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Isolation and characterization of a rice cDNA encoding the γ-subunit of translation elongation factor 1B (eEF1Bγ)

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Abstract We isolated a rice cDNA clone (*refg*) encoding the γ-subunit of translation elongation factor 1B (eEF-1Bγ; the old designation was EF-1γ). The *refg* encodes an open reading frame of 419 amino acids which shows a similarity to the equivalent sequences from animals and yeast. Complex formation analysis, which showed the recombinant protein of *refg* (His-eEF1Bγ) and formed a complex with GST-eEF-1Bβ, indicated that the *refg* encodes rice eEF1Bγ of the eEF1Bαβγ complex. Expression analysis showed that *refg* mRNA is very abundant in suspension-cultured cells during the exponential phase of growth. A DNA blot analysis indicated that *refg* is located at a single locus in the rice genome.

Key words: Elongation factor 1γ; eEF1Bγ; cDNA; Translation; Rice

1. Introduction

eEF1Bγ is part of the eukaryotic translation elongation factor-1 complex (eEF1) which plays a central role in the elongation step of protein biosynthesis. eEF1 (the old designation was EF-1) consists of four different subunits (eEF1A, eEF1Bα, eEF1Bβ and eEF1Bγ) in the higher eukaryotes. They had been termed EF-1α, EF-1β′, EF-1β and EF-1γ in plants, respectively [1]. eEF1A catalyzes the GTP-dependent binding of aminoacyl-tRNA to the acceptor site on the 80S ribosome concomitant with the hydrolysis of GTP. The resulting eEF1A·GDP is catalytically converted to eEF1A·GTP by the guanine-nucleotide exchange factor eEF1B (complex of eEF1Bα, eEF1Bβ and eEF1Bγ). Both eEF1Bα and eEF1Bβ have been known to possess GDP/GTP exchange activity. However, the function of eEF1Bγ at present is still unknown.

Recently, some interesting data, which shows the potential abilities of eEF1Bγ, have been reported. eEF1Bγ is phosphorylated by the M-phase promoting factor, a universal regulator of the G2 to M transition of the cell cycle, in *Xenopus laevis* [2]. The phosphorylation of eEF1Bγ may be related to the regulation of the elongation step in protein biosynthesis. Furthermore, it has been reported that eEF1Bγ contains an N-terminal domain similar to the domain of the class θ glutathione *S*-transferase (GST) [3]. These data suggest that eEF1Bγ may be a multi-functional protein like eEF1A [4] and extensive analysis on the molecular level could be a key to understanding the unknown function of eEF1Bγ.

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The nucleotide sequence data of *refg* will appear in the DDBJ, EMBL and GenBank database under the accession number D89802.

The isolation of the cDNA clone, which covers a full-length amino acid coding region of eEF1Bγ, has not yet been reported from any plant sources though a partial nucleotide sequence of *Arabidopsis* eEF1Bγ has been registered in the DNA data bank (accession number Z25640). In this paper, we report the isolation and characterization of a rice cDNA clone encoding eEF1Bγ. This is the first report to show the molecular structure and characterization of the eEF1Bγ sequence from plants.

2. Materials and methods

2.1. Plant materials and growth conditions

Suspension-cultured cells of rice (*Oryza sativa* L. var. Yamahoushi) were maintained in AA liquid medium [5]. The cultures were incubated in a gyratory shaker (100 rpm) at 25°C and subcultured every two weeks. Cells at the exponential phase of growth (3 days after subculture) were harvested and utilized for the RNA isolation. Rice plants were grown in soil in a greenhouse. Rice seedlings were grown by hydroponics under continuous light (10 000 lux) in an incubator at 28°C.

2.2. DNA and RNA preparation

The total DNA of *A. thaliana* from mature leaves and rice (*Oryza sativa* L. var. Yamahoushi) from suspension-cultured cells were isolated according to Shure et al. [6]. The total RNA of rice cells was isolated by the phenol-SDS method [7].

2.3. Generation of EF1Bγ probes, screening of cDNA libraries and nucleotide sequencing

The EF1Bγ specific probe based on the partial sequence of *Arabidopsis* eEF1Bγ (accession number Z25640) was obtained by PCR from *Arabidopsis* total DNA using primers AtEFgF (5′-GATGGGCGTCACTAAC-3′) and AtEFgR (5′-CCTCAAGTCCTCTCTTCAATGCAG-3′). Amplified PCR fragments (313 bp) were labeled by random oligonucleotide priming and used to screen the rice cDNA library. The rice (*Oryza sativa* L. var. Hayayuki) cDNA library (λZAP, mRNA from anther) was a generous gift from H. Uchimiya (Tokyo University). Approximately 2×10^4 recombinants were screened by plaque hybridization and the hybridizing plaques were isolated. After three cycles of plaque purification, in vivo excisions of the pBluescript plasmids were performed in the *Escherichia coli* K-12 strain XL-1 Blue.

Deletion mutants were generated by exonuclease III digestion using the Kilo-Sequence Deletion Kit (Takara). The nucleotide sequences of the inserts were determined using the *Bca*BEST Sequencing Kit (Takara) with M13 universal and reverse primers.

2.4. Expression analysis by RT-PCR

For the first strand cDNA synthesis, one μg of total RNA was annealed with 20 pmol of oligo-d$(T)_{20}$ primer and extended using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Toyobo) at 37°C for 1 h in 20 μl of 1×M-MLV-RT buffer containing 10 mM DTT, 0.5 mM dNTPs and 40 units rRNasin (Promega). One-twentieth of the reaction products was used as a template in the 20-μl PCR reactions containing 10 mM Tris-HCl (pH 8.0), 50 mM KCl, 1.5 mM $MgCl_2$, 4 mM dNTPs, 0.2 unit of Ex Taq polymerase (Takara) and 10 pmol of each gene-specific forward and reverse [γ-^{32}P]-labeled primers: refg-F (5′-CTAAGGCAGCTGAGAAAC-

PII: S0014-5793(98)01014-X

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   1                                                  CGGCCGCCTTGCTGCTCCCTCCACTGCTCGC
  32  CTCGCCTCCGCCACCATCTGAAATCGAGTGAGTGGGAGCTAAGGCGGCGGCCCCGTCGAG
  92  ATGGCGCTCGTATTGCATACTTTCGATGGAAACAAGAATGCATTCAAGGCACTCATTGCT
      M  A  L  V  L  H  T  F  D  G  N  K  N  A  F  K  A  L  I  A    20
 152  GCCGAGTACTCTGGTGTCAAGGTTGAGTTGGCAAAGAACTTTCAGATGGGTGTCTCCAAC
      A  E  Y  S  G  V  K  V  E  L  A  K  N  F  Q  M  G  V  S  N    40
 212  AAGACTCCTGAGTATCTCAAGATGAATCCTATTGGGAAGGTCCCTATTCTAGAGACTCCT
      K  T  P  E  Y  L  K  M  N  P  I  G  K  V  P  I  L  E  T  P    60
 271  GATGGTCCTGTTTTTGAAAGCAATGCGATTGCACGATATGTTACTCGCTCGAAGTCTGAC1
      D  G  P  V  F  E  S  N  A  I  A  R  Y  V  T  R  S  K  S  D    80
 332  AACCCACTCTATGGGTCTTCACTGATTGAATATGCCCACATTGAGCAGTGGATTGACTTT
      N  P  L  Y  G  S  S  L  I  E  Y  A  H  I  E  Q  W  I  D  F   100
 392  TCAGCCACAGAGGTTGATGCTAATACTGGAAAATGGCTCTTCCCACGTCTTGGATTTGCT
      S  A  T  E  V  D  A  N  T  G  K  W  L  F  P  R  L  G  F  A   120
 452  CCTTATGTTGCTGTCAGTGAGGAAGCAGCTATTGCTGCTTTGAAGAGATCATTGGGTGCC
      P  Y  V  A  V  S  E  E  A  A  I  A  A  L  K  R  S  L  G  A   140
 512  CTCAACACACACCTTGCATCAAACACATACCTTGTTGGCCATTCAGTGACTCTTGCTGAT
      L  N  T  H  L  A  S  N  T  Y  L  V  G  H  S  V  T  L  A  D   160
 572  ATTGTGATGACATGCAACCTCTACATGGGCTTTGCTCGGATCATGACCAAGAATTTTACT
      I  V  M  T  C  N  L  Y  M  G  F  A  R  I  M  T  K  N  F  T   180
 632  TCTGAGTTCCCTCATGTTGAGAGGTACTTCTGGACCATGGTTAACCAGCCAAACTTTAAG
      S  E  F  P  H  V  E  R  Y  F  W  T  M  V  N  Q  P  N  F  K   200
 692  AAAGTCATGGGTGATGTGAAGCAGGCAGATTCTGTCCCACAAGTTCAAAAGAAGGCTGCA
      K  V  M  G  D  V  K  Q  A  D  S  V  P  Q  V  Q  K  K  A  A   220
 752  GCACCAAAGGAGCAGAAGCCAAAGGAAGCCAAGAAAGAGGCCCCAAAAGAAGCTCCAAAG
      A  P  K  E  Q  K  P  K  E  A  K  K  E  A  P  K  E  A  P  K   240
 812  CCTAAGGCAGCTGAGAAACCAGAGGAGGAAGAGGAAGCACCAAAGCCAAAGCCAAAGAAT
      P  K  A  A  E  K  P  E  E  E  E  E  A  P  K  P  K  P  K  N   260
 872  CCTCTTGATTTGCTCCCTCCAAGCAAAATGATCCTTGATGAGTGGAAGAGGTTATACTCA
      P  L  D  L  L  P  P  S  K  M  I  L  D  E  W  K  R  L  Y  S   280
 932  AACACAAAAACAAACTTCCGTGAGGTTGCTATCAAGGGTTTCTGGGATATGTATGACCCA
      N  T  K  T  N  F  R  E  V  A  I  K  G  F  W  D  M  Y  D  P   300
 992  GAAGGTTACTCCCTGTGGTTCTGCGACTACAAATACAATGATGAGAACACCGTGTCCTTC
      E  G  Y  S  L  W  F  C  D  Y  K  Y  N  D  E  N  T  V  S  F   320
1052  GTGACCATGAACAAGGTTGGTGGGTTCCTGCAGCGAATGGACCTGTGCCGCAAATATGCC
      V  T  M  N  K  V  G  G  F  L  Q  R  M  D  L  C  R  K  Y  A   340
1112  TTCGGGAAGATGCTTGTGATCGGCTCTGAGCCGCCATTCAAGGTGAAGGGTCTTTGGCTC
      F  G  K  M  L  V  I  G  S  E  P  P  F  K  V  K  G  L  W  L   360
1172  TTCCGTGGCCCCGAGATCCCCAAGTTCGTCATGGATGAGGTCTACGACATGGAGCTCTAT
      F  R  G  P  E  I  P  K  F  V  M  D  E  V  Y  D  M  E  L  Y   380
1232  GAGTGGACCAAGGTTGACATCTCAGATGAGGCCCAGAAGGAGCGCGTCAGCGCCATGATT
      E  W  T  K  V  D  I  S  D  E  A  Q  K  E  R  V  S  A  M  I   400
1292  GAGGACCTTGAGCCATTTGAGGGCGAGGCTTTGCTGGATGCGAAATGCTTCAAGTGAGGC
      E  D  L  E  P  F  E  G  E  A  L  L  D  A  K  C  F  K  *      419
1352  GTCGTTGGAGCAAGGATACCGTGGAAAAATGAGCTATTTAGGTTTTGGATTTACCGTTCG
1412  AAAATAGTTCATGATTCTGAAAAAGAAAACACTATCAGCTTGTGGTTATATTAGTCTATA
1472  TTGAGTGTGCTCTCGTTTCTGTGTGCTGTTTTTAAGAGTTTTGGCCCTTCTATCTTGTAG
1532  TTCTTAGTTGTTAAAGACACTATCCATCGAATCATCATCGGAAACACACCATTCCTTGTG
1592  AAAAAAAAAA
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Fig. 1. The nucleotide and deduced amino acid sequences of *refg* encoding rice eEF1Bγ. The nucleotide sequence data of *refg* appear in the DDBJ, EMBL and GenBank database under the accession number D89802.

CA-3′) and refg-R (5′-TTTCCACGGTATCCTTGCTC-3′) for rice eEF1Bγ gene (*refg*), refb′-F (5′-CAGTTGGAGTGAATCTGCCT-3′) and refb′-R (5′-TGCTTCCAGAACAACCAGTC-3′) for rice eEF1Bα gene [8], refb-F (5′-CAGCTTCAACCCCTGATGTT-3′) and refb-R (5′-ATCGCCATCACCTGACTCAA-3′) for rice eEF1Bβ gene [9] and refa-F (5′-CCAACCTTGACTGGTACAAG-3′) and refa-R (5′-CGTTCTTGATGACGCCAACA-3′) for four rice eEF1A genes [10]. Thermal cycling was as follows: denaturing at 94°C for 1 min, followed by 21 cycles of denaturing at 94°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 1 min. The resulting products (10 μl) were separated using 6% polyacrylamide gel electrophoresis and visualized by autoradiography of the dried gels. Twenty-one cycles of PCR, which stay at an exponential phase, were determined by a preliminary experiment.

2.5. Southern blot analysis

The total DNA (3 μg) of rice digested with restriction enzymes (*Bam*HI, *Eco*RI, *Sac*I or *Xho*I) was electrophoresed on a 0.7% agarose gel. The gel was then treated with 0.4 M NaOH, and denatured DNA was blotted onto a nylon membrane (Hybond-N Plus, Amersham). Hybridization was carried out with an [α-^{32}P]-labeled probe (full-length cDNA inserts of *refg*) at 65°C. The nylon membrane was washed at 65°C (2×30 min) with 2×SSC containing 0.1% SDS and exposed to X-ray film with an intensifying screen at −80°C.

Table 1
Amino acid sequence similarities of eEF1Bγ from various organisms

	O.s	*O.c*	*H.s*	*A.s*	*X.l*	*T.c*	*S.p*
O. sativa	–	–	–	–	–	–	–
O. cuniculus	36.9	–	–	–	–	–	–
H. sapiens	36.2	98.2	–	–	–	–	–
A. salina	35.9	58.7	58.7	–	–	–	–
X. laevis	38.1	76.0	75.7	58.3	–	–	–
T. cruzi	34.6	30.6	30.9	30.0	29.6	–	–
S. pombe	30.4	32.9	32.4	33.8	35.3	27.7	–
S. cerevisiae	31.5	33.4	32.9	30.7	32.8	25.5	40.1

Sources include: *Oryza sativa*; *Oryctolagus cuniculus* [12]; *Homo sapiens* [13]; *Artemia salina* [14]; *Xenopus laevis* [15]; *Trypanosoma cruzi* [16]; *Schizosaccharomyces pombe* [17]; and *Saccharomyces cerevisiae* [18].

2.6. Expression of eEF1Bα, eEF1Bβ and eEF1Bγ in Escherichia coli and complex formation analysis by polyacrylamide gel electrophoresis.

The coding region of the *refg* and previous identified rice cDNA clone for eEF1Bα [8] was amplified by PCR using each gene-specific primer: refg-atg (5′-CGGGATCCGATGGCGCTCGTATTGCATACT-3′) and refg-tga (5′-CGGGATCCTCACTTGAAGCATTTCGCATCC-3′) for *refg*, refb′-atg (5′-CGGGATCCCGATGGCCGTGACCTTCACCG-3′) and refb′-taa (5′-CGGGATCCTTAAATCTTGTTGAACGCA-3′) for the rice eEF1Bα gene. These PCR products were digested with *Bam*HI and gel-purified. The purified fragments for eEF1Bγ were cloned into the *Bam*HI site of the *E. coli* expression vector pET15b, and the purified fragments for eEF1Bα were cloned into the *Bam*HI site of the *E. coli* expression vector pGEX3x. For the construction of eEF1Bβ, the previous identified rice cDNA clone for eEF1Bβ [9] was digested with the restriction enzymes, *Kpn*I and *Xba*I. After the blunting, it was cloned into *Sma*I site of the *E. coli* expression vector pGEX3x. These constructs were introduced into the *E. coli* BL21 (DE3) strain. Cells were grown in LB medium at 37°C until the OD_{600} reached 0.6. IPTG was added

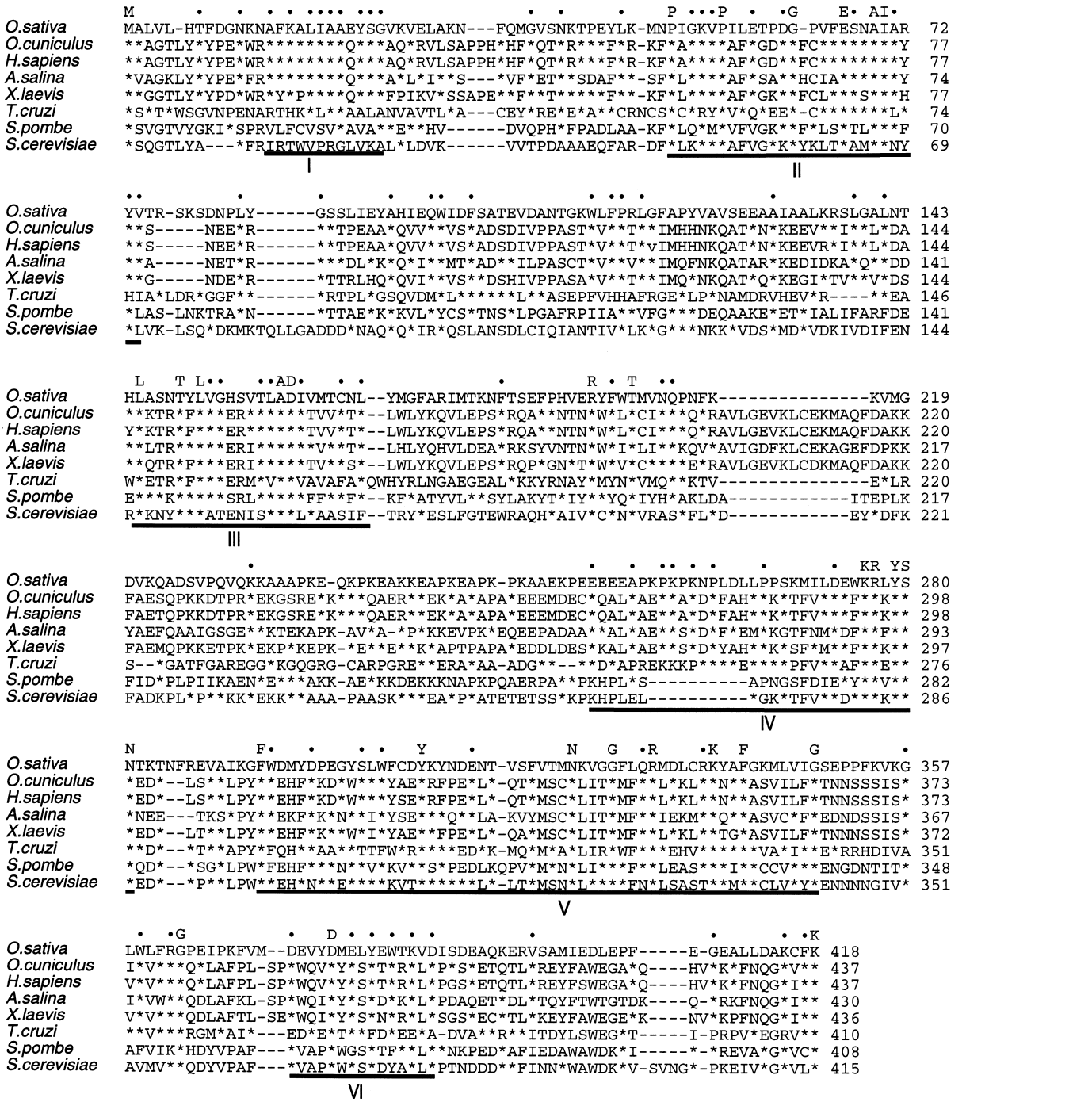

Fig. 2. Alignment of amino acid sequences for eEF1Bγ from rice (*O. sativa*), *Oryctolagus cuniculus* (*O. cuniculus*) [12], *Homo sapiens* (*H. sapiens*) [13], *Artemia salina* (*A. salina*) [14], *Xenopus laevis* (*X. laevis*) [15], *Trypanosoma cruzi* (*T. cruzi*) [16], *Schizosaccharomyces pombe* (*S. pombe*) [17], and *Saccharomyces cerevisiae* (*S. cerevisiae*) [18]. Each amino acid sequence is represented by the standard single letter code. Gaps are introduced to obtain maximum similarity. The identical amino acids in all sequences are shown at the top of the alignment. Positions with at least four sequences identical to the rice eEF1Bγ sequence are indicated by the closed circles. The homologous regions are underlined. The shaded boxes indicate the potential phosphorylation site by *cdc2* kinase.

to a final concentration of 1 mM and the cultures were grown at 30°C for 4 h. After the purification, fusion proteins (His-eEF1Bγ, GST-eEF1Bα and GST-eEF1Bβ) were dialyzed against buffer (50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM 2-mercaptoethanol) and used for the complex formation analysis. His-eEF1Bγ (7 μg) was mixed with GST-eEF1Bα (7 μg) or GST-eEF1Bβ (7 μg) and reacted for 10 min at 0°C. After the reaction, the mixture was subjected to 6.0% native polyacrylamide gel electrophoresis and visualized by Coomassie blue-staining.

3. Results and discussion

3.1. Isolation and characterization of cDNA clone encoding rice eEF1Bγ

Plaque hybridization of a rice anther cDNA library identified 10 positive clones. Among them, only one clone had an insert longer than 1.5 kb. This cDNA fragment was subcloned into the *Eco*RI site of pBluescript SK$^-$ for determination of the nucleotide sequence. Fig. 1 shows the entire nucleotide and deduced amino acid sequences for rice eEF1Bγ, designated *refg*. *refg* consists of 1601 bases with part of the poly(A) tail (a stretch of 10 adenine residues). The open reading frame starts at base 92 and ends with the TGA stop codon at base 1346, and is capable of encoding for a 418 amino acid polypeptide. A calculated molecular mass of 47.5 kDa and a predicted p*I* of 6.11 were approximately the same as the characterization of the previous purified eEF1Bγ from rice embryo (M_r 53 kDa, p*I* 6.7) [1,11]. Isolation of the gene encoding eEF1Bγ has not been reported in plants, therefore, this is the first report of the eEF1Bγ sequence in plants. The rice eEF1Bγ showed relatively low degrees of similarity to the other reported eEF1Bγs in animals and yeast. It shares only a 36.9%, 36.2%, 35.9%, 38.1%, 34.6%, 30.4% and 31.5% amino acid identity with that of *Oryctolagus cuniculus* [12], *Homo sapiens* [13], *Artemia salina* [14], *Xenopus laevis* [15], *Trypanosoma cruzi* [16], *Schizosaccharomyces pombe* [17] and *Saccharomyces cerevisiae* [18], respectively (Table 1). Interestingly, the calculated amino acid identities among other identified eEF1Bγs were also very low except for the case between mammals such as *O. cuniculus* and *H. sapiens*. These degrees of similarities in the eEF1Bγ sequences were extremely low as compared with the case of the other subunits of the eEF1 complex (eEF1A, eEF1Bα and eEF1Bβ). These variations in the amino acid sequence for eEF1Bγ may suggest that eEF1Bγ is not the essential component of the translation machinery. Fig. 2 shows an alignment of the deduced amino acid sequences of eEF1Bγ from rice, *O. cuniculus*, *H. sapiens*, *A. salina*, *X. laevis*, *T. cruzi*, *S. pombe* and *S. cerevisiae*. The alignment shows the existence of some highly conserved regions in all the eEF1Bγs (regions I–VI in Fig. 2). One of them, region IV, includes the potential phosphorylation site (residues 276–280 of *refg*) by PKC, PKA and PKG. This site is completely conserved in all the aligned amino acid sequences. Additionally, two highly conserved potential phosphorylation sites by *cdc2* kinase (T/SPx) were found in aligned sequences (shaded boxes in Fig. 2). In practice, eEF1Bγ is phosphorylated by *cdc2* kinase in *X. laevis* [19]. The phosphorylation by protein kinases may be related to the function of eEF1Bγ. Other putative functional domains were not found in the other conserved regions using computer analysis. The binding domains of eEF1Bγ to eEF1A or eEF1Bα, however, are probably located in their conserved regions.

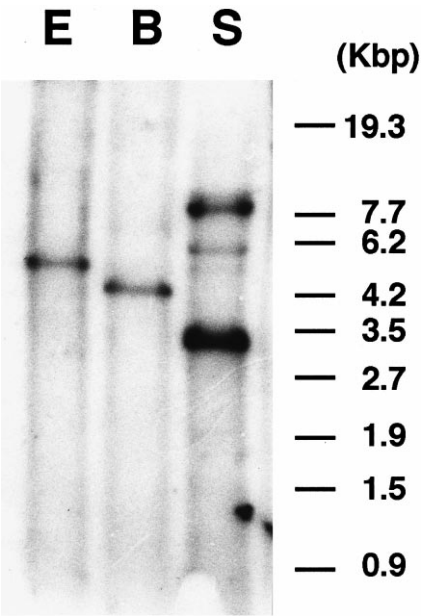

Fig. 3. Southern blot analysis of *refg* gene organization. Rice genomic DNA (3 μg) was digested with *Eco*RI (E), *Bam*HI (B) or *Sac*I (S). Digested DNA was electrophoresed on a 0.7% agarose gel and then blotted onto a nylon membrane. An [α-^{32}P]-labeled full-length cDNA insert of *refg* was used as a probe. λ DNA digested with *Eco*T14I was used as a size marker.

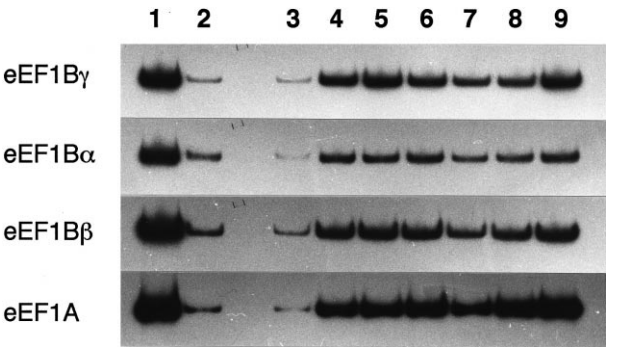

Fig. 4. mRNA accumulation analysis of genes encoding eEF1Bγ and other eEF1B constitutive subunits (eEF1Bα and eEF1Bβ) at different developmental stages. One μg of each total RNA sample was annealed to 20 pmol of cDNA priming primer (RT) and extended by reverse transcriptase. The resulting first-strand cDNA was amplified by each [γ-^{32}P]-labeled forward and reverse primer. Products were separated by 6% polyacrylamide gel electrophoresis and visualized by autoradiography. Lane 1: Suspension cultured cells (3 days after sub-culture); lane 2: dormant seeds; lane 3: seeds soaked in water for 24 h; lane 4: 5-day-old seedlings; lane 5: roots of mature plants; lane 6: leaf sheaths of mature plants; lane 7: youngest (top) leaf blades of mature plants; lane 8: oldest (bottom) leaf blades of mature plants; lane 9: endosperm of immature seeds (10 days after anthesis).

3.2. Southern blot analysis

Southern blot analysis was carried out to confirm the organization of the *refg* gene in the rice genome. Rice total DNA was digested with restriction enzymes (*Eco*RI, *Bam*HI and *Sac*I) and hybridized with the *refg* specific probe (full-length cDNA inserts of *refg*). As seen in Fig. 3, this probe hybridized to one fragment in the *Eco*RI or *Bam*HI digested total DNA, and three fragments (two major bands and one

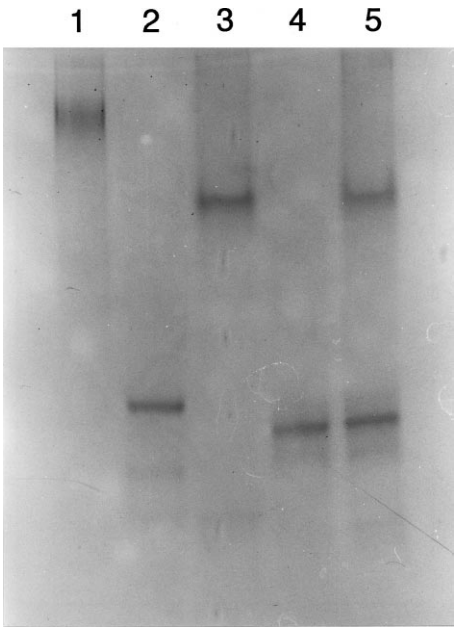

Fig. 5. Complex formation analysis by polyacrylamide gel. His-eEF1Bγ (7 μg) was mixed with GST-eEF1Bα (7 μg) or GST-eEF1Bβ (7 μg), and reacted for 10 min at 0°C. After the reaction, the mixture was subjected to 6.0% native polyacrylamide gel electrophoresis and visualized by staining with Coomassie blue. Lane 1: His-eEF1Bγ and GST-eEF1Bβ; lane 2: GST-eEF1Bβ; lane 3: His-eEF1Bγ; lane 4: GST-eEF1Bα; and lane 5: His-eEF1Bγ and GST-eEF1Bα.

weak band) in the *Sac*I digested total DNA. There are no restriction sites for *Eco*RI or *Bam*HI and one site for *Sac*I in the full-length *refg* cDNA used as a probe. This hybridization pattern suggested that *refg* is present as a single-copy gene in the rice genome.

3.3. Expression analysis

The mRNA expression of the *refg* gene was determined by RT-PCR with genes encoding the other subunits of the eEF1 complex (eEF1A, eEF1Bα and eEF1Bβ). Fig. 4 shows the analysis of *refg* transcript accumulation at specific developmental stages and in specific tissues. Suspension-cultured cells at the exponential phase of growth were the most abundant in the *refg* mRNA. The mRNA level was also high in 5-day-old seedlings and each tissue of mature rice plants including endosperm of immature seeds (10 days after anthesis). However, the mRNA level was relatively low in dormant seed and seeds which were soaked in water for 24 h to induce germination. The expression pattern of *refg* in various rice tissues was similar to that of the other genes for the eEF1 complex. These results were reproduced using three independently isolated RNA samples.

3.4. Complex formation ability of recombinant eEF1Bγ with recombinant eEF1Bα or recombinant eEF1Bβ

The *refg* showed low amino acid sequence similarities to other eEF1Bγ sequences identified in animals and yeast. This result raised a question of whether an isolated *refg* clone encodes an eEF1Bγ. To address this question, we made a recombinant protein of *refg* (His-eEF1Bγ) and examined its complex formation ability to other subunits of eEF1B (eEF1Bα and eEF1Bβ). His-eEF1Bγ was able to form the complex with GST-eEF1Bβ, but it was not able to form the complex with eEF1Bα (Fig. 5). This result corresponds to our previous reconstruction analysis data of rice eEF1 using each purified subunit of eEF1 from rice embryo [11]. It showed that rice eEF1Bγ directly forms a complex with eEF1Bβ, but does not form a complex with eEF1Bα. Furthermore, His-eEF1Bγ cross-reacted with polyclonal antibodies against the eEF1 complex of wheat (data not shown). These results led us to conclude that *refg* encodes a eEF1Bγ which constitutes a rice eEF1 complex.

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