Developmentally Regulated Expression of Soybean Proline-Rich Cell Wall Protein Genes

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Previously, we reported the characterization of a developmentally regulated proline-rich cell wall protein (SbPRP1) gene of soybean; the encoded protein is represented by a consensus amino acid repeat structure of Pro-Pro-Val-Tyr-Lys [Hong, J.C., Nagao, R.T., and Key, J.L. (1987). J. Biol. Chem. 262, 8367-8376]. Two other closely related members of this family of proline-rich protein (PRP) genes (SbPRP2 and SbPRP3), which differ from the extensin family of cell wall proteins, have been characterized (J.C. Hong, R.T. Nagao, and J.L. Key, unpublished results). Here we report studies on the regulation of expression of this gene family during soybean development by analyzing various plant organs, including leaves, stems, and roots of etiolated seedlings and light-grown plants, as well as young and mature pods, seed coats, and cotyledons. These organs were tested at different stages of development (young and fully mature tissue). Although a high level of sequence homology is observed at the nucleotide and amino acid sequence level among these three PRP genes/proteins, there are marked differences in the patterns of expression of each gene in different plant organs and organ regions. SbPRP1 is highly expressed and is the predominant form of PRP transcript in the mature hypocotyl, root, and immature seed coat. SbPRP2 is the major form of PRP in the apical hypocotyl and young suspension culture cells. SbPRP3 is the major form of PRP gene expressed in aerial parts; it is highly expressed in leaves, although no expression is detected in the roots. These data demonstrate that proline-rich protein genes are developmentally regulated, showing both dramatic stage-specific and organ-specific expression.

INTRODUCTION

Three classes of structural cell wall protein genes have been described in dicotyledonous plants: (1) extensin, the hydroxyproline-rich glycoprotein (HRGP) of cell wall, to date the best-characterized cell wall protein (Chen and Varner, 1985a); (2) glycine-rich protein (GRP) (Condit and Meagher, 1986; Keller, Sauer, and Lamb, 1988); and (3) proline (or hydroxyproline)-rich protein (PRP) (Chen and Varner, 1985b; Hong, Nagao, and Key, 1987). A recent review summarized the current knowledge on cell wall proteins (Cassab and Varner, 1988). These proteins are characterized by basic repeat motifs that differ among the different classes of cell wall proteins: Ser-(Hyp), for extensin, (Gly-X), for GRP, and Pro-Pro-Val-X-Y for PRP. Sequence analysis of cDNA and genomic clones isolated from a few plant species indicates that these cell wall proteins are encoded by small gene families.

The isolation and characterization of a developmentally regulated proline-rich protein gene, SbPRP1, of soybean was recently reported (Hong, Nagao, and Key, 1987). This proline-rich protein gene is a member of a gene family that includes at least three proline-rich protein genes (SbPRP1, SbPRP2, and SbPRP3) (J.C. Hong, R.T. Nagao, and J.L. Key, unpublished results). The nucleotide sequence of these three PRP genes predicts novel proteins containing highly repeated amino acid sequences in the coding region. The basic amino acid repeats observed are Pro-Pro-Val-Tyr-Lys for SbPRP1, an alternating repeat of Pro-Pro-Val-Tyr-Lys and Pro-Pro-Val-Glu-Lys for SbPRP2, and a more variable and shorter coding region of Pro-Pro-Val-Tyr-Lys for SbPRP3. Differences occur in the basicity and tyrosine content as well as the size of the mature protein. Recently, Averyhart-Fullard, Datta, and Marcus (1987) reported the isolation of a proline-rich protein from the cell wall of cultured soybean cells that has an amino acid composition identical to that of SbPRP1; 50% of the prolines of the protein are hydroxylated.

RNA gel blot analyses of RNA isolated from soybean hypocotyls demonstrated differential expression of the PRP gene family. The SbPRP1 mRNA (1220 nucleotides) represents the major form of PRP RNA in mature tissue, while SbPRP3 mRNA (650 nucleotides) represents a minor form (Hong, Nagao, and Key, 1987). SbPRP2 mRNA (1050 nucleotides) is the major PRP RNA in the apical hypocotyl region. In this study we report on a more detailed investi-
gation of PRP gene expression by analyzing various organs and organ regions at different stages of development using gene-specific probes. This report demonstrates that SbPRP genes are developmentally regulated, showing both dramatic stage-specific and organ-specific expression.

RESULTS

Developmental Regulation of the SbPRP Gene Family

Figure 1A presents RNA gel blot analyses of RNAs isolated from three different hypocotyl sections of soybean seedlings using SbPRP gene-specific probes. Three blots were hybridized separately with each of the SbPRP probes. It can be seen that each PRP probe hybridized specifically to the appropriate corresponding mRNA band on RNA gel blots. The RNA gel blot patterns were uniformly consistent with the dot-blot analyses presented in Figure 2B. Because RNA gel and dot-blot analyses were performed;

Extensins

A E M

SbPRP1

SbPRP2

SbPRP3

Figure 1. RNA Gel Blot Analysis of the SbPRP Gene Family in Soybean Hypocotyl Using Gene-Specific Probes.

(A) Fifteen micrograms of total RNA isolated from apical (A), elongating (E), and mature (M) sections of hypocotyl was separated by electrophoresis on 6% formaldehyde-2% agarose gel, transferred to nitrocellulose, and each hybridized to 1.5 million cpm per milliliter of \(^{32}\)P-labeled gene-specific probes for SbPRP1, SbPRP2, or SbPRP3. Blots were hybridized separately to each gene-specific probe and are presented as a composite figure.

(B) The extensin family of genes was analyzed by hybridizing to 3 million cpm per milliliter of a mixture of \(^{32}\)P-labeled soybean extensin cDNA probes that were isolated from a soybean cDNA library using carrot extensin probe pDC5A1 (provided by J. Varner). Three major RNA bands hybridizing to extensin probes are indicated by the arrowheads on the right.

Experiments and yielded similar results, only dot-blot analyses are presented here.

Extensin mRNAs were also detected in the normal unwounded hypocotyl and accumulated to high levels in mature hypocotyl (Figure 1B); three major RNA bands were observed. Multiple RNA bands were identified on RNA gels hybridized to the mixed extensin probes that are developmentally regulated in organs other than hypocotyl (data not shown). These extensin RNAs seem to represent a complex gene family. Partial sequence analysis of several cDNA clones homologous to carrot extensin probe pDC5A1 revealed Ser-(Pro), as a major amino acid repeat structure in the coding region (J.C. Hong, R.T. Nagao, and J.L. Key, unpublished results). Because gene-specific probes were not available, preliminary estimation of the change in the relative level of the extensin mRNAs was made by dot-blot hybridization using multiple soybean extensin cDNAs as probes.

The relative quantitative changes in each mRNA level were analyzed by dot-blot hybridization. All RNA samples were loaded onto each single filter and hybridized to each SbPRP gene-specific probe. The length of DNA insert used for the preparation of \(^{32}\)P-labeled probe ranged from 158 bp (SbPRP2) to 254 bp (SbPRP1). Equal amounts of \(^{32}\)P-labeled DNA of approximately the same specific activity were used to evaluate the relative change of mRNA abundance among the SbPRP genes. Evaluation of comparative message abundance based on the difference in dot size can be applied only to the SbPRP gene family, although the comparison among probes is less definitive than a comparison of the same probe hybridized to the various RNA samples. There is no basis for comparing extensin mRNA levels with SbPRP mRNA levels.

Organ-Specific Regulation of SbPRP Gene Expression in Soybean Seedlings

Figure 2B shows a dot-blot analysis of RNA isolated from various parts of the soybean seedling. RNA isolated from 3-day-old suspension-cultured soybean cells is also included for comparison. In hypocotyl, the same pattern of SbPRP gene expression is observed as seen in the earlier limited RNA gel blot analyses (Hong, Nagao, and Key, 1987). In cotyledons of germinated soybean seedlings, the SbPRP2 transcript is present as a major form of PRP mRNA, with the SbPRP3 transcript representing a minor form. The SbPRP3 transcript was the only PRP mRNA detected in etiolated plumules. SbPRP1 mRNA was not detected in either cotyledons or plumules. In seedling root, SbPRP1 was the major form of PRP mRNA expressed throughout the entire root, independent of the stage of development. This pattern of PRP gene expression in the root is very different from that observed in the hypocotyl, where SbPRP1 RNA is not detected in the young expanding cells. SbPRP3 RNA was not detected in any root tissue.
Proline-Rich Protein Gene Expression

SbPRPi SbPRP?

SDRP3

-0.3 cm

4 Day Old Seedling

Tissue Cultured Cells (Tc)
(3 Days After Transfer)

Figure 2. Organ-Specific Expression of SbPRP Genes in Soybean Seedlings.

(A) Four-day-old etiolated soybean seedlings were dissected into hypocotyl, cotyledon, plumule, and root for the preparation of RNA. Hypocotyl and root were further divided into apical (A), elongating (E), and mature (M) sections. Suspension-cultured cells 3 days after transfer, a relatively young stage, were included for reference.

(B) Five micrograms of total RNA isolated from each sample was applied to nitrocellulose and hybridized to 2 million cpm per milliliter of $^{32}$P-labeled gene-specific probe. For extensin gene expression, a mixture of soybean extensin cDNA probes was used. Quantification based upon sizes of dots is valid only among SbPRP gene family members.

In suspension cultured cells, all three SbPRP genes are expressed, with SbPRP1 and SbPRP2 transcripts more abundant than SbPRP3.

Stage-Specific Expression of SbPRP Genes in Soybean Hypocotyl

During hypocotyl development, the relative abundance of the three SbPRP mRNAs changed, the SbPRP2 form most abundant in young cells and SbPRP1 becoming most abundant as cells reached the fully elongated state (Hong, Nagao, and Key, 1987). To obtain more detailed information on the SbPRP mRNA changes during hypocotyl development, dot-blot analyses of RNAs isolated from the transition zone between elongating and fully mature regions were performed. Five-day-old soybean hypocotyls were serially dissected for the preparation of RNA, as diagramed in Figure 3A.

Figure 3B presents the pattern of PRP mRNA accumulation during hypocotyl development. The level of SbPRP2 RNA gradually decreased from section E and reached a basal level (section M4) as organs underwent further maturation. Meanwhile, SbPRP1 RNA levels began to increase from section M1, which is 3 cm below the elongating region, and reached maximum levels in the basal section (M6). Thus, the major change in expression from SbPRP2 to SbPRP1 occurs about 3 cm below the elongating section (between sections M3 and M4). Interestingly, SbPRP3 mRNA accumulation was initiated in the elongating region and reached maximum levels in the region where the switch in expression from SbPRP2 to SbPRP1 occurred. SbPRP3 mRNA levels decreased as hypocotyl sections approached the roots.

Differential Expression of SbPRP Genes in Soybean Plants

To determine whether SbPRPs are differentially expressed in other organs and to determine the pattern of PRP gene expression throughout plant development, PRP gene expression in major organ types was investigated. Figure 4B shows the dot-blot analysis of RNA isolated from the leaves, stems, and roots of 3-week-old soybean plants. Leaves and stems were subdivided into three different stages of development: rapidly expanding (L1 and S1), fully expanded (L2 and S2), and mature (L3 and S3) (see diagram in Figure 4A).

In leaves, SbPRP3 mRNA was the major form of PRP, with SbPRP2 mRNA being a minor form. SbPRP1 mRNA
Figure 4. SbPRP Gene Expression in Light-Grown Soybean Plants.

(A) Three-week-old soybean plants grown in a growth chamber with a 14-hr light and 10-hr dark cycle were dissected as illustrated to isolate RNA. The conditions for dot-blot analysis were the same as described for Figure 2. Apical and mature RNAs of etiolated 4-day-old hypocotyls (Figure 2) were loaded onto the same filter for comparative purposes.

(B) The conditions for dot-blot analysis were the same as described for Figure 2. Apical and mature RNAs of etiolated 4-day-old hypocotyls (Figure 2) were loaded onto the same filter for comparative purposes.

PRP Gene Expression during Seed Development in Soybean Plants

To analyze PRP gene expression during soybean seed development, seed pods were subdivided into three tissue parts: pod (P), seed cotyledon (Sd), and seed coat (Sc). These parts were further subdivided into different stages of development as follows: (1) for pod, P1 (6 days to 11 days after anthesis (daa)), P2 (12 daa to 15 daa), and P3 (22 daa to 26 daa), (2) for seed cotyledon and seed coat, Sd1 and Sc1 (young stage of 16 daa to 19 daa) and Sd2 and Sc2 (fully grown, mature stage of seed 24 daa to 28 daa).

Figure 5. SbPRP Gene Expression during Soybean Seed Development.

(A) Soybean fruit parts were subdivided into three tissue parts: pod (P), seed cotyledon (Sd), and seed coat (Sc). These parts were further subdivided into different stages of development as follows: (1) for pod, P1 (6 days to 11 days after anthesis [daa]), P2 (12 daa to 15 daa), and P3 (22 daa to 26 daa), (2) for seed cotyledon and seed coat, Sd1 and Sc1 (young stage of 16 daa to 19 daa) and Sd2 and Sc2 (fully grown, mature stage of seed 24 daa to 28 daa).

(B) Hybridization conditions were the same as described for Figure 2.
An intriguing observation in this experiment is the accumulation of SbPRP2 RNA only in the seed coats of maturing seeds, which contrasts markedly with the pattern of expression of the other two PRP genes. Overall, the data indicate that SbPRP1 is one of the highly expressed forms of cell wall proteins at this stage of seed coat development.

DISCUSSION

In the present study, earlier preliminary observations (Hong, Nagao, and Key, 1987) on the expression of PRP genes (SbPRP1, SbPRP2, and SbPRP3) were extended by analyzing mRNA abundance in various organs and organ regions of etiolated soybean seedlings and green plants at different stages of development.

Experiments were made to determine whether the pattern of PRP expression in other organs was similar or different from the expression described for cultured cells or hypocotyl. Results from these studies indicate that there is no simple correlation between the major form of PRP expressed and the growth stage of an organ. There is instead a complex pattern of organ-specific and developmentally regulated expression for each of the three PRP mRNAs.

In soybean, the organ-specific expression of the PRP gene family can be summarized as follows: (1) whereas SbPRP1 is the major form of PRP in the root, mature hypocotyl, and in the early stages of seed coat development; it is not (or only weakly) expressed in leaves and stems; (2) SbPRP2 is expressed in mature leaf, stem, and seed coat, and this is the major form of PRP gene expressed in apical regions of hypocotyls, in the cotyledons of germinated seedlings, and in young cultured suspension cells; and (3) SbPRP3 is not expressed in the root, but is the major form of PRP in aerial parts of soybean plants including leaf, stem, and pod at different stages of development. Collectively, SbPRP mRNAs were detected in all organs (except in developing embryos), whereas the major form of SbPRP expressed changes dramatically among the various organs and stages of development.

One interesting observation of PRP gene expression in (etiolated) soybean seedlings is the stage-specific switch in the major form of PRP mRNA that accumulates during hypocotyl development (Figure 3). Apical and elongating regions produce a high level of SbPRP2 mRNA that decreases as maturation proceeds, reaching a basal level at the M4 section. Concomitant with this decline, SbPRP1 becomes detectable in section M4, accumulating to high levels in the mature tissue of M6. In contrast, SbPRP3 mRNA is abundant throughout the mature sections (M1 to M5), declining somewhat in the most mature section, M6.

Developmentally regulated patterns of extensin mRNA accumulation were observed in this study, showing differences in the abundance and in the size of mRNAs hybrid-izing to the extensin probes at different stages of development (data from RNA gel blots not presented).

Extensin has been shown to be an abundant cell wall HRGP in soybean seed coat (Cassab et al., 1985). With protein gel blots using extensin antibody, extensin was first detected at 16 days to 18 days after anthesis and increased during development to very high levels at 24 days after anthesis (Cassab and Varner, 1987). In young seed coat at early phases of cotyledon enlargement (examined at 16 days to 19 days after anthesis), extensin RNAs were detected as expected. SbPRP1 mRNA accumulated to high levels in the seed coat at this same stage, probably to higher levels than extensin mRNAs at this stage. The large accumulation of SbPRP1 mRNA suggests that the SbPRP1 plays an important role in early development of the seed coat. The early accumulation of salt-extractable hydroxyproline-containing proteins in soybean seed coats, before the appearance of extensin (Cassab and Varner, 1987), is consistent with the large accumulation of SbPRP1 mRNA in the young seed coat.

There are several examples of differential expression for individual members of a gene family in different tissues or organs (Coruzzi et al., 1984; Dean et al., 1985; Hightower and Meagher, 1985). However, the highly regulated accumulation of SbPRP mRNAs in different organs at different stages of tissue differentiation reported here represents one of the most dramatic examples of changing patterns of mRNA expression for a plant gene family.

In conclusion, these results show that proline-rich proteins of the SbPRP gene family are developmentally regulated, showing dramatic organ-specific and stage-specific expression. These data indicate that the PRP gene family may play an important role in plant development. As one approach to assessing the possible role of the different PRP proteins, studies on the in situ localization of each PRP mRNA and protein are in progress.

METHODS

Plant Material

To obtain seedlings, soybean (Glycine max merr., cv Wayne) seeds were germinated and grown for 4 days to 5 days in moist vermiculite at 28°C to 30°C in the dark. To obtain tissues from whole plants, soybean seeds were planted in sterilized soil and grown in a growth chamber (Conviron E15, Controlled Environments Inc.) with a 14-hr light (28°C) and 10-hr dark (20°C) cycle for 3 weeks and then changed to an 11-hr light and 13-hr dark cycle to induce flowering. All plants were watered to saturation daily and fertilized weekly with 20-20-20 fertilizer (Peters Co., Allentown, PA).

RNA Isolation and Organ Preparation

Total RNA from etiolated soybean seedlings or green plants was isolated as described previously (Hong, Nagao, and Key, 1987).
RNA of tissue-cultured cells was isolated as described by Ulrich and Key (1987). In this study, organs were collected as described below, frozen quickly in liquid nitrogen, and stored at -80°C until the RNA was extracted.

Plants from four different stages of development were used in these studies: 4-day-old and 5-day-old etiolated seedlings, 3-week-old green plants, and flowering plants bearing developing pods. Each set of plants was sectioned according to the diagrams in part A of Figures 2 to 5.

Four-day-old etiolated seedlings were sectioned into hypocotyl, root, seed cotyledon, and plumule (Figure 2A). Hypocotyl and root tissues were further sectioned into apical, elongating, and mature regions. In this study, apical root refers to a 2-mm to 3-mm section from the apex including the root cap. For a more detailed study of PRP gene expression in the maturing region of the hypocotyl, 5-day-old etiolated hypocotyls were serially sectioned: three 1-cm-long sections from the apex down, two sections of 2 cm, and a 3-cm-long hypocotyl, respectively (Figure 3A). Two grams to 50 g of organ were collected depending on the organ section.

Three-week-old soybean plants were divided into three major organ systems (leaf, stem, and root) and examined according to the stage of development (Figure 4A). This included a stage in which cells were undergoing enlargement, a stage of full cell expansion, and a stage in which cells were fully matured but photosynthetically active. In this study, leaf refers to leaf blade without petiole, and stem refers to internode. The bulk of root, including root hairs, was washed free of soil before sampling; the tap root was excluded from the root preparation prior to RNA extraction.

Developing seed pods were collected and separated into pod, seed coat, and seed cotyledon (Figure 4A). Pod seeds were collected at roughly three stages: very young pods of approximately 1 cm to 2 cm in length, which were collected 6 days to 11 days after anthesis, young pods of about 3 cm to 4 cm, taken at 12 days to 15 days, and fully mature pods of 5 cm to 6 cm at 23 days to 26 days. Two stages of seed development were examined for the seed cotyledon and seed coat: (1) early stage of cell enlargement [16 days to 19 days after anthesis, or stages H, I, and J according to Meinke, Chen, and Beachy (1981)] and (2) mature green stage in which cells are fully expanded and undergoing cell maturation (24 days to 26 days after anthesis, or stage N). Seeds collected from 16-day-old to 19-day-old pods were designated young seed cotyledon and young seed coat. Seeds from 24-day-old to 28-day-old, fully mature pods were designated old seed cotyledon and old seed coat.

RNA Gel Blot and Dot-Blot Hybridization Analyses

For RNA gel blot hybridization, 15 µg of total RNA was electro- phoresed on a 2% agarose gel containing 6% formamide using Mops (pH 7.0) as the electrophoresis buffer (Maniatis, Fritsch, and Sambrook, 1982) and transferred to nitrocellulose as previously described (Hong, Nagao, and Key, 1987). For dot-blot hybridization, 5 µg of total RNA was denatured and applied to nitrocellulose using a manifold (Bethesda Research Laboratories) according to the method of Galau, Bijaisoradat, and Hughes (1987). After transfer, the blots were prehybridized and hybridized in a solution of 50% (v/v) formamide, 5 x SSC, 50 mM sodium phosphate, 0.1% (w/v) gelatin, 0.1% (w/v) Ficoll 400, 0.1% (w/v) polyvinylpyrrolidone 360, 100 µg/mL RNA, 100 µg/mL sheared salmon sperm DNA, and 0.2% (w/v) SDS at 42°C for 18 hr to 24 hr as previously described (Hong, Nagao, and Key, 1987). After hybridization, filters were washed three times at room temperature for 10 min in 2 x SSC, 0.1% SDS, followed by two washes in 2 x SSC, 0.1% SDS at 60°C, and one wash in 0.2 x SSC, 0.1% SDS at 60°C for 15 min. Dot blots and RNA gel blots were hybridized with 2 million cpm and 1.5 million cpm per milliliter of 32P-labeled PRP gene-specific probe, respectively (see below).

Preparation of 32P-Labeled DNA Probes

Recently, we isolated and sequenced two more SpPRP genes, SpPRP2 and SpPRP3, which encode mRNAs of 1050 nucleotides and 650 nucleotides in length, respectively (J. C. Hong, R. T. Nagao, and J. L. Key, unpublished results). These members of the soybean PRP gene family (SpPRP1, SpPRP2, and SpPRP3) have high sequence homology at both the DNA and amino acid sequence levels throughout the entire transcription unit. However, there was sufficient DNA sequence difference in the 3'-untranslated region of each gene to permit the use of gene-specific probes. The 254 bp of Hpall (+714)/HaelI (+968) fragment of the SpPRP1 gene, 158 bp of NdeI (+667)/HindIII (+825) of SpPRP2, and 252 bp of Rsal (+273 to +525) of SpPRP3 were cloned into Bluescript KS+ plasmid (Stratagene). The cloned insert DNAs were twice-purified by agarose gel electrophoresis and labeled by the random oligonucleotide priming method of Feinberg and Vogelstein (1983) for the preparation of gene-specific probes.

Soybean extensin cDNAs were isolated from a cDNA library made from mature hypocotyl RNA and screened with the carrot extensin probe pDC5A1 (Chen and Varner, 1985a), provided by J. Varner. Several cDNA clones were identified during colony screening that showed varying levels of hybridization signal using this carrot extensin probe. Since no information was available about the nucleotide sequence of those clones, a mixture of cDNA inserts in equal proportions was used as hybridization probes after random primer labeling.

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REFERENCES


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