-1β of *A. salina* [3,5], human [6–8] and *Xenopus laevis* [9,10], EF-1β of *Saccharomyces cerevisiae* [11], and EF-1β' of silkworm [12]. In addition to the conserved phosphorylation site, EF-1δ in *A. salina*, human, and *X. laevis* possess a leucine zipper motif in the N-terminal region [8,9]. Recently, we have found that rice and wheat EF-1β' do not contain a serine residue corresponding to the CK II phosphorylation site of *A. salina* [13,14]. Wheat EF- 1β' was not phosphorylated by purified CK II, whereas serine residue(s) in wheat EF-1β was phosphorylated [15].

To investigate the molecular structure of the plant EF-1β, we cloned the cDNA of EF-1β from rice. We show here the first plant cDNA sequence encoding EF-1β, and find that the sequence has no conserved phosphorylation site and no leucine zipper motif.

2. Materials and methods

Rice EF-1β subunit was isolated from rice (*Oryza sativa* L., var. Toyonishiki) embryo according to the method of Ejiri [16]. The subunit was cleaved with cyanogen bromide or lysylendopeptidase, and the resulting peptides were separated by reverse-phase HPLC using an ODS-120T column (Tosoh Corp.) in 0.1% TFA with an acetonitrile gradient of 0–80% in 80 min. The amino acid sequences of the fragments were analyzed with a gas phase protein sequencer (Shimazu Corp., Model PSQ-1).

A λgt10 rice (*Oryza sativa* L., var. Hayayuki) cDNA library provided by Dr. K. Toriyama was screened with a ^{32}P-labeled 470-bp fragment corresponding to nucleotides 94–563 of rice EF-1β' cDNA[13], and five positive plaques were obtained. After plaque purification, the inserts were subcloned into the *Eco*RI site of the phagemid Bluescript II KS$^+$ vector. The sequences were determined using the Sequenase version 2.0 kit applied to double stranded DNA (USB Corp.) [17].

For Southern analysis, DNA digested with a restriction enzyme was electrophoresed on a 0.7% agarose gel, then transferred to a nylon membrane (Gene Screen Plus, New England Nuclear) essentially by the procedure of Reed and Mann [18]. The filter was hybridized with random primed radiolabeled fragments [19] in 6 × SSC (1 × SSC is 0.15

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M NaCl, 0.015 M sodium citrate) and 1% SDS, and following hybridization washed with 2 × SSC and 1 % SDS at 65 °C.

3. Results and discussion

3.1. Isolation of a cDNA clone encoding rice EF-1β

A λgt10 rice cDNA library containing 1×10^5 recombinants, which had been amplified once, was screened at low stringency with a 470 bp PCR-amplified fragment encoding a part of rice EF-1β′ cDNA. We obtained five positive clones. Four clones were found to contain inserts of about 1.0 kb corresponding to EF-1β′ cDNA, as judged by restriction map analysis. The other clone, named RB, and which carried a cDNA insert of about 1.4 kb possibly encoding EF-1β, was analyzed further.

3.2. Characterization of rice EF-1β cDNA and protein

Fig. 1 shows the nucleotide and deduced amino acid sequences of the cDNA. The cDNA insert (1420 bp)

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GCCAGCAGCCGCGCCGCTGCCTCTCCTCCTCCTCCCCTCGCCGCCGATCCAATCCGGTCA   60
CTTTCAGTCTTTTTTCTTGAGGGGGAGATGGCGGTTTCTTTCACCAACGTTAGCTCAGAG  120
                    M  A  V  S  F  T  N  V  S  S  E              11

GCAGGCCTCAAAAAGCTCGATGAGTACCTTCTCACTCGCAGCTACATCTCTGGGTACCAA  180
A  G  L  K  K  L  D  E  Y  L  L  T  R  S  Y  I  S  G  Y  Q      31

GCCTCCAACGATGACTTGGCTGTGTACTCTGCATTTTCAACTGCGCCCTCTTCAAGCTAT  240
A  S  N  D  D  L  A  V  Y  S  A  F  S  T  A  P  S  S  S  Y     51

ACCAATGTTGCTAGGTGGTTTACTCACATTGATGCACTCCTACGTCTGAGTGGAGTTACT  300
T  N  V  A  R  W  F  T  H  I  D  A  L  L  R  L  S  G  V  T     71

GCTGATGGTCAAGGCGTAAAGGTCGAGTCGACAGCTGTTCCTTCAGCTTCAACCCCTGAT  360
A  D  G  Q  G  V  K  V  E  S  T  A  V  P  S  A  S  T  P  D     91

GTTGCTGATGCAAAGGCTCCTGCAGCTGATGATGACGATGATGACGATGTTGACCTTTTT  420
V  A  D  A  K  A  P  A  A  D  D  D  D  D  D  D  V  D  L  F     111

GGTGAGGAGACTGAAGAGGAGAAGAAGGCAGCTGAGGAGCGTGCTGCTGCTGTCAAGGCT  480
G  E  E  T  E  E  E  K  K  A  A  E  E  R  A  A  A  V  K  A     131

TCTGGCAAGAAGAAGGAATCTGGGAAGTCCTCAGTGTTGCTTGATGTCAAACCATGGGAC  540
S  G  K  K  K  E  S  G  K  S  S  V  L  L  D  V  K  P  W  D     151

GATGAGACTGACATGACCAAATTGGAAGAAGCTGTGAGGAATGTTAAGATGGAAGGCCTC  600
D  E  T  D  M  T  K  L  E  E  A  V  R  N  V  K  M  E  G  L     171

CTTTGGGGCGCATCCAAGCTTGTCCCGGTTGGTTACGGTATCAAGAAATTGCAAATCATG  660
L  W  G  A  S  K  L  V  P  V  G  Y  G  I  K  K  L  Q  I  M     191

ATGACCATTGTCGATGATCTTGTGTCGGTTGATAGTCTGATTGAGGACTACTTCTACACC  720
M  T  I  V  D  D  L  V  S  V  D  S  L  I  E  D  Y  F  Y  T     211

GAACCAGCGAATGAGTACATCCAGAGCTGCGACATTGTTGCGTTCAACAAGATCTAGATC  780
E  P  A  N  E  Y  I  Q  S  C  D  I  V  A  F  N  K  I  *        229

TTCCTTGAGTCAGGTGATGGCGATCGGTGCAGCGGCCGCCGCGCCACGCACGCACCGTCA  840
GGCATCGACGACGAGCGAGACGCCCTCGGCGCAGAAGACACTCACGGCGATGGCAGGTGA  900
CGAGGACGGCGCTGCTGGTGGTGTTGGTAGCGGGGGCGATGACGATGACGATGCGCGGGG  960
CGGAGGCGCAGAGCCGAGCTGCGCGGCGCAGCTCACGCAGCTGGCGCCGTGCGCGCGAGT 1020
CGGCGTGGCGCCGCCGGGGCAGCCGCTGCGAGCCCCCGGCGGAGTGCTGCCTGCTGGGCG 1080
CCGTGTCGCACGACTGCCGTGCGGCACGCTCGACATCATCAACAGCCTCCCGCCAAGTGC 1140
GGCCTCCCGCGCGTCACCTGCCAGTGATGGAGATGGTGTGCCAAGGTAATTGCGTTTGCT 1200
CGTGCGAGGATGAGAAGAGAAGATTGAATAAGATGTTTGATGGCAACAAGTCATCAGGCG 1260
ATCCGATCCCTGCAGCTATGAATGGGGTATACGTAGTAGTGGTCTCGTTAGCATCTGTGT 1320
GTCGCATATCAGCGCCGTGCGTGCCGTGTCTGTCCTGCTTGCTCTGCTGATCGTTCAATG 1380
AACGACAAATTAATCTAACTCTGGAGTGACAAGTCGTTCG                       1420
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Fig. 1. Nucleotide and deduced amino acid sequences of the rice EF-1β cDNA. The underlined amino acids were confirmed by protein sequencing. The asterisk indicates stop codon.

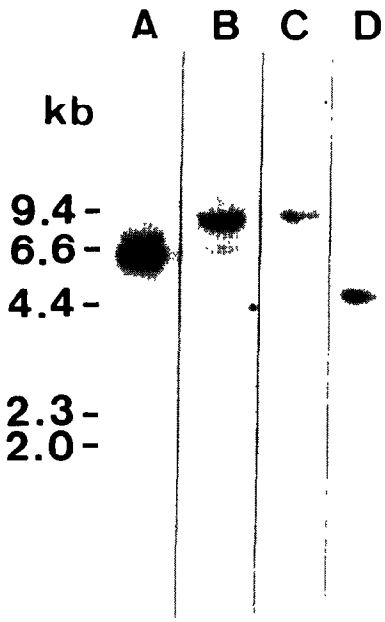

Fig. 2. Southern blot analyses of *Eco*RI (lanes A, C) or *Bam*HI (lanes B, D) digested rice (*Oryza sativa* L., var. Hayayuki) DNA (10 μg) with the *Kpn*I/*Hin*dIII fragment of rice EF-1β cDNA (nucleotides 178–616, Fig. 1) (lanes A, B) or the *Eco*RI fragment of rice EF-1β′ cDNA (nucleotides 1–980) [13] (lanes C, D) as probes.

contains 687 bp of coding region encoding 229 amino acids, which is 6 amino acids longer than rice EF-1β′ protein. By contrast, human EF-1δ and *X. laevis* EF-1δ are 281 and 265 amino acids long, respectively. The putative initiation codon ATG at position 88–90 is not preceded by a stop codon, but it is the first ATG in the sequence. The purine residue in position 85 and the G/C-rich sequence surrounding the first ATG conforms to the consensus eukaryotic initiation sequence [20]. The sequences determined at the protein level, 62 amino acids in total, were found within the amino acid sequence predicted from the cDNA (Fig. 1), save for one exception. We found phenylalanine instead of tryptophan at position 173 when the protein sequence of EF-1β was determined. The difference may be due to DNA polymorphisms between cultivars used. The termination codon TAA is present at positions 775–777, followed by the 3′-untranslated region which contains no consensus polyadenylation signal AATAAA. The signal is also not found in rice EF-1β′ [13]. The calculated molecular weight of 24,861 Da is smaller than that of 28,000 Da determined by SDS-PAGE. Similar results were also observed in rice EF-1β′[13], wheat EF-1β′ [14], *A. salina* EF-1β [5] and human EF-1δ [8]. A *Kpn*I/*Hin*dIII fragment of rice EF-1β cDNA (position 178–616, Fig. 1) or an *Eco*RI fragment of rice EF-1β′ cDNA (position 1–980) [13] was hybridized to *Eco*RI or *Bam*HI digests of the rice (*Oryza sativa* L., var. Hayayuki) genomic DNA. The Southern blot analyses suggest that rice EF-1β and EF-1β′ are single-copy genes (Fig. 2). The minor bands

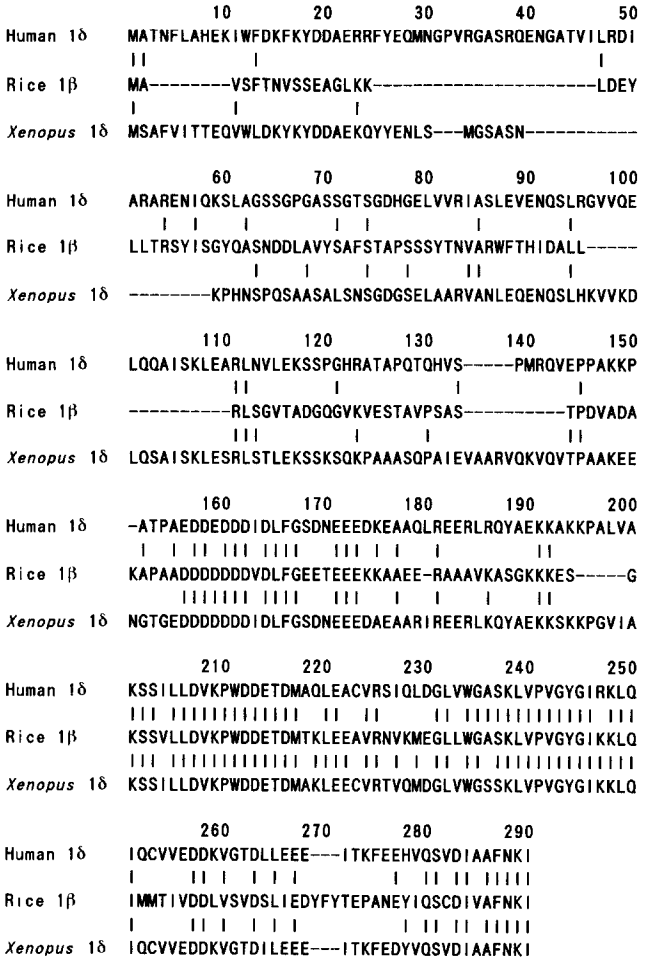

Fig. 3. Comparison of the amino acid sequences. The presented sequences are: Human 1δ, EF-1δ from human [8]; Rice 1β, EF-1β from rice; *Xenopus* 1δ, EF-1δ from *Xenopus laevis* [9]. Gaps introduced to optimize alignments are presented with dashes.

may be attributed to small exons or distantly related genes.

3.3. Comparison of amino acid sequences from different sources

A comparison of the deduced amino acid sequence of rice EF-1β with that of human EF-1δ and *X. laevis* EF-1δ reveals 38 and 44% identical residues, respectively (Fig. 3, Table 1). Amino acids 136–229 of rice EF-1β which correspond to the C-terminal region, show higher similarity with residues 189–281 of human EF-1δ (59%), 133–225 of human EF-1β (60%), 173–265 of *X. laevis* EF-1δ (64%), and 135–227 of *X. laevis* EF-1β (60%). The C-terminal region shows 51–77% identical residues to that of EF-1β from *S. cerevisiae* and *A. salina*, and EF-1β' from silkworm and wheat. Since the C-terminal region of EF-1β in *A. salina* retains the full guanine nucleotide exchange activity [3], it is likely that the region of rice EF-1β possesses a similar function. It is noteworthy that residues 148 to 154 (KPWDDET) are completely conserved among eukaryotes. Some of these residues are likely to participate in the GDP/GTP exchange reaction.

The serine residue at position 89 of *A. salina* EF-1β was phosphorylated by CK II [4]. The sequence around this residue (position 85–95, DLFGSDEEDEE) is well conserved in human EF-1δ (position 158–168, DLFGSDNEEED) and *X. laevis* EF-1δ (position 104–114, DLFGSDNEEED) (Fig. 3). Although the consensus sequence DLFG-EETEEE (position 109–118) is well conserved in rice EF-1β protein (Fig. 3), the serine residue itself is absent. This serine is also missing in rice EF-1β' (DLFG-DETEED) and wheat EF-1β' (DLFG-DETEED) [13,14]. Wheat EF-1β' containing the threonine residue in the sequences DLFG-DETEED were not phosphorylated by purified CK IIs [15]. Since one or more serine residues in wheat EF-1β, but not threonines, were phosphorylated by purified CK II [15], the threonine residue in the rice EF-1β sequence (DLFG-EETEEE) might not be used as a phosphorylation site. Since serine residues in rice EF-1β, but not EF-1β', are phosphorylated in vitro (unpublished results), another phosphorylation site may exist. The existence of this site is now under investigation.

Besides the difference in phosphorylation sites between plant EF-1β and animal EF-1δ, a leucine zipper motif in animal EF-1δs (e.g. human EF-1δ, position 80–115; *X. laevis* EF-1δ, position 58–93; *A. salina* EF-1δ, position 58–93 [8]) is not present in rice EF-1β (Fig. 3). The function of the leucine zipper therefore appears not to be universal among eukaryotes.

Both EF-1β and EF-1β' from plants share a similar percentage identical amino acids with *S. cerevisiae* EF-1β (41–43%) as with animal EF-1$\delta\beta$bs (36–53%) (Table 1). In contrast, the similarity with EF-Ts from *E. coli* is only 20–22% (Table 1). On the other hand, the similarity between rice EF-1β' and wheat EF-1β' (79%) is higher than that of rice EF-1β and rice EF-1β' (60%) (Table 1). Similarly, homology between human EF-1δ and *X. laevis* EF-1δ or human EF-1β and *X. laevis* EF-1β is higher than that of human EF-1δ and human EF-1β or *X. laevis* EF-1δ and *X. laevis* EF-1β (Table 1). These results suggest that EF-1β and EF-1β' in plants, or EF-1δ and EF-1β in animals, probably arose before eukaryotes diverged into plant and animal species.

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Table 1
The extent of amino acid homology (%) between various EF-1$\beta\beta'$ (or $\delta\beta$)s[a]

EF-1$\beta\beta'$ ($\delta\beta$)	Rice β'	Wheat β'	*Artemia* β	Human δ	Human β	Silkworm β'	*Xenopus* δ	*Xenopus* β	Yeast β	EF-Ts[b]
Rice β[c]	59.7	56.8	42.9	37.7	47.0	49.4	43.5	45.5	43.1	19.6
Rice β'[d]		79.1	43.0	36.7	45.9	51.1	40.9	46.8	40.6	20.1
Wheat β'[e]			43.0	36.9	45.2	50.2	41.2	46.1	42.3	19.7
Artemia β[f]				40.4	52.9	59.5	44.5	53.7	53.1	20.6
Human δ[g]					49.1	44.1	60.6	45.2	35.7	20.8
Human β[h]						60.2	50.9	84.6	46.9	21.6
Silkworm β'[i]							48.7	59.0	50.7	22.0
Xenopus δ[j]								52.3	38.3	21.4
Xenopus β[k]									46.5	20.1
Yeast β[l]										20.5

[a] The identity of the deduced amino acid sequences of various EF-1$\beta\beta'$ ($\delta\beta$) s was based upon an alignment of sequence using the GENETYX-MAC Ver. 5.0 software system (Software Development Co., Tokyo, Japan).
The presented sources are:
[b] *Escherichia coli* EF-Ts, [21].
[c] Rice EF-1β,this report.
[d] Rice EF-1β', [13].
[e] Wheat EF-1β', [14].
[f] *Artemia salina* EF-1β, [3,5].
[g] Human EF-1δ, [8].
[h] Human EF-1β, [6,7].
[i] Silkworm EF-1β', [12].
[j] *Xenopus laevis* EF-1δ, [9].
[k] *Xenopus laevis* EF-1β, [10].
[l] *Saccharomyces cerevisiae* EF-1β, [11].

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