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Cloning and characterization of the elongation factor EF-1β homologue of *Saccharomyces cerevisiae*

EF-1β is essential for growth

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A *Saccharomyces cerevisiae* cDNA homologue of the elongation factor EF-1β was found among the clones obtained by immunoscreening of a yeast cDNA expression library with an antibody against calmodulin affinity-purified proteins. The cDNA encoded a protein of 206 amino acids which was highly homologous (about 70% homology) with *Artemia salina* and human EF-1β. A protein with an apparent molecular mass of 33,000, significantly larger than that expected from the gene, was identified by Western blotting. Gene disruption experiments indicated that EF-1β is essential for growth.

Elongation factor 1β; Calmodulin binding protein; Gene disruption; *Saccharomyces cerevisiae*

1. INTRODUCTION

The elongation factor 1 (EF-1) is responsible for the GTP-dependent binding of aminoacylated tRNA to the ribosomal 'A' site in chain elongation, and also participates in proof reading of the codon–anticodon match (for a review, see [1]). EF-1 from mammalian cells consists of three subunits, α (50 kDa), β (30 kDa) and γ (48 kDa). EF-1α, a protein analogous in function to bacterial Tu, is responsible for the binding of amino acyl-tRNA to the 80 S ribosome and recycles via a process in which EF-1βγ catalyzes the exchange of GDP for GTP on EF-1α [2,3]. Several signal transduction pathways are thought to utilize EF-1 as a target for the control of protein synthesis [1]. Protein phosphorylation of the subunits is responsible for the regulation of EF-1 activity [4,5]. EF-1 exists as a monomer, as well as a high molecular weight complex, and the complex form of EF-1 has higher peptide elongation activities [6].

The yeast, *Saccharomyces cerevisiae*, facilitates the elucidation of molecular and physiological functions of cloned genes. Of the yeast genes that encode subunits of EF-1, only the EF-1α genes, *TEF1* and *TEF2*, have been cloned and characterized [7,8]. The deduced protein products of the two unlinked EF-1α genes have completely identical amino acid sequences [7,8]. The yeast proteins show 80% identities with EF-1α from *Artemia salina* and humans. The disruption of both *TEF* genes is lethal, but either *TEF* gene alone is sufficient for normal growth. With cloned *TEF* genes, it has been shown that the level of EF-1α is critical for translational fidelity [9]. Biochemical studies of yeast EF-1βγ have demonstrated that the nucleotide exchange activity resides in EF-1β, but the monomeric EF-1α itself is active in vitro without EF-1βγ [10]. Thus, the physiological function of EF-1βγ is uncertain. In the course of screening a *S. cerevisiae* cDNA expression library with an antibody against yeast calmodulin binding proteins, we obtained cDNA clones highly homologous with EF-1α and -1β. In the present report, we describe cloning and molecular analysis of the putative gene for EF-1β, and show that the yeast EF-1β gene is essential for growth.

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2. MATERIALS AND METHODS

2.1. *Yeast strains*

A diploid strain of *S. cerevisiae* strain W303 (*MAT*a/*MAT*α *ade2-1*/*ade2-1 can1-100*/*can1-100 trp1*/*trp1 leu2-3,112*/*leu2-3,112 his3-11,15*/*his3-11,15 ura3*/*ura3*) [11] was used for gene disruption. A protease-deficient mutant, 20B-12 (*MAT*α *pep4-3 trp1*) [12] was used for the preparation of cell extracts for Western blotting.

2.2. *Cloning and nucleotide sequence determination*

Cloning and sequencing procedures for the cDNA clone will be described elsewhere (K. Hiraga et al., manuscript in preparation).

2.3. *Gene disruption*

A disruption plasmid was constructed in pBluescript by replacing the *Eco*RI–*Nco*I fragment within the ORF with the yeast *HIS3* gene. The plasmid was digested with *Hpa*I and *Vsp*I, and the resulting 2.4

kb fragment was purified. The linearized DNA was used to transform the heterozygous *his3/his3* diploid strain W303. The one-step gene disruption method was used [13].

2.4. *Western blotting*

The EFB1 protein was expressed in *E. coli* as a fusion protein with glutathione S-transferase (GST) [14]. The entire coding sequence of *EFB1* was fused to the GST gene (pGEX-*EFB1*). *E. coli* NM522 cells carrying pGEX-*EFB1* were grown at 37°C and treated with 1 mM IPTG for 1 h. Cells were collected and suspended in buffer containing 20 mM Tris-HCl (pH 7.0), 5 mM EDTA and 1 mM PMSF. Cells were disrupted by passage through a French press at 7,000 psi. Triton X-100 was added to a final concentration of 1% (w/v) and the mixture was centrifuged at 15,000 × *g* for 30 min. The supernatant was isolated and the fusion protein was purified by an affinity column of glutathione–agarose. The GST portion of the isolated fusion protein was removed by digestion with thrombin. The EFB1 protein was purified by preparative SDS-polyacrylamide gel. The protein was electroeluted from the gel and used for immunization in mice, as described previously [15]. The procedures for preparation of yeast cell extracts and Western blotting were described previously [15,16], and the EFB1 protein was detected with the antiserum, as described previously [15].

3. RESULTS

3.1. *Cloning and sequencing of the gene encoding yeast homologue of EF-1β*

We have been cloning and analyzing *S. cerevisiae* genes that encode calmodulin binding proteins. The gene described in the present study was originally found in cDNA clones obtained by screening with antiserum against calmodulin binding proteins (K. Hiraga et al., to be published). In brief, a *S. cerevisiae* cDNA expression library constructed in λ-ZAP was immunoscreened by plaque staining. From the recombinant phage, a subclone was obtained by in vivo excision of pBluescript from λ-ZAP. A plasmid that contained an insert of about 400 bp was obtained. The nucleotide sequence of this clone was determined, but an initiation codon was not found at the N-terminus. Using the insert of this clone as a probe, cDNA and genomic DNA libraries were screened for full-length DNAs. The nucleotide sequence of a 698 bp cDNA insert contained a 618 bp open reading frame (ORF). The nucleotide sequences of the 1.2 kb insert of the chromosomal gene was also determined. Alignments of the chromosomal and the cDNA copy indicated that the protein coding region of the gene is interrupted by an intron. The nucleotide sequence of the genomic DNA and the protein sequence deduced from the cDNA are shown in Fig. 1. The translational initiation site was assigned to the methionine codon at nucleotide position 181–183 of the genomic DNA insert. An intron of 366 bp long was located at position 261–626. The intron had the sequences GAAGG at the 5′ splice junction, TACTAACA at the branch site for lariat formation and TAG at the 3′ splice acceptor site. These sequences showed homology to the consensus splice signals emerging from *S. cerevisiae*, except for the consensus sequence of the 5′ splice site GTAPyGT [17]. The ORF terminated with the codon TAA at position 1,165–1,167. The polyadenylation signal, CAAATAAA, was found at nucleotide 1,229–1,236 of the genomic DNA [18]. The deduced ORF contained 206 amino acids corresponding to a protein of 22,600 Da. Screening of the DDBJ data base with the deduced amino acid sequence of the ORF revealed that the gene was not previously identified, but has no homology to the EF-1β translation factor from *Artemia salina* (brine shrimp) [19] and human [20]. The yeast protein shares 55% identity (70% homology) with *S. salina* EF-1β, and 50% identity (69% homology) with human EF-1β, respectively, at the amino acid level, suggesting that EF-1β is highly conserved in eukaryotes (Fig. 2). The yeast gene was designated *EFB1* (elongation factor 1 beta).

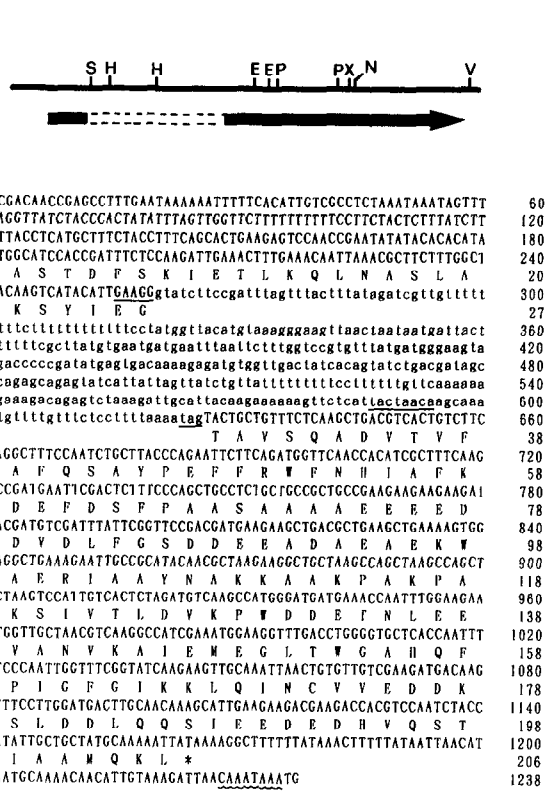

Fig. 1. Restriction endonuclease cleavage site map (A), nucleotide sequence and deduced amino acid sequence (B) of *S. cerevisiae EFB1* gene. (A) Sites of restriction endonuclease cleavage in a 1.2 kb genomic DNA fragment. The arrow indicates the ORF of the *EFB1* gene deduced from the genomic DNA and cDNA sequences. Restriction sites: E, *Eco*RI; H, *Hin*cII; N, *Nco*I; P, *Pvu*II; S, *Sau*IIIAI; X, *Xba*I; V, *Vsp*I. (B) Presumed intron inferred from the sequences of the genomic DNA and cDNA is shown in lower-case letters, and the suggested splicing signals are underlined. The polyadenylation signal is indicated by a wavy line.

Southern blot analyses of genomic DNA digested with various restriction enzymes was carried out to determine if *EFB1* is a single-copy gene (Fig. 3). One fragment with the expected size was produced with the enzymes *Hpa*I, *Eco*RV, *Bam*HI, *Hin*dIII and *Pst*I that did not cleave the *EFB1* gene; and two fragments were

Fig. 2. Comparison of the deduced amino acid sequences of *S. cerevisiae* EFB1 protein with *Artemia salina* and human EF-1β. Amino acid sequences deduced from the *S. cerevisiae* cDNA for *EFB1* (residue 1–206) are aligned with those of *A. salina* EF-1β (residues 1–207) and human EF-1β (residue 1–225) to maximize the homology. Identical residues are boxed. Hyphens have been introduced for optimal alignment.

detected with *Xba*I for which a single restriction site was predicted by the cloned gene. These results indicated that *EFB1* is a single-copy gene, in contrast to the occurrence of two homologous genes for EF-1α in *S. cerevisiae* [7,8]. To locate the *EFB1* gene on the yeast chromosomes, Southern blotting was performed with chromosomes separated by CHEF electrophoresis. The result showed that *EFB1* is located on chromosome I (data not shown).

3.2. *Effect of gene disruption*

The effect of gene disruption of *EFB1* was examined. A disruption plasmid was constructed by replacing the *Eco*RI–*Nco*I fragment within the ORF with the *HIS3* gene (Fig. 4A). Using the disruption plasmid, a diploid strain W303, which is heterozygous at the *EFB1* locus, was constructed. Gene disruption was confirmed by Southern hybridization of DNA from the diploid cells, as shown in Fig. 4B. Tetrad analysis of the diploid was carried out upon sporulation. Heterozygous diploids were induced to sporulate and tetrads were dissected (Fig. 4C). In each tetrad, only two spores formed colonies and all of these colonies were histidine auxotrophs (data not shown), indicating that *EFB1* is essential for spore viability.

3.3. *Immunological identification of* EFB1 *gene product*

The GST gene was fused with the entire coding sequence of *EFB1* as described in section 2. The fused protein produced in *E. coli* was affinity-purified on a glutathione–Sepharose column. The GST portion of the fusion protein was removed by digestion with thrombin. From the gene construct, the EFB1 protein thus prepared was expected to contain an extra 7 amino acids

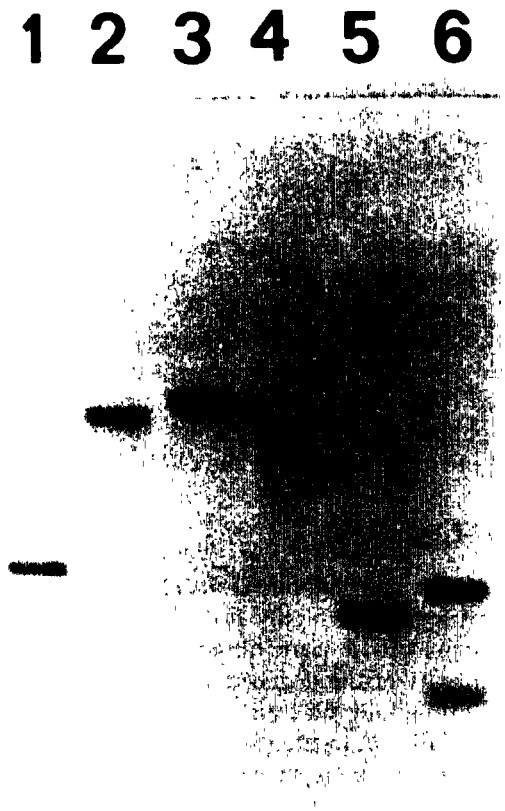

Fig. 3. Southern blot analysis of genomic *EFB1*. Genomic DNA from *S. cerevisiae* strain W303 (10 μg) was digested with various restriction enzymes and used for hybridization with labeled genomic *Hpa*I–*Vsp*I fragment of *EFB1* as probe. Digestion was with 1, *Hpa*I; 2, *Eco*RV; 3, *Bam*HI; 4, *Hin*dIII; 5, *Pst*I; 6, *Xba*I. Hybridization was at 42°C.

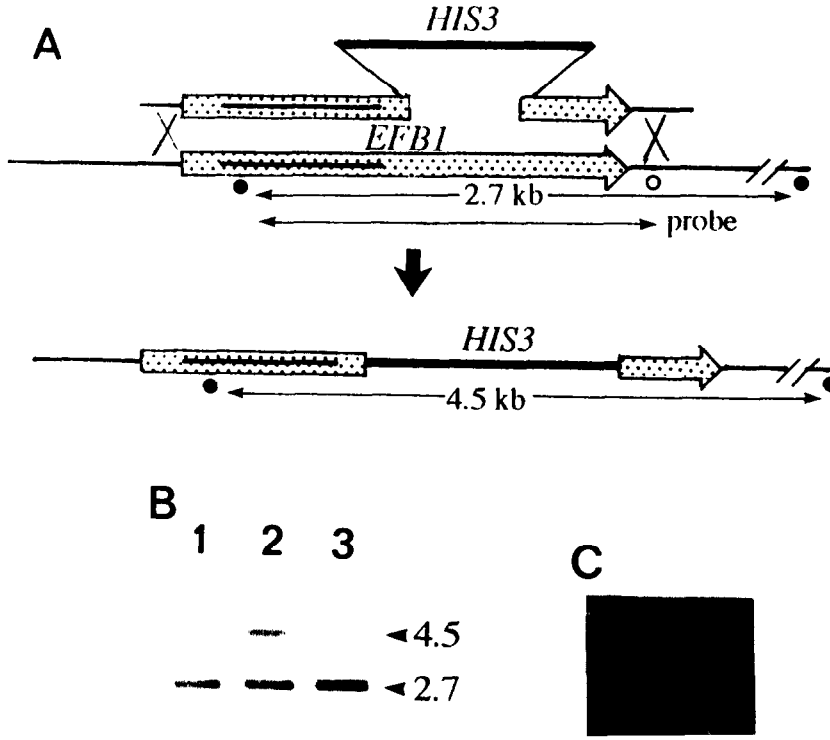

Fig. 4. Disruption of the *EFB1* gene. (A) The deletion allele of *EFB1* was constructed by replacing a 246 bp *Eco*RI–*Nco*I region within the coding sequence of genomic *EFB1* with the 1.8 kb *HIS3* gene. The intron is indicated by the bar in the *EFB1* gene. (B) Genomic yeast DNA (5 μg) was digested with *Hpa*I (●) and prepared for hybridization with the *Hpa*I (●)–*Vsp*I (○) fragment as shown in (A). Lane 1, DNA from wild-type diploid; lane 2, DNA from *EFB1*-disrupted diploid; lane 3, DNA from a viable haploid segregant of *EFB1*-disrupted diploid. The numbers are in kb. (C) Tetrad analysis of the diploid strain, W303, heterozygous for the *EFB1* locus. Haploid segregants were dissected and incubated on YPD medium at 28°C for 3 days. Spores from each ascus were lined in vertical rows.

due to the linker sequences at the N-terminus. This protein was used to raise antiserum in mice, and the EFB1 protein was detected by Western blotting of the cell extracts prepared from *S. cerevisiae* (Fig. 5). A band with an apparent molecular weight of 33,000 Da was detected. The molecular weight determined on the gel was significantly higher than the deduced molecular weight of this protein from the ORF (22,600 Da).

4. DISCUSSION

The cDNA and genomic DNA clones of *S. cerevisiae* encoding a protein highly homologous with *Artemia* and human EF-1β were obtained and characterized. The similarity of the deduced amino acid sequence extended throughout the entire sequences (Fig. 2). Ser-89 of *Artemia* EF-1β, located N-terminal to an acidic cluster of amino acids, is suggested to be involved in the regulation of catalytic activity of EF-1α. Phosphorylation of this residue has been demonstrated to reduce the nucleotide exchange activity of EF-1α [5]. A serine residue (Ser-86) was conserved in the yeast counterpart at a similar position with respect to the acidic cluster (Fig. 2).

The physiological function of EF-1β is uncertain [10]. Thus, it was of interest to test the effect of *EFB1* gene disruption. The *EFB1* gene turned out to be essential for growth. The growth of spores, as observed under a phase-contrast microscope, stopped with a very small extrusion, indicating that EF-1β is depleted early after spore germination in the gene disruptants. In vitro, EF-1β stimulates the exchange reaction of EF-1α·GDP to EF-1α·GTP [2,3], while a non-enzymatic exchange reaction also occurs in vitro [2,3,10]. The results of the gene disruption experiments suggest that EF-1β is required for the conversion of EF-1α·GDP to EF-1α·GTP in vivo.

The molecular weight of *S. cerevisiae* EF-1β (33,000 Da) identified by Western blotting agreed well with purified EF-1β from *S. cerevisiae* (Fig. 5) [21]. However, the apparent molecular weight determined by SDS-gel electrophoresis was larger than that expected from the ORF by about 10,000 Da. The cause for this discrepancy is unknown, but may reflect some unusual structural features of this protein. This possibility was supported by our observation that the thrombin-digested fusion protein, which contains an extra 7 amino acids at the N-terminus of the *EFB1* protein, had an apparent molecular weight of 33,000 Da on the gel, similar in size to the *S. cerevisiae* protein, but different from the calcu-

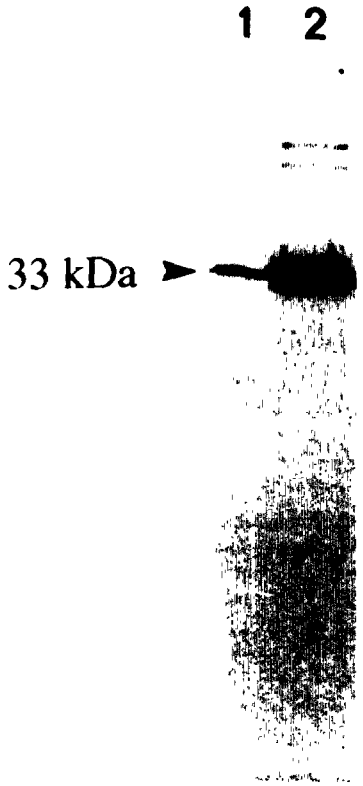

Fig. 5. Immunochemical identification of the *EFB1* gene products. Extracts from *S. cerevisiae* (1) and GSH-EF-1β fusion protein digested with thrombin (2) were separated by 10% acrylamide SDS-polyacrylamide gel electrophoresis and analyzed by immunoblotting with antiserum against EF-1β proteins.

lated molecular weight of ca. 23,000 Da (Fig. 5). A similar discrepancy in the molecular weight of *Artemia* EF-1β was also noted, although the difference (2,800 Da) was smaller in this case [19].

An increasing number of observations, as summarized in [22], suggest that cellular Ca^{2+} is involved in the regulation of post-transcriptional protein synthesis in a variety of eukaryotic cells. Normal protein synthesis attenuates in response to various stresses, such as heat shock, various chemicals and virus infection [1]. Ca^{2+} is a candidate for the stress signal, and Ca^{2+} regulation of translational initiation has been suggested [23]. The gene encoding a ribosomal calmodulin binding protein has been isolated, and its involvement in protein synthesis was suggested [24]. EF-1 may play a role in the quantitative regulation of translation. During our cloning experiments, we also obtained cDNA clones coding for EF-1α. Purified EF-1α produced in *E. coli* bound calmodulin when analyzed by calmodulin affinity chromatography and a gel over-lay method (Hiraga, unpublished result). Thus, EF-1β may have been obtained in our cloning procedures by its association with EF-1α. These results support a role for Ca^{2+}-regulation in protein synthesis at the level of elongation.

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