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Cloning and sequencing of the cDNA encoding rice elongation factor 1β′

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A cDNA clone coding for rice elongation factor 1β′ (EF-1β′) was isolated from a rice anther cDNA library. The clone, named RB′, was 980 bp long and contained a single open reading frame coding for 223 amino acids; the first 31 amino acids, except for the first methionine, which is absent in the mature protein, are identical to those of the purified protein determined with a protein sequencer. The amino acid sequence of rice EF-1β′ shows homology to the C-terminal half of *Artemia salina* EF-1β (59%) and human EF-1β (63%), but might not have a phosphorylation site for casein kinase II which has been conserved in *Artemia salina* EF-1β and EF-1δ, human EF-1β and silkworm EF-1β′.

cDNA cloning; Elongation factor 1β′; Rice; Translation

1. INTRODUCTION

Elongation factor 1 (EF-1) catalyzes the GTP-dependent binding of aminoacyl-tRNA to the aminoacyl site on ribosomes concomitant with the hydrolysis of GTP. EF-1 from various eukaryotes, such as rice, wheat, and cauliflower [1], or silkworm and *Artemia salina* [2], is composed of four non-identical subunits; α, β, β′ and γ. EF-1α and EF-1ββ′γ correspond in function to prokaryotic EF-Tu and EF-Ts, respectively, in that EF-1α reacts with GTP and aminoacyl-tRNA to form a ternary complex, while the EF-1ββ′γ catalyzes the exchange of GDP bound to EF-1α with exogenous GTP and stimulates the EF-1α-dependent aminoacyl-tRNA binding to ribosomes.

Recently, van Damme et al. demonstrated that *Artemia salina* EF-1 is composed of four subunits, α, γ, δ and β [3], which correspond to α, γ, β̂ and β′, respectively, from their order by molecular weight.

It was also demonstrated that phosphorylation of a serine residue at position 89 in *Artemia salina* EF-1β by endogenous casein kinase II affects the GDP/GTP exchange rate on EF-1α [4]. The consensus sequences for the phosphorylation of *Artemia salina* EF-1β are conserved in all of the eukaryotic EF-1s examined so far, e.g. human EF-1β [5], *Artemia salina* EF-1δ [6] and silkworm EF-1β′ (unpublished results). We found that wheat EF-1β, but not EF-1β′, was phosphorylated in vitro [7]. To determine the difference in structure, especially the phosphorylation sites, between EF-1β′ and EF-1β in plants, we have cloned the cDNA of EF- 1β′ from rice. We show here the first plant cDNA sequence encoding an EF-1β′ which does not have the conserved phosphorylation site.

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2. MATERIALS AND METHODS

Rice EF-1β′ subunit was isolated from rice embryo according to the method of Ejiri [1], and the N-terminal amino acid sequence was determined with a gas-phase protein sequencer (ABI Corp., Model 473A). For PCR analysis, four sets of mixed oligonucleotides, 5′-CTSAARGCNCTNGARCARCA-3′ (RN-1), 5′-CTSWSNGGNA-ARACNTAYGT-3′ (RN-2), 5′-TTCATRTCNGTYTCRTCRTC-CCA-3′ (M-1), and 5′-TGGCCCCANGTTARNCCYTCCA-3′ (M-2), (N = A/G/C/T; R = A/G; S = C/G; W = A/T; Y = C/T), were synthesized with a DNA synthesizer (ABI Corp., Model 381A). RN-1 and RN-2 are complementary to the amino acid sequences of rice EF-1β′ from residues 14–20 (LKALEQH) and 21–27 (LSGKTYV), respectively (Fig. 1). M-1 and M-2 correspond to the wheat EF-1β′ peptide sequences, WDDETDMK and MEGLTWGH, respectively, which were determined with the protein sequencer. Both sequences are well conserved among *Artemia salina* EF-1β, human EF-1β and silkworm EF-1β′. 50 μl of a λgt10 rice cDNA library provided by Dr. K. Toriyama [8] was heated to 90°C for 10 min, and 2 μl, which contained 7.8×10^7 amplified recombinants, was used as the templates for PCR.

PCR was performed in a total volume of 100 μl containing the template, 1.5 μM primers, 200 μM deoxynucleotides, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% (w/v) gelatin and 2.5 U *Taq* polymerase under the following conditions: 1.5 min at 93°C, 3 min at 55°C and 2 min at 70°C for 35 cycles followed by an extension for 10 min at 70°C. The amplified fragments were separated on a 1% agarose gel, and the major band of 470 bp was electroeluted, then subcloned into a T vector [9].

The λgt10 rice cDNA library was screened with α-^{32}P-labeled 470 bp fragment [10], and a positive plaque was picked. After plaque purification, the insert was subcloned into the *Eco*RI site of the phagemid, Bluescript II KS$^+$, and named RB′. The sequences of both strands were determined using the Sequenase version 2.0 kit applied to double-stranded DNA (USB Corp.) [11].

For Northern analysis, 10 μg total RNA from rice leaves were electrophoresed in a 1.5% agarose gel containing 2.2 M formaldehyde,

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and blotted to a nylon membrane (GeneScreen Plus, New England Nuclear). Final washing was performed with 2 × SSC (1 × SSC is 0.15 M NaCl/15 mM sodium citrate, pH 7.0) containing 1% SDS at 65°C.

3. RESULTS AND DISCUSSION

3.1. *Cloning of rice EF-1β′ cDNA*

PCR amplifications were performed using RN-1 as the upstream primer and M-1 or M-2 as the downstream primers, and the rice cDNA library as the template. Two PCR products of 420 and 470 bp, derived from RN-1/M-1 and RN-1/M-2, respectively, were obtained and hybridized to the 5′-end labelled RN-2 with [γ-^{32}P]ATP and polynucleotide kinase. The 470 bp fragment was subcloned into the T vector and sequenced to confirm that it corresponded to the EF-1β′ gene. Using the 470 bp fragment as a probe, the λgt10 rice cDNA library containing 5 × 10^3 unique recombinants were screened. Finally, a single positive clone, named RB′, which carried a cDNA insert of about 1.0 kb, was obtained.

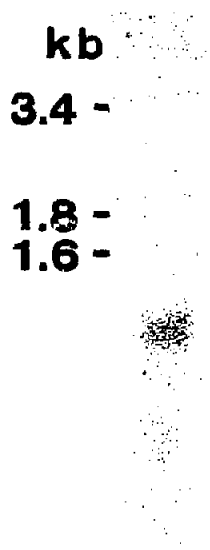

Fig. 2. Northern blot analysis of total RNA from rice. Total RNA (10 μg) was electrophoresed and blotted to a nylon membrane, and hybridized with an α-^{32}P-labeled cDNA insert.

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GCGGCCGCGCGCCTTCGACGAGCTCCAGCCCGTAGACCTCGCCGGATCTCCCCGATGGCC   60
                                                M  A            2

GTGACCTTCACCGACCTCCACACCCCCGACGGGCTCAAAGCCCTCGAGCAGCACCTCTCC  120
V  T  F  T  D  L  H  T  A  D  G  L  K  A  L  E  Q  H  L  S     22

GGCAAGACCTACGTCTCCGGAAACGCGATCAGCAAGGACGACATCAAGGTGTTCGCCGCC  180
G  K  T  Y  V  S  G  N  A  I  S  K  D  D  I  K  V  F  A  A     42

GTGCCATCAAAGCCTGGAGCTGAGTTCCCGAACGCTGCTCGCTGGTATGATACCGTCGCT  240
V  P  S  K  P  G  A  E  F  P  N  A  A  R  W  Y  D  T  V  A     62

GCGGCCCTCGCTTCAAGGTTCCCTGGCAAGGCAGTTGGAGTGAATCTGCCTGGAGGAGGT  300
A  A  L  A  S  R  F  P  G  K  A  V  G  V  N  L  P  G  G  G     82

GCGGCATCGTCTGCTGCAGCGGCTGCGCCAGCTGCTAAGGATGCTGATGAAGATGATGAT  360
A  A  S  S  A  A  A  A  A  P  A  A  K  D  A  D  E  D  D  D     102

GACCTGGATCTTTTTGGTGATGAGACCGAGGAAGACAAGAAAGCAGCAGATGAGCGTGCA  420
D  L  D  L  F  G  D  E  T  E  E  D  K  K  A  A  D  E  R  A     122

GCTTCCAAGGCCTCTTCAAAGAAGAAAGAAAGCGGAAAATCATCTGTCCTGTTGGATGTC  480
A  S  K  A  S  S  K  K  K  E  S  G  K  S  S  V  L  L  D  V     142

AAACCATGGGACGACGAGACTGATATGAAGAAGTTGGAGGAGGCTGTCCGCAGTGTGCAG  540
K  P  W  D  D  E  T  D  M  K  K  L  E  E  A  V  R  S  V  Q     162

ATGGAGGGTCTCACCTGGGGAGCATCAAAGCTTGTGCCTGTGGGTTACGGGATCAAGAAG  600
M  E  G  L  T  W  G  A  S  K  L  V  P  V  G  Y  G  I  K  K     182

TTGCAGATCATGTTGACCATTGTTGATGACTTGGTGTCTGTGATAGCCTATCGAAGACAT  660
L  Q  I  M  L  T  I  V  D  D  L  V  S  V  I  A  Y  R  R  H     202

CTGACTGAAGAGCCGATCAACGAATTTGTCCAGAGCTGCGACATCGTTGCGTTCAACAAG  720
L  T  E  E  P  I  N  E  F  V  Q  S  C  D  I  V  A  F  N  K     222

ATTTAAAATATCAAAAGGGGATGTTGCTACTTATCCAAAAACTGGGACTGGGACTGGTTG  780
I  *                                                           223

TTCTGGAAGCATTTTTCATCCCATCTTATTATCCTTCACAGAAATGCTAGTTTGAGTTTA  840
TTTTTGAGTGATGCCTGTCATTAGTGCCTCTTATCTTTAAGTAGTTGAACTGCAGAGCGG  900
CTTGTAATGGCGAAACATATGCTATGTCAAAGTACATCCGTCCATTTTTACTGTGCAAAA  960
AAAAAAAAAAAAAAAAAAAA                                          980
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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 the stop codon.

3.2. *Primary structure of rice EF-1β′*

The cDNA insert contains 669 bp of coding region encoding 223 amino acids, 54 bp of 5′ untranslated region, and 257 bp of 3′ untranslated region. Silkworm EF-1β′ cDNA codes for the same number of amino acids, while wheat EF-1β′, human EF-1β and *Artemia salina* EF-1β codes for 216, 225 and 207 amino acids, respectively. The nucleotide and deduced amino acid sequences of the cDNA insert is shown in Fig. 1. The 5′ untranslated region is G+C-rich (76%), and the putative initiation codon, ATG, at position 55–57, is present just before the alanine codon, GCC, which is the N-terminal amino acid of the purified EF-1β′ protein from rice embryo. N-Terminal methionine is also absent in the mature protein of *Artemia salina* EF-1β [6], pig EF-1β [12] and wheat EF-1β′ (Oizumi, Matsumoto, Taira and Ejiri, unpublished). The first 31 amino acids except for the first methionine (position 2–32) predicted by the nucleotide sequences are all identical with those of the purified EF-1β′ protein (Fig. 1). The termination codon, TAA, is present at position 724–726, followed by the 3′ untranslated region which does not include a poly adenylation signal, AATAAA. The size of the transcript (about 1,000 nucleotides; Fig. 2) was very close to that of the cDNA insert in RB′. These results prove that the protein coded by the cDNA in RB′ represents rice EF-1β′. The calculated molecular weight of 23,771 kDa is smaller than the molecular weight of 27,000 kDa determined by SDS-PAGE. Although the reason is not clear at present, similar results were also observed in

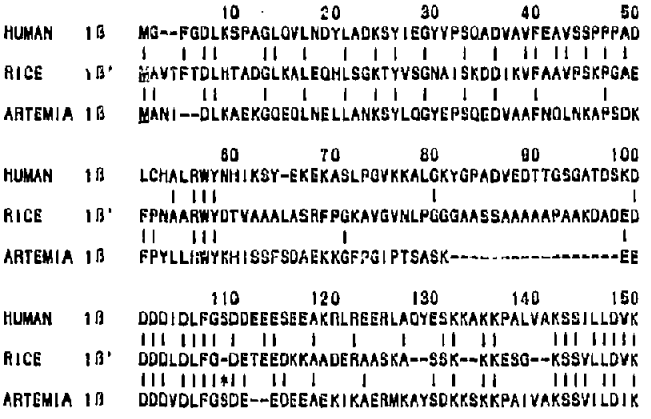

Fig. 3. Comparison of the amino acid sequences. The sequences presented are: Human 1β, EF-1β from human [5,12]; Rice 1β', EF-1β' from rice; Artemia 1β, EF-1β from *Artemia salina* [6]. Gaps introduced to facilitate alignment are presented with dashes. N-Terminal methionines which are absent in the mature protein are underlined. The serine phosphorylated by casein kinase II in the *Artemia salina* sequence is indicated by an asterisk.

Artemia salina EF-1β [6] and wheat EF-1β' (Oizumi, Matsumoto, Taira and Ejiri, unpublished).

3.3. *Comparison of amino acid sequences from different sources*

A comparison of the deduced amino acid sequences of rice EF-1β' with that of wheat EF-1β' (Oizumi, Matsumoto, Taira and Ejiri, unpublished) reveals 79% identical residues, suggesting both proteins possess a similar function in the GDP/GTP exchange reaction. Fig. 3 shows the alignment of EF-1β' and EF-1β sequences from different sources. Rice EF-1β' shows 47% sequence identity with human EF-1β, and 46% with *Artemia salina* EF-1β. Residues 131–223 of rice EF-1β' (Fig. 1) which correspond to the C-terminal half, show higher homology with residues 133–225 of human EF-1β (63%) and residues 115–207 of *Artemia salina* EF-1β (59%). Since the C-terminal region (residues 115–207) of *Artemia salina* EF-1β retains the guanine nucleotide exchange activity [3], it is likely that this region is well conserved among various eukaryotes. The phosphorylation of a serine residue at position 89 in *Artemia salina* EF-1 affects the guanine nucleotide exchange activity [4]. The consensus sequence for the target of casein kinase II contains a serine residue followed by clusters of acidic amino acid residues [4]. This sequence is well conserved among *Artemia salina* EF-1β (DLFGSDEEDEE), human EF-1β (DLFGSDDEEE), and silkworm EF-1β' (DLFGSGDEEED). Although the consensus sequence (except the serine residue), DLFG–DETEED, is well conserved in rice EF-1β' protein, the serine residue to be phosphorylated by casein kinase II is absent. The fact that the sequence, DLFG–DETEED, is also present in wheat EF-1β' at the corresponding region of rice EF-1β' (Oizumi, Matsumoto, Taira and Ejiri, unpublished) suggests that this is a characteristic of plant EF-1β'. Just as wheat EF-1β' was not phosphorylated in vitro [7], rice EF-1β' also seems not to be phosphorylated. The EF-1β' subunit of plants seems to receive no effect of phosphorylation by casein kinase II. However, it remains possible that a threonine residue in the sequence, DLFG–DETEED, might be phosphorylated by casein kinase II. Since wheat embryo EF-1β is probably regulated by the phosphorylation, elucidation of the structural differences between EF-1β and EF-1β' is proceeding.

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