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Interaction of PRK1 Receptor-like Kinase with a Putative eIF2B β-Subunit in Tobacco

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PRK1, a receptor-like kinase that is expressed in pollen, pollen tubes, and ovaries, has been shown to play important roles in pollen development and embryo sac development in *Petunia inflata*. We have used the kinase domain of PRK1 as a bait in the yeast two-hybrid system to identify PRK1-interacting proteins. The screening resulted in isolation of a cDNA encoding a protein highly homologous to the human and yeast β-subunit of translation initiation factor 2B (eIF2B-β), which was designated *NeIF2Bβ*. eIF2B is a guanine nucleotide exchange protein that functions in the regulation of translation in eukaryotic cells. Deletion mutants of NeIF2Bβ were analyzed for their interaction with PRK1, and the results suggested that the N-terminal half of NeIF2Bβ, especially the region between residue 103 and 235, is important for the interaction. This protein association was confirmed by *in vitro* binding assay of the recombinant NeIF2B*β* and PRK1 proteins. Despite high sequence homology between NeIF2Bβ and its yeast counterpart, the *NeIF2Bβ* cDNA could not rescue the phenotype of the yeast mutant strain lacking the *GCD7* gene encoding eIF2B-β, when transferred into the mutant strain.

Keywords: β-Subunit of Eukaryotic Translation Initiation Factor 2B; *In vitro* Protein Binding Assay; PRK1 Receptor-like Kinase; Yeast Two Hybrid Screening.

Introduction

During pollen development and pollen tube growth, numerous genes become differentially expressed. It was estimated that about 20,000 different genes are present in pollen grain at anthesis, and among them about 10% are pollen-specific (Willing and Mascarenhas, 1984; Willing *et al*., 1988). In addition, translational control of presynthesized mRNAs also appears to play a role during pollen germination and pollen tube growth, as shown by work using inhibitors of RNA synthesis (Mascarenhas, 1993). Although the translational control mechanism during pollen development and pollen tube growth has not been revealed, it may involve translation of a subset of mRNAs required for the processes being controlled by developmental signaling cues.

PRK1, a pollen-expressed receptor-like kinase of *Petunia inflata*, plays an essential role in postmeiotic development of pollen (Lee *et al*., 1996; Mu *et al*., 1994). PRK1 contains leucine-rich repeats in the extracellular domain, and its kinase domain autophosphorylates on serine and tyrosine (Mu *et al*., 1994). Subsequent analysis revealed that PRK1 also appears to be involved in embryo sac development in the postmeiotic stage, especially during embryo sac maturation (Lee *et al*., 1997). Though these results indicate that PRK1 is required for postmeiotic gametophyte development, molecular mechanisms of PRK1 action in the processes are entirely unknown. Here, we report that PRK1 interacts with a plant homologue of the β-subunit of eIF2B, designated NeIF2Bβ. eIF2B is a guanine nucleotide exchange protein that plays a role in regulation of translation in animal and yeast cells. The interaction between PRK1 receptor-like kinase and the translation initiation factor suggests that the PRK1-mediated signaling mechanism may involve regulation of translation machinery.

Materials and Methods

Plasmid constructs For pLexA/PRK1 cloning, a partial cDNA corresponding to the kinase domain of PRK1 was digested with SmaI/SalI and cloned into SmaI/SalI-digested

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pLexA (Clontech, USA). The C-terminal and N-terminal deletion constructs of the NeIF2Bβ cDNA in pB42AD vector (Clontech, USA) were generated as follows. For pB42AD-NeIF2Bβ $^{(1\text{-}330)}$, the pB42AD-NeIF2Bβ $^{(1\text{-}415)}$ was digested with *Eco*RI and *Xho*I, was made blunt-ended, and was self-ligated. In a similar manner, pB42AD-NeIF2Bβ$^{(1\text{-}415)}$ was digested with *BglII*/*Xho*I and *Hinc*II/*Xho*I for pB42AD-NeIF2Bβ $^{(1\text{-}235)}$ and pB42AD-NeIF2Bβ$^{(1\text{-}288)}$, respectively. Then they were made blunt-ended and were self-ligated. For construction of two N-terminal deletion mutants, pB42AD-NeIF2Bβ$^{(103\text{-}415)}$ and pB42AD-NeIF2Bβ$^{(173\text{-}415)}$, the PCR-amplified DNA fragments containing a blunt end and an *Xho*I site in their 5′ and 3′ ends, respectively, were cloned into *Eco*RI(Klenow-filled)/*Xho*I-digested pB42AD plasmid.

For GST-NeIF2Bβ cloning, the entire NeIF2Bβ coding region was PCR-amplified using ADB1 primer(GCCTCTCC-CGGATCCGGCACGAG) and LPJG primer(GGCAAGG-TAGACAAGCCGACAACC) with *NeIF2Bβ* cDNA as a template. The resulting PCR product was digested with *Bam*HI and *Xho*I, then cloned into *Bam*HI/*Sal*I-digested pGEX-KG (Clontech, USA) to generate in-frame fusion of GST and NeIF2Bβ. For MBP-PRK1 cloning, the kinase domain of PRK1 (residues 421-720) was PCR-amplified using PR16 primer (AGAGGATCCAGGCCACGATTTGAT) and PR26R primer (TGGTCGACGTCAAACTCCAGCATC) with *PRK1* cDNA as a template. The resulting PCR product was digested with *Bam*HI and *Sal*I and cloned into *Bam*HI/*Sal*I digested pMAL^{TM}c2 vector (New England Biolab, UK) to generate in-frame fusion of MBP and PRK1.

Screening of yeast two-hybrid library The MATCHMAKER LexA two-hybrid system (Clontech, USA) was used to screen a tobacco flower cDNA library (complexity of 5×10^5 total recombinants) constructed in pB42AD plasmid with the cytoplasmic kinase domain of PRK1 (residue 409-720; Mu *et al*., 1994) as described by Yoon and Lee (1999). The bait plasmid was constructed in pLexA using a partial cDNA corresponding to the PRK1 kinase domain. A total of 10^6 yeast cotransformants were obtained. After amplification, a total of 2×10^7 transformants were screened, which yielded 158 independent clones (blue, Leu$^+$). PCR-amplification and sequencing of 42 independent clones revealed that they were divided into four different classes, and one class consisting of 16 clones encoded a polypeptide which was highly homologous to the rat, human and yeast β-subunit of eIF2B. The clone was designated *NeIF2Bβ*. To verify the interaction, the pB42AD plasmid containing the NeIF2Bβ was transformed back into the EGY48[p8op-lacZ] strain and the EGY48[p8op-lacZ] strain containing the pLexA/PRK1 plasmid, and the transformants were tested for β-galactosidase activity. To test the interaction of different regions of NeIF2Bβ with the kinase domain of PRK1, EGY48[p8op-lacZ] strains containing pLexA/PRK1 plasmid were transformed with pB42AD plasmid containing a partial sequence of NeIF2Bβ (two N-terminal deletions and three C-terminal deletions). The transformants were grown on selection plates and were assayed for β-galactosidase activity.

β-Galactosidase activity levels were determined in accordance with the manual of the MATCHMAKER LexA two-hybrid system. Three separate colonies assayed for each construct. β-Galactosidase activity was determined as the OD_{420} per hour.

***In vitro* protein binding assay** After induction of *E. coli* cells (BL21-DE3 strain) containing the recombinant plasmid or the vector alone, the GST-NeIF2Bβ fusion protein and GST alone were purified using a glutathione agarose column following the manufacturer's instructions (Sigma, USA). MBP and MBP-PRK1 fusion proteins were purified from *E. coli* following the manufacturer's instructions (New England Biolab, UK).

The binding assay was performed by mixing 10 μg of MBP-PRK1 fusion protein attached to amylose beads with either 10 μg of purified GST or GST-NeIF2Bβ fusion protein in the presence of 300 μl of binding buffer (20 mM HEPES, pH 7.5, 1 mM EDTA, 5 mM $MgCl_2$, and 1 mM DTT). Samples were rotated for 2 h at 4°C, were pelleted, and were washed three times with the binding buffer. The samples were suspended in protein sample buffer, were boiled, and were separated on 10% SDS-PAGE gels. The gel was blotted to nitrocellulose membrane and incubated with the GST antibody (1:1,000 dilution; Clontech, USA). They were then reacted with horseradish peroxidase-conjugated goat antimouse IgG secondary antibody (1:1,000 dilution; Sigma, USA), and the signal was detected by ECL + Plus (Amersham, USA).

Complementation assay Yeast H2220 strain [MATa leu ura trp gcn3△ gcd7△::hisG p1108(GCN4-lacZ TRP1) pJB99 (GCD7 URA)] (Hinnenbusch *et al*., 1994) was transformed with pYEGα-NeIF2Bβ, a multicopy LEU2 plasmid containing the *NeIF2Bβ* cDNA fused under GAL10 promoter, or pYEGα plasmid alone. After selection on Leu$^-$ plates, the transformants were grown on an induction medium (Leu$^-$ Ura$^+$ Gal/Raf). After four times of cell culture to delete the pJB99 (GCD7 URA) plasmid from the yeast cells, the cell suspension was spread on selection plates (Leu$^-$ Ura$^+$) containing 1 mg/ml 5-FOA (5-fluoro-orotic acid).

Results

Screening of yeast two-hybrid library to identify PRK1-interacting proteins To isolate proteins that interact with the kinase domain of PRK1, the yeast two-hybrid system that uses LexA recognition sites to regulate expression of both *LEU2* and *lacZ* genes was used. A partial cDNA corresponding to the PRK1 kinase domain (residue 409-720; Mu *et al*., 1994) was cloned in pLexA as the bait plasmid. A tobacco flower cDNA library was constructed in pB42AD plasmid by fusing the cDNAs to an 88-residue acidic *E. coli* peptide (B42) that activates transcription in yeasts.

Approximately 2×10^7 transformants were screened on the basis of Leu$^+$ and β-galactosidase activity. The LexA-PRK1 fusion protein did not itself activate transcription of reporter genes. A total of 158 positive colonies that activated transcription of *LEU2* and *lacZ* genes only in the presence of LexA-PRK1 were

obtained. Among them, 42 colonies were chosen randomly, and their cDNA inserts in pB42AD plasmid were PCR-amplified and sequenced. Those cDNA clones fell into four classes on the basis of the sequence, and only one class represented by 16 colonies is described in this study. The cDNA encoded the amino acid sequence that was highly homologous to the β-subunit of translation initiation factor eIF2B from animals and yeast. The cDNA was designated *NeIF2Bβ*.

***NeIF2Bβ* encodes a plant homologue of the β-subunit of eIF2B** The *NeIF2Bβ* cDNA derived from the pB42AD two hybrid plasmid was 1,754 bp in length, with the translation start codon ATG in the 83rd nucleotide position from the 5′ end of the cDNA. *NeIF2Bβ* encoded a protein of 415 amino acids with a predicted molecular mass of 44,713 Da.

The deduced amino acid sequence of NeIF2Bβ showed high homology to eIF2B-β from animals and yeast, suggesting that it encodes a plant homologue of the β-subunit of eIF2B. An essential step during translational initiation is the formation of a ternary complex composed of eIF2-GTP-Met-tRNA$_i$, which binds to 40S ribosomal subunits and mRNA. The binding requires GTP hydrolysis and yields an intermediate eIF2-GDP complex that must be recycled to eIF2-GTP for another round of initiation to start. The eIF2B is responsible for this guanidine nucleotide recycling. Because regeneration of active eIF2 is essential for continued protein synthesis and because eIF2B is present in limiting amounts compared with eIF2, it was speculated that it is a major regulator of overall rates of translation initiation (Kleijn *et al*., 1998). eIF2B is a multimeric protein consisting of five subunits (α, β, γ, δ, and ε) in yeast and mammals. This is the first identification of an *eIF2B* gene in the plant kingdom.

Figure 1 shows the deduced amino acid sequence of NeIF2Bβ and the alignment with eIF2B-β sequences from rat, rabbit, and yeast. It displayed 39% identity to eIF2B-β from rat (Price *et al*., 1996; BLASTP score of 221/ probability of 1e-56), 38% identity to eIF2B-β from rabbit (Craddock *et al*., 1995; BLASTP score of 218/ probability of 9e-56), and 36% identity to eIF2B-β from *Saccharomyces cerevisiae* (Bushman *et al*., 1993; BLASTP score of 243/ probability of 2e-63). The alignment of the NeIF2Bβ sequence with eIF2B-β sequences from yeast and animals revealed conserved regions, particularly in the C-terminal half, among eIF2B-β sequences. In the middle of the eIF2B-β sequence, there is a sequence segment that is highly specific in NeIF2Bβ (residues 88-166). Actually a part of the divergent region was absent in mammalian and yeast eIF2B-β sequences. In the C-terminal region, there are additional sequence segments missing in animal and yeast eIF2B-β. Those regions may constitute specific domains subject to plant-specific regulation or function.

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NeIF2Bβ      -------MPDMNTLVNDFIIKLKKRKIEGSKATAKLTAEV  33
Rat          MPGAAAKGSELSERIESFVETLKRG--GQRRTSEDMARET  38
Rabbit       MPGATEKGSELSERIESFVEALKRG--GQQRSSEDMARET  38
Yeast        --MSTINVEHTYPAVSSLIADLKSR--K-VQGPFAVAVET  35

NeIF2Bβ      LRSCISQQRLPHTNQAAALIDAIRTIGEKLIAANPVELAV  73
Rat          LGLLRRLITDHHWNNAGDLMDLIRREGRRMTAAHPPETTV  78
Rabbit       LGLLRRIITDHRWSNAGELMELIRREGRRMMAAQPSETTV  78
Yeast        ALVMRQVISQTRWSTVDQLIDTVRAVGSTLVKAQPTEFSC  75

NeIF2Bβ      GNIVRRVLHIIREEDVSLTAAAVGGLAVLAGSDDEDDFKQ 113
Rat          GNMVRRVLKIIREE-----------YGRLHGRSDESDQQE 107
Rabbit       GNMVRRVLKIIREE-----------YGRLHGRSDESDQQE 107
Yeast        GNIIRRILRLIREEYQELLKTADENEKLIVSSSNSSSPSQ 115

NeIF2Bβ      DDHPDLSAAAVAAASRSTLRPPSLQTLLEDIPQSTAAPHT 153
Rat          ------------------------------------SLHKL 112
Rabbit       ------------------------------------SLHKL 112
Yeast        KRD---------IPSNEKLVQSHEPVSVQMYSSMLNLLGR 146

NeIF2Bβ      SSSGGDSEGKSKSADKNTASPKLKHNIIEAVNELIQDIAT 193
Rat          LTSGG------LSEDFSFHYAPLKSNIIEAINELLVELEG 146
Rabbit       LTSGG------LSEDFSFHYAQLQSNIVEAINELLVELEG 146
Yeast        PTLESPTHSKTVGDSRVTGGMDMRAVIISGIQDVIDELDK 186

NeIF2Bβ      CHEQIAEQAVEHIHHNEVILTLGNSRTVMEFLCAAKEKKR 233
Rat          TTENIAAQALEHIHSNEVIMTIGFSRTVEAFLREAAQK-R 185
Rabbit       TTENIAAQALEHIHSNEVIMTIGLSRTVEAFLREAARK-R 185
Yeast        INTDIEVQSMDHLHSNEIILTQGCSKTVEAFLRFAAKK-R 225

NeIF2Bβ      SFRVFVAEGAPRYQG--HALAKELVARGLQTTVITDSAIF 271
Rat          KFHVIAAECAPFCQG--HEMAVNLSEAGIETTVMTDAAIF 223
Rabbit       KFHVIVAECAPFCQG--HEMAVNLSKAGIETTVMTDAAIF 223
Yeast        PKFSVIVAEGFNNQKGSHAMAKRLAQAGIDTTVISDATIF 265

NeIF2Bβ      AMISRVNMVVVGAHAVMANGGVIAPVGMNMVALAAQRHAV 311
Rat          AVMSRVNKVIIGTKTILANGSLRAVAGTHTLALAAKHHST 263
Rabbit       AVMSRVNKVIIGTKTILANGALRAVTGTHTLALAAKHHST 263
Yeast        AIMSRVNKVILGTHAILGNGGLVTYSGAQLVAQAARHHAT 305

NeIF2Bβ      PFVVLAGTHKLCPLYPHNPEVLLNELRSPAELLDFGEFSD 351
Rat          PLIVCAPMFKLSPQFPS-EEDSFHKFVAPEEVLPFTEGDI 302
Rabbit       PLIVCAPMFKLSPQFPN-EEDSFHKFVAPEEVLPFTEGDI 302
Yeast        PVVVCSGIYKLSPVYPY-DLESIIQLSSPDKIMSFNEGDL 344

NeIF2Bβ      CLDFGSSSGSPILHVVNPAFDYVPPNLVSLFITDTGGHNP 391
Rat          LEKVS---------VHCPVFDYVPPDLITLFISNIGGNAP 333
Rabbit       LDKVG---------CHCPVFDYVPPELITLFISNIGGNAP 333
Yeast        ISRAE---------ILNPYYDYIPPDLVDLFITNLGGYPP 375

NeIF2Bβ      SYMYRLIADYYSADDFVVKQSSIS 415
Rat          SYVYRLMSELYHPDDHVL------ 351
Rabbit       SYIYRLMSELYHPEDHVL------ 351
Yeast        SYLYRIMNDTYDASDTIL------ 393
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Fig. 1. Deduced amino acid sequence of NeIF2Bβ and alignment with other eIF2B-β sequences. The NeIF2Bβ (accession number AF137288) is aligned with eIF2B-β of rat (Price *et al*., 1996), rabbit (Craddock *et al*., 1995), and *S. cerevisiae* (Bushman *et al*., 1993). The number on the right indicates the amino acid residues. Gaps which were introduced to maximize alignment are indicated by dashes (-). Residues conserved among at least three of the sequences compared are highlighted by shades.

Interaction of the kinase domain of PRK1 with NeIF2Bβ To verify the interaction, the library-derived pB42AD plasmid containing NeIF2Bβ was isolated and reintroduced into yeast cells either alone or with the pLexA/PRK1 plasmid, and the transformants were tested for β-galactosidase activity. They showed β-galactosidase activity only in the presence of both pB42AD/NeIF2Bβ and pLexA/PRK1 plasmids. Yeast cells expressing PRK1 together with NeIF2Bβ grew on selective media (Leu-, Trp-, Ura-, His-) and showed the strong expression of the *LacZ* reporter gene (Table 1 and Fig. 2). To examine the specificity of the interaction

Table 1. Yeast two-hybrid assay of interactions between NeIF2Bβ and various signaling components including PRK1 kinase domain.

Bait	Prey	Growth (Leu^-)	Leu^+/X-Gal
pPRK1	pB42AD	−	White
pPRK1	pNeIF2Bβ	+	Blue
pCHRK1	pNeIF2Bβ	−	White
PPKC	pNeIF2Bβ	−	White
pENV4	pNeIF2Bβ	−	White
pMyb	pNeIF2Bβ	−	White
pPP2A	pNeIF2Bβ	−	White

Yeast cells (EGY48) containing various combinations of baits and preys were grown for 2 d at 30°C either on galactose media lacking leucine for detection of the LEU2 reporter expression or on galactose media containing X-Gal for detection of β-galactosidase activity.

between PRK1 and NeIF2Bβ, the kinase domain of CHRK1 encoding a chitinase-related receptor-like kinase in tobacco (Kim *et al*., 2000) was used for the same assay. The PKC, Myb, and PP2A proteins from human were also examined for their interaction with NeIF2Bβ as negative controls. CHRK1 and other signaling proteins tested did not interact with NeIF2Bβ, as shown by the inability to grow on Leu^- and the lack of β-galactosidase activity (Table 1).

To determine if any specific region(s) of NeIF2Bβ is involved in the interaction with the kinase domain of PRK1, five deletion constructs of NeIF2Bβ (two N-terminal deletions and three C-terminal deletions) were fused separately to B42AD and introduced into yeast cells together with PRK1 fused with the LexA DNA binding domain. Among the C-terminal deletion mutants, the yeast strains expressing B42AD-NeIF2Bβ$^{(1\text{-}330)}$ and B42AD-NeIF2Bβ$^{(1\text{-}288)}$ showed a significant decrease of β-galactosidase activity (Fig. 2); however, the yeast strain containing B42AD-NeIF2Bβ$^{(1\text{-}235)}$ lacking 180 amino acids at the C-terminus exhibited strong interaction as shown by high β-galactosidase activity (Fig. 2). These results indicate that the C-terminal half of NeIF2Bβ is not essential for the interaction with PRK1. The poor interaction of B42AD-NeIF2Bβ$^{(1\text{-}330)}$ and B42AD-NeIF2Bβ$^{(1\text{-}288)}$ with PRK1 may be due to instability or abnormal structural conformation of the polypeptides. One of the N-terminal deletion mutants containing B42AD-NeIF2Bβ$^{(103\text{-}415)}$, which lacks 102 amino acids at the N-terminus, exhibited strong β-galactosidase activity; however, the yeast strain containing B42AD-NeIF2Bβ$^{(173\text{-}415)}$, which carries a further N-terminal deletion of 70 amino acids, did not exhibit β-galactosidase activity (Fig. 2). These results indicate that the N-terminal half of NeIF2Bβ, especially the region between residue 103 and residue 235, is important for the *in vivo* interaction of NeIF2Bβ with PRK1. Interestingly, this region contains the sequence segment

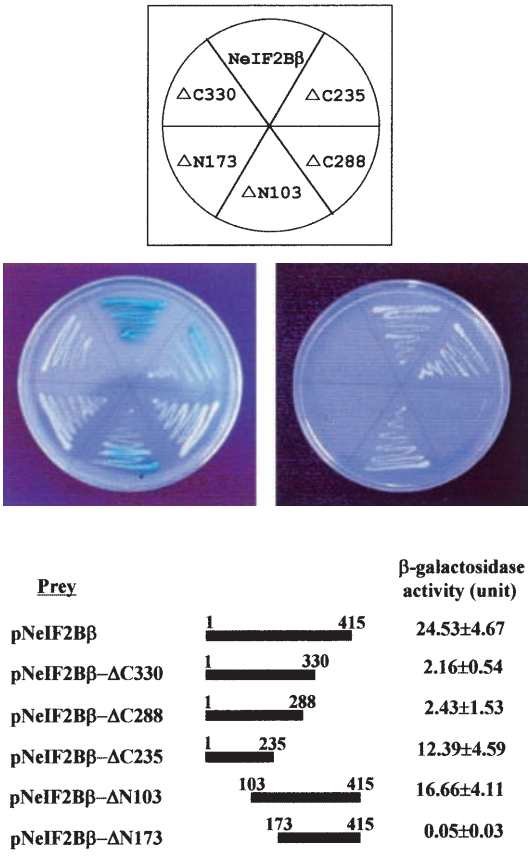

Fig. 2. Mapping of the NeIF2Bβ domain involved in interaction with PRK1. LexA-PRK1 was combined with B42AD fusions of NeIF2Bβ polypeptides carrying various N-terminal or C-terminal deletions. Three separate colonies per construct were picked up to examine β-galactosidase activity. β-Galactosidase activity was determined as the OD_{420} per hour.

that is highly divergent among plant, yeast and mammalian eIF2B-β sequences (Fig. 1).

***In vitro* interaction between NeIF2Bβ and the kinase domain of PRK1** To confirm that a direct interaction occurs between NeIF2Bβ and the kinase domain of PRK1, *in vitro* binding assays were carried out using MBP-PRK1 fusion proteins bound to the amylose resin. The purified GST or GST-NeIF2Bβ fusion protein was incubated with the resin-bound MBP-PRK1 protein or MBP. After washing, the matrix-bound fractions were eluted and separated by SDS-PAGE, then stained with Coomassie, which shows the relative levels of the MBP and MBP-PRK1 proteins in the binding reactions. Separately, the bound fractions, eluted and separated by SDS-PAGE, were analyzed by Western blotting with the GST antibody to detect the presence of bound GST-NeIF2Bβ fusion protein. As shown in Fig. 3, the GST-NeIF2Bβ protein showed preferential binding to the MBP-PRK1 kinase. Binding of GST-NeIF2Bβ protein to MBP was not detected. The GST protein by itself did not bind to MBP-PRK1 fusion protein. As a control for

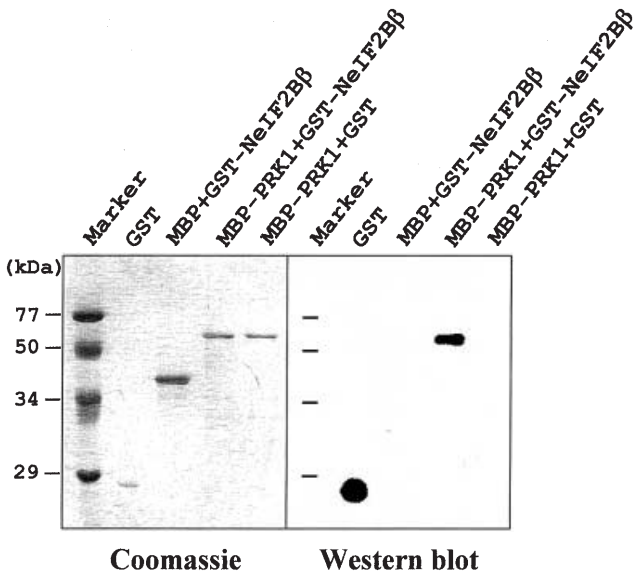

Fig. 3. Interaction of PRK1 with NeIF2Bβ *in vitro*. GST and GST-NeIF2Bβ was incubated with immobilized maltose binding protein (MBP) and MBP-PRK1 fusion protein. After washing, the amylose resin-bound fractions were separated by SDS-PAGE. The separated proteins were visualized by Coomassie staining or transferred to the membrane for Western blotting with GST antibody to detect the GST fusion protein.

the GST antibody, purified GST protein was also included in immunoblotting. These results suggest that the NeIF2Bβ protein directly interacts with the kinase domain of PRK1.

Yeast complementation assay We examined whether NeIF2Bβ could complement the lethal phenotype of yeast mutant lacking eIF2B-β encoded by *GCD7*. Yeast H2220 strain (Pavitt *et al.*, 1997), which carries chromosomal deletion of *GCD7*, also carries a single copy plasmid (pJB99) containing the *GCD7* gene. The loss of *GCD7* resulted in the lethal phenotype in yeast (Bushman *et al.*, 1993). We examined if NeIF2Bβ rescues the lethal phenotype of the H2220 strain when the plasmid carrying the *GCD7* gene is deleted from the strain. To carry out the complementation assay, the *NeIF2Bβ* cDNA corresponding to the coding region was amplified with PCR and cloned into a multicopy plasmid (pYEGα) under GAL10 promoter. Yeast H2220 cells were transformed with either the vector (pYEGα) or the recombinant plasmid (pYEGα-NeIF2Bβ). The resulting transformants were grown on a selection plate (Ura^+ Gal/Raf) to induce the expression of NeIF2Bβ. After repeated growth in the same media to delete the pJB99 (GCD7) plasmid from the yeast cells, the cell suspension was spread on Ura^+ plates containing 5-FOA to kill Ura^+ cells. No colonies were formed from the transformants carrying either the pYEGα-NeIF2Bβ plasmid or the pYEGα vector on the plates containing 5-FOA, suggesting that NeIF2Bβ cannot substitute for GCD7 in the yeast eIF2B complex or rescue the lethal phenotype of the yeast mutant lacking GCD7.

Discussion

PRK1 has been shown to play important roles in postmeiotic development of pollen and the embryo sac (Lee *et al.*, 1996; 1997; Mu *et al.*, 1994). To understand a molecular mechanism of PRK1-mediated signaling, we attempted to identify signaling components that interact with the PRK1 kinase domain using yeast two-hybrid screening. The search resulted in the isolation of a cDNA clone encoding a putative eIF2B-β, the β-subunit of translation initiation factor 2B. This is the first identification of eIF2B subunit genes in the plant kingdom. This finding indicates the possibility that plant cells also use the eIF2B protein complex for guanine nucleotide exchange for translation initiation.

eIF2B, the guanine nucleotide exchange factor, is present in limited amounts compared with eIF2 in animal systems (Kleijn *et al.*, 1998), and it is thought to be a major regulator of overall rates of translation initiation. Mammalian eIF2B is a complex composed of 26-(α), 39-(β), 58-(γ), 67-(δ), and 82-(ε) kDa subunits (Merrick and Hershey, 1996). Although the specific mechanism of eIF2B-facilitated guanine nucleotide exchange is not entirely clear (Trachsel, 1996), it was suggested that the ε-subunit is the active component in GTP/GDP exchange, while the β-, γ-, and δ-subunits control the overall activity of the complex (Kleijn *et al.*, 1998). The activity of eIF2B changes in response to various extracellular stimuli, including growth factors, serum deprivation, heat shock, and Ca^{2+}-mobilizing agents (Webb and Proud, 1997), though the mechanism of how eIF2B activity is modulated by these factors, which in turn regulates the cellular protein synthesis rate, is not well known. The α-subunit is the subunit of eIF2B that can be phosphorylated, and its phosphorylation state may regulate the activity of eIF2B in response to various stimuli (Kleijn *et al.*, 1998). However, there have been no reports implicating of phosphorylation of the eIF2B β-subunit or an interaction between the subunit and a protein kinase. The finding of the interaction between PRK1 and NeIF2Bβ may indicate a novel signaling pathway that links stimulus recognition in the cell surface to modulation of translation activity in the cytoplasm, although the mechanism of the signaling cascades mediated by the association is not currently known.

Recently, the α-subunit of eIF2B was shown to be associated with a subset of G protein-coupled receptors, including α_{2A}-, α_{2B}-, and α_{2C}- and β_2-adrenergic receptors in human cells (Klein *et al.*, 1997). The interaction of the eIF2B-α and C-terminal cytoplasmic tail of these receptors *in vivo* was shown by co-immuoprecipitation

and colocalization in specialized regions of the cell membrane (Klein *et al*., 1997). While eIF2B has a well established role in regulating translation initiation in the cytoplasm, the association of these initiation factors with signaling components suggests that these initiation factor subunits may have additional roles; however, more studies are necessary to elucidate precisely how eIF2B-α associates with the plasma membrane and enhances receptor signaling (Klein *et al*., 1997). Additionally, a homologue of eukaryotic elongation factor-1α (LIEF-1α1) was found to interact with a Ca^{2+}/calmodulin-dependent protein kinase (CCaMK) in lily by using a yeast two-hybrid system (Wang and Poovaiah, 1999). CCaMK could phosphorylate LIEF-1α1 *in vitro* and those two proteins were coimmunoprecipitated from lily anther, implicating their *in vivo* interaction.

Our study demonstrated that the kinase domain of PRK1 interacts with eIF2B-β in yeast and *in vitro*. *NeIF2Bβ* mRNA was abundantly expressed in plant reproductive organs including pollen and the pistil, while its expression was also detected in vegetative tissues. It has been shown that gene expression during pollen development is at least partially under translational control (Mascarenhas, 1993). Mature pollen stores a large amount of RNA, and inhibitor studies demonstrated that germination and early pollen tube growth are dependent on translation but not on transcription (Mascarenhas, 1993). Interestingly, eIF-4A becomes phosphorylated upon pollen tube germination in tobacco (op den Camp and Kuhlemeier, 1998). In yeast, increased translation of *GCN4* mRNA under starvation or stress conditions, while translation of other mRNAs is being reduced, is a well-known case of translational regulation in eukaryotic cells (Hinnebusch, 1994). GCN4, in turn, activates transcription of at least 40 different genes encoding amino acid biosynthesis genes (Hinnebusch, 1994). Similarly, the initial PRK1-mediated signaling may involve translation of a specific subset of pollen genes, which, in turn, regulate processes required for pollen development. Alternatively, eIF2B-β may enhance or desensitize PRK1-mediated signaling during pollen development through the direct interaction with PRK1.

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