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Plant Gene Register

Characterization of PRP1 and PRP2 from *Medicago truncatula*[1]

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An unusual family of extracellular PRPs has recently been described in plants (for reviews, see Keller, 1993; Showalter, 1993). The PRPs have been best characterized in soybean (Hong et al., 1990), in which three distinct cell-wall proteins are composed almost entirely of two repetitive pentapeptides: POVYK and POVEK (O = 4-*trans*-hydroxy-L-pro); or PPVYK and PPVEK in the protein sequences deduced from nucleotide sequence data. Each soybean *prp* gene shows a distinct pattern of expression during plant development. Based on the observed sequences and patterns of gene expression, PRPs are hypothesized to be structural molecules that may play roles in growth control, vascular differentiation, and/or disease resistance. The expression of several related *prp* genes in legumes is induced early in the development of symbiotic root nodules where the Pro-rich gene products called ENODs may play roles in nodule morphogenesis, infection, and/or physiology (reviewed by Franssen et al., 1992).

The *prp* gene family has not previously been characterized in *Medicago truncatula*, a useful model host plant for studies of legume-*Rhizobium* symbiosis (Barker et al., 1990). We screened a mature *M. truncatula* root nodule cDNA library with a Pro-rich early nodulin probe (Mtprp4; R.C. Wilson, F. Long, E.M. Maruoka, and J.B. Cooper, unpublished data) and isolated two cDNA clones, Mtprp1 and Mtprp2, encoding homologs of sbPRP1 and sbPRP2, respectively. Each *prp* gene is expressed in both hypocotyls and roots, in addition to mature root nodules. The PRPs encoded by these cDNAs both have extremely biased amino acid compositions, with five amino acids (P, V, Y, E, and K) making up 88 mol% (PRP1) and 92 mol% (PRP2) of the polypeptide backbones. Like the homologous PRPs in soybean, MtPRP1 and MtPRP2 are both composed almost entirely of the two repetitive Pro-rich pentapeptides, PPVYK and PPVEK. The MtPRP1 translation product is a 23.3-kD polypeptide containing 22 PPVYK repeats, 4 PPVVK repeats, and 7 PPVEK repeats. Thus, MtPRP1 resembles sbPRP1, which contains primarily PPVYK repeats. MtPRP2 is a 42-kD translation product containing 36 PPVYK and 31 PPVEK repeats. Like sbPRP2, and unlike either MtPRP1 or sbPRP1, nearly all of the repetitive pentapeptides in MtPRP2 are arranged into larger decapeptide repeats (PPVEKPPVYK).

Table I. *Characteristics of Mtprp1 and Mtprp2 cDNAs from Medicago truncatula*

Organism:
Medicago truncatula cv Jemalong.
Source:
cDNA library in λZAPII constructed using poly(A)$^+$ RNA isolated from mature symbiotic root nodules formed by *Rhizobium meliloti* strain 1021.
Clone Types:
942- and 1436-bp cDNAs cloned in pBluescript SK$^-$.
Techniques:
Library screened with a coding region probe from the Pro-rich early nodulin gene *Mtprp4* (R.C. Wilson, F. Long, E.M. Maruoka, and J.B. Cooper, unpublished data). DNA sequence determined by complete dideoxy sequencing of both strands using exonuclease III-generated nested deletions and analyzed using Genetics Computer Group software. Expression characteristics were determined using poly(A)$^+$ RNA resolved on denaturing agarose gels and probed with single-stranded RNA probes. Transcript sizes determined using RNA size markers.
Method of Identification:
Sequence homology with soybean Pro-rich proteins sbPRP1 and sbPRP2.
Features of cDNA Structures:
Mtprp1 is a 942-bp cDNA clone containing a 90-bp 5′ nontranslated region, a 199-nucleotide 3′ nontranslated region, and a 35-nucleotide poly(A) tail. Mtprp2 is a 1436-bp cDNA clone containing a 55-bp 5′ nontranslated region, a 210-nucleotide 3′ nontranslated region, and a 58-nucleotide poly(A) tail.
Expression Characteristics:
Polyadenylated transcripts of approximately 950 and approximately 1450 nucleotides detected in RNA isolated from *M. truncatula* hypocotyls, roots, and mature root nodules.
Structural Features of Deduced Proteins:
Open reading frame of Mtprp1 encodes a 206-amino acid polypeptide, M_r 23,321. Open reading frame of Mtprp2 encodes a 371-amino acid polypeptide, M_r 42,051. Both proteins contain a conserved 22-amino acid N-terminal signal peptide.

The coding sequences of these two *M. truncatula* PRPs are approximately 80% homologous at the nucleotide level and about 90% homologous at the amino acid level (Table I). Both PRPs have virtually identical N-terminal signal peptides

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Abbreviation: PRP, proline-rich protein.

(20 of 22 identical amino acids with two conservative substitutions), which are also homologous with the soybean PRP signal peptides. Despite the difference in size of MtPRP1 and MtPRP2, approximately 40 amino acids at both the N- and C-terminal ends of the mature proteins are also conserved (the C-terminal 40 amino acids are identical, and the N-terminal 44 amino acids have a single conservative substitution). Even the 3′ nontranslated sequences of *Mtprp1* and *Mtprp2* show about 90% sequence identity, indicating that these genes may have evolved from a recent gene duplication or that there may be some functional selection for these specific 3′ nontranslated sequences. Comparisons with sequences in the GenBank data base indicate that *Mtprp1* and *Mtprp2* are 70 to 80% homologous with the soybean PRPs at the nucleotide level and 85 to 90% homologous at the amino acid level. The primary difference between the homologous PRPs in *Medicago* and soybean is the predicted size of the secreted polypeptides: MtPRP1 is 21 kD and sbPRP1 is approximately 27 kD, whereas MtPRP2 is approximately 40 kD and sbPRP2 is approximately 24 kD. MtPRP1 and MtPRP2 are also 50 to 60% homologous (at the amino acid level) with both MtENOD12 (Pichon et al., 1992) and MsENOD10 from alfalfa (Löbler and Hirsch, 1993).

The two constitutive *prp* genes that we cloned and characterized do not appear to be related to some of the other "*prp*" genes that have been cloned during the past several years. For example, *Pvprp1* from bean encodes a novel protein containing a signal peptide (with no homology to the signal peptides from soybean or *Medicago* PRPs) fused to a protein with an N-terminal domain consisting of unique Pro-rich decapeptide repeats and a nonrepetitive C-terminal domain that is not Pro rich (Sheng et al., 1991). *Naprp3* from *Nicotiana alata* encodes the polypeptide backbone of a tobacco extensin glycoprotein (Chen et al., 1992). A consistent nomenclature for the *prp* gene family may help avoid future unnecessary confusion in the cell-wall protein literature.

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