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Plant Gene Register

PZF, a cDNA Isolated from *Lotus japonicus* and Soybean Root Nodule Libraries, Encodes a New Plant Member of the RING-Finger Family of Zinc-Binding Proteins

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Low-stringency screening of a soybean root nodule cDNA library (10^6 recombinants screened) with a probe encoding the bovine rasGAP catalytic domain (Marshall et al., 1989) permitted the isolation of a rare cDNA that contains a 500-bp region of 68% similarity to the rasGAP cDNA. This partial soybean cDNA was used to isolate a full-length cDNA of 2.1 kb from the root nodule cDNA library of the model legume *Lotus japonicus* (Handberg and Stougaard, 1992). The full-length cDNA contains only one long ORF coding for a translation product of 60 kD. Searching the GenBank and EMBL data bases with FASTA (Pearson and Lipman, 1989) and BLAST (Altschul et al., 1990) revealed only little overall similarity to known proteins. The most prominent characteristic is the presence of a putative C3HC4 Zn-finger domain, the RING-finger domain (Lovering et al., 1993), suggesting that the plant proteins deduced from the cDNA sequences have a regulatory function. Consequently, the soybean and *L. japonicus* cDNAs were designated *Gmpzf* and *Ljpzf*, respectively. The deduced proteins were named Pzf (Table I).

Most RING-finger family members appear to be regulatory transcription factors or DNA handling tools. The group comprises several mammalian oncoproteins, homeotic gene products in *Drosophila*, and DNA repair proteins in yeast and mammals. Furthermore, many members are encoded by immediate-early genes of a variety of viruses (Lovering et al., 1993).

Biochemical evidence for Zn^{2+} and DNA binding due to the C3HC4 domain was established for some members, such as Mel18 (Tagawa et al., 1990), RING1 (Lovering et al., 1993), and COP1 (von Arnim and Deng, 1993).

The only plant RING-finger family member identified so far is *At*COP1 from *Arabidopsis thaliana* (Deng et al., 1992). This putative transcription factor also possesses homology to β subunits of heterotrimeric G proteins. Thus, COP1 may link phytochrome-mediated signal reception directly to regulation of transcriptional activities.

The *Ljpzf* protein sequence contains an upstream HKRK motif (aa 141–145) that might serve as a NLS. The protein also contains other regions of clustered basic residues that might be involved in nucleic acid binding. The protein is highly hydrophilic; however, the putative NLS neighbors a Pro-rich sequence, aa 81 to 125, containing sequences reminiscent of the SH3-interactive domains described by Yu et

Table I. *Characteristics of the Pzf-cDNAs from Lotus japonicus and Glycine max*

Organism:	
	Lotus japonicus var Gifu B-129; *Glycine max* var Evans.
Gene Function:	
	Putative transcription factor.
Techniques:	
	mRNA used for cDNA cloning isolated from mature root nodules. *G. max* nodule cDNA library was screened with a ras GAP probe (courtesy of Dr. J. Gibbs). A single clone containing 1.3 kb was sequenced by double-stranded dideoxy chain termination. *L. japonicus* nodule cDNA library was screened with the *Gmpzf* cDNA fragment. One full-length clone of 2.1 kb with an 11-nucleotide poly(A)$^+$ sequence at the 3′ end was also sequenced. mRNA analysis by northern blotting of 2 μg of poly(A)$^+$ RNA from leaf, root, nodule, and callus. Southern blotting of restriction enzyme-digested genomic DNA (*Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I, *Pst*I, and *Xba*I).
Expression and Regulation:	
	In *G. max* low levels of a 2.1-kb mRNA can be detected in poly(A)$^+$ mRNA from all the organs examined. Slightly enhanced in nodules.
Gene Copy Number:	
	In *G. max* three hybridizing fragments (*Eco*RI fragment probe) were seen in all digests of genomic DNA and in *L. japonicus* only one. Both *Gmpzf* and *Ljpzf* contain identically situated internal *Xba*I sites; therefore, extra band appears in *Xba*I digests.
Features of Nucleotide Sequences:	
	Gmpzf, Partial clone of 1317 bp, 1167 bp at 5′ end, all within one ORF. *Ljpzf*, One ORF between bp 299 and bp 1946. Polyadenylation site at 2093 out of 2103. Homology of *Gmpzf* to *Ljpzf* is 78% in compared parts at the nucleotide level.
Features of the Deduced Amino Acid Sequences:	
	Homology of *Gm*pzf to *Lj*pzf is 70% at the aa level in compared parts. *Lj*pzf protein consists of 549 aa and has a calculated molecular mass of 60 kD. C-terminal RING-finger at aa 496 to 537. Putative NLS at aa 141 to 145. Other clusters of basic residues in and around the RING-finger may also serve this purpose.

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Abbreviations: aa, amino acid; NLS, nuclear localization signal; ORF, open reading frame; RING, really interesting new gene.

al. (1994) and containing homology to residues 326 to 370 of the mitosis inhibitor protein wee1 from *Schizosaccharomyces pombe* (Russell and Nurse, 1987). The putative NLS is contained within a short stretch (aa 135–145) of homology to the loop region of the *Drosophila melanogaster Asc* T4 helix-loop-helix domain (Murre et al., 1989). Overall, the aa 75 to 150 domain may be considered a plausible candidate for involvement in protein-protein interactions. Furthermore, the Pzf protein contains a region, centered around residues aa 440 to 450, with superficial homology to parts of the ligand binding domain of the human retinoid X receptor rxrβ (Leid et al., 1992).

Taken together, these observations suggest that Pzf could be involved in regulated transcriptional activities.

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The EMBL/GenBank accession numbers for the sequences reported in this article are Z36749 (*Gmpzf*) and Z36750 (*Ljpzf*).

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