

REVIEW ARTICLE

Structure and Function of Plant Cell Wall Proteins

Allan M. Showalter

Department of Environmental and Plant Biology, Molecular and Cellular Biology Program, Ohio University, Athens, Ohio 45701

INTRODUCTION

Plant cell walls are amazingly complex amalgams of carbohydrates, proteins, lignin, water, and incrusting substances such as cutin, suberin, and certain inorganic compounds that vary among plant species, cell types, and even neighboring cells. Developmental events and exposure to any of a number of abiotic and biotic stresses further increase this compositional and structural variation. Moreover, the dynamic nature and functions of plant cell walls in terms of growth and development, environmental sensing and signaling, plant defense, intercellular communication, and selective exchange interfaces are reflected in these variations. Much is currently known about the structure and metabolic regulation of the various cell wall components, but relatively little is known about their precise functions and intermolecular interactions.

In this review, I will discuss the accumulated structural and regulatory data and the much more limited functional and intermolecular interaction information on five plant cell wall protein classes. These five protein classes, listed in Table 1, include the extensins, the glycine-rich proteins (GRPs), the proline-rich proteins (PRPs), the solanaceous lectins, and the arabinogalactan proteins (AGPs). These five proteins may be evolutionarily related to one another, most obviously because each of them, with the exception of the GRPs, contains hydroxyproline, and less obviously in the case of the GRPs because this class has nucleotide sequence similarity to the extensins. For completeness, I should mention that these are not the only cell wall proteins that are known. Others exist, such as cysteine-rich thionins, 28- and 70-kD water-regulated proteins, a histidine-tryptophan-rich protein, and many cell wall enzymes such as peroxidases, phosphatases, invertases, α -mannosidases, β -mannosidases, β -1,3-glucanases, β -1,4-glucanases, polygalacturonase, pectin methyl esterases, malate dehydrogenase, arabinosidases, α -galactosidases, β -galactosidases, β -glucuronosidases, β -xylosidases, proteases, and ascorbic acid oxidase (Varner and Lin, 1989). However, the above five classes generally represent the most abundant, and to date, the most well-studied and widely documented, plant cell wall proteins.

Before describing these five wall protein classes, I should point out that research on these individual proteins has occurred in several plant species, but relatively few examples exist where these cell wall proteins have been studied together

in one plant, let alone in one particular plant organ or type of cell. Thus, data from one plant species are often extrapolated to represent the situation in other plant species. Although such extrapolations are usually valid, enough variations are now known that caution should be exercised in making or believing such claims.

EXTENSINS

Structure

Extensins are a family of hydroxyproline-rich glycoproteins (HRGPs) found in the cell walls of higher plants. In dicots, extensins are particularly abundant and are generally characterized by the following: they are rich in hydroxyproline and serine and some combination of the amino acids valine, tyrosine, lysine, and histidine; they usually contain the repeating pentapeptide motif Ser-Hyp₄, often within the context of other, larger repeating motifs; most of the hydroxyproline residues are glycosylated with one to four arabinosyl residues, and some of the serine residues are glycosylated with a single galactose unit; they are basic proteins with isoelectric points of ~ 10 due to their high lysine content; they generally assume a polypyrrolidone II helical structure in solution; and they have a rodlike appearance when viewed in the electron microscope. These characteristics are exemplified by numerous dicot extensins (Stuart and Varner, 1980; Leach et al., 1982; Mellon and Helgeson, 1982; Smith et al., 1984, 1986; van Holst and Varner, 1984; Cassab et al., 1985; Esquerré-Tugayé et al., 1985; Stafstrom and Staehelin, 1986; Biggs and Fry, 1990; Kawasaki, 1991) and extensin cDNA and genomic clones (Chen and Varner, 1985a, 1985b; Showalter et al., 1985, 1991; Corbin et al., 1987; Showalter and Varner, 1987; Keller and Lamb, 1989; Evans et al., 1990; Gatehouse et al., 1990; Sauer et al., 1990; De Loose et al., 1991; Adams et al., 1992; Zhou et al., 1992).

A recently characterized extensin from sugar beet, a primitive dicot, provides an interesting contrast to the many extensins characterized from advanced herbaceous dicots (Li et al., 1990). This extensin apparently lacks the repeating pentapeptide motif Ser-Hyp₄, but it does contain a repeating variation

Table 1. Five Major Classes of Structural Plant Cell Wall Proteins and Some Distinguishing Properties

Protein Class	% Protein	% Sugar	Abundant AAs ^a	AA Motifs	Selected Prior Reviews
Extensins (dicot)	~45	~55	O,S,K,Y,V,H	SOOOOSOSOOOYYK SOOK SOOOTOVYK SOOOVYKYK	Showalter and Rumeau (1990) Showalter and Varner (1989) Cassab and Varner (1988)
"Extensins" (monocot)	~70	~30	O,T,S,P,K	TPKPTOOTYTOSOKPO ATKPP	Showalter and Rumeau (1990) Kieliszewski and Lamport (1988)
"Extensins" (Chlamydomonas)	25-50	50-75	O,S	PPP and PP SP PXP and PXXP	Adair and Snell (1990)
"Extensins" (Volvox)	~30	~70	O,S	S(O) ₃₋₇	None
GRPs (dicot)	~100	~0	G	GX	Condit and Keller (1990)
GRPs (monocot)	~100	~0	G	GG and GGY	None
PRPs	80-100	0-20	O,P,V,Y,K	PPVYK and PPVEK	Marcus et al. (1991) Showalter and Rumeau (1990)
PRPs (nodulins)	? ^b	?	O?,P,E,Y,H,K	PPHEK and PPPEYQ	Showalter and Rumeau (1990)
Solanaceous lectins	~55	~45	O,S,G,C	?	Showalter and Varner (1989)
AGPs	2-10	90-98	O,S,A,T,G	AO	Showalter and Varner (1989) Fincher et al. (1983)

^a O = hydroxyproline; AA = amino acid.

^b ? = unknown.

of this motif, namely Ser-Hyp₂[X]-Hyp₂-Thr-Hyp-Val-Tyr-Lys, where [X] represents a Val-His-Glu/Lys-Tyr-Pro "insertion sequence." Table 2 shows that this sugar beet sequence motif is similar to peptide repeats found in tomato P1, carrot, tobacco, and petunia extensins.

In monocots, somewhat different versions of extensin apparently exist. For example, in the graminaceous monocot maize, both a threonine-hydroxyproline-rich glycoprotein (THRGP) and a histidine-hydroxyproline-rich glycoprotein (HHRGP), which is also rich in alanine, are present (Kieliszewski and Lamport, 1988). The THRGP is particularly well characterized by both protein (Kieliszewski and Lamport, 1987; Kieliszewski et al., 1990) and molecular cloning studies (Stiefel et al., 1988, 1990). Such studies show that the THRGP is distinct from a typical dicot extensin in that it is rich in threonine and proline in addition to hydroxyproline, lysine, and serine; it contains two novel amino acid repeat motifs, Thr-Pro-Lys-Pro-Thr-Hyp-Hyp-Thr-Tyr-Thr-Hyp-Ser-Hyp-Lys-Pro-Hyp and Ala-Thr/Ser-Lys-Pro-Pro but only one Ser-Hyp₄ sequence; the serine residues and approximately half of the hydroxyproline residues are not glycosylated; and it is predicted to exist in a random coil conformation, not in a polyproline II helix. Another, apparently related THRGP is also found in maize, but it has yet to be cloned (Hood et al., 1988). Cloned extensin sequences from two other graminaceous monocots, sorghum and rice, show clear sequence similarity to the known maize THRGP sequence (Raz et al., 1991; Caelles et al., 1992).

Amino acid sequence analysis of a number of chymotryptic peptides of the maize HHRGP has demonstrated the presence of several amino acid sequence repeats including Ala-Hyp₃, Ala-Hyp₄, and Ser-Hyp₁₋₃ repeats; however, at this point it is

still unclear how these repeat motifs are arranged (Kieliszewski et al., 1992b). Recently, another group has cloned an apparent HRGP from maize that is expressed specifically in the pollen. This maize HRGP cDNA clone specifies several Lys-Ser-Ser/Pro-Pro₃-Ala-Pro-X-Ser₂-Pro₄-X repeats, in which X represents some hydrophobic amino acid (A. Broadwater, A. Rubinstein, K. Lowrey and P. Bedinger, personal communication).

Gymnosperms also contain cell wall HRGPs. Examination of salt eluates of Douglas fir cell suspension cultures has revealed at least two distinct HRGPs. One of these HRGPs has sequence characteristics very similar to the PRPs and will be discussed later (Kieliszewski et al., 1992a), whereas the other appears to contain both Ser-Hyp₄ and Ala-Hyp repeat units (Fong et al., 1992), which are typically thought to be characteristic of extensins and AGPs, respectively. Indeed, this latter HRGP is extraordinarily interesting as it tends to blur the distinction between extensins and AGPs and indicates the possible shuffling of diverse repeat domains that may occur in evolution. Bao et al. (1992) have also found that wood from loblolly pine contains a cell wall HRGP with 24% proline and 11% hydroxyproline; amino acid sequences from this interesting protein remain to be elucidated.

Lower plants appear to have yet other versions of extensin or, more generally, HRGPs. For example, the green alga *Chlamydomonas* contains at least two sets of HRGPs (Adair and Snell, 1990). One set is characteristically distributed in the cell walls of vegetative and gametic cells, whereas the other set is found in the cell walls of zygotic cells. Both sets of HRGPs appear as rodlike molecules in electron micrographs, with the former set containing more bends in the fibrous structure. Further, molecular cloning has shown that the zygotic cells contain

Table 2. Some Intriguing Amino Acid and Nucleotide Sequence Repeat Similarities among the Various Structural Cell Wall Proteins^a

Cell Wall Protein	Amino Acid Sequence Repeat ^b	No. of Repeats	Nucleotide Sequence Repeat ^c	Reference
Tomato extensin P1	S0000TOVYK	ND ^d	NA ^e	Lampert (1977); Smith et al. (1986)
Carrot extensin gene	SPPPPTVYKYK	7	TCT CCA CCA CCA ACA CCG GTT TAC AAG TAC AAP	Chen and Varner (1985b)
Tobacco extensin cDNA	SPPPPTVYK	6	TCN CCA CCA CCA ACT CCC GTT TAC AAG	J. Memelink, personal communication
Petunia extensin cDNA	SPPPPTVYK	1	TCC CCA CCA CCA ACC CCA GTT TAC AAG	C. Hironaka, personal communication
Tomato extensin P1	S0000VKPHPTLOVTK	ND	NA	Smith et al. (1986)
Sugar beet extensin	SOOVHEYP00TOVYK	ND	NA	Li et al. (1990)
Tomato extensin P1	S0000TOVYK	ND	NA	Lampert (1977); Smith et al. (1986)
Carrot extensin gene	SPPPPTVYKYK	7	TCT CCA CCA CCA ACA CCG GTT TAC AAG TAC AAP	Chen and Varner (1985b)
Tobacco extensin cDNA	SPPPPTVYK	6	TCN CCA CCA CCA ACT CCC GTT TAC AAG	J. Memelink, personal communication
Petunia extensin cDNA	SPPPPTVYK	1	TCC CCA CCA CCA ACC CCA GTT TAC AAG	C. Hironaka, personal communication
Maize THRGP	SPKPPPTP--KPTPPTVYK	6	TCC CCC AAG CCT CCG AGC CCC --- AAG CCG ACC CCG CCG TAC ACC CCT	Stiefel et al. (1988, 1990)
Maize THRGP	SPKPPPTP--KPTPPTVYK	5	AGC CCC AAG CCA CCG ACA CCT --- AAG CCG ACC CCG CCG TAC ACT CCA	Stiefel et al. (1988, 1990)
Maize THRGP	SOAKP0IP--KPT00TYTO	ND	NA	Kieliszewski et al. (1990)
Tomato extensin P3	S0000S0S0000VYK	ND	NA	Lampert (1977); Epstein and Lampert (1984)
Tomato extensin cDNA	SPPPPSPPPPVYK	3	TC3 CCA CCA CCA TCS CCA TCT CCT CCR CCA TAC TAC TAC AAP	Showalter et al. (1991)
Petunia extensin cDNA	SPPPPSPPPPVYK	3	TCA CCA CCA CCA TCA CCA TCA CCC CCA CCT CCC TAC TAC TAC AAP	C. Hironaka, personal communication
Bean extensin cDNA	SPPPPSPPPPVYK	6	TCA CCT CCR CCA CCA TC3 CCA CCA CCR CCG TAC TAC TAC AAP	Corbin et al. (1987)
Bean extensin cDNA	SPPPPSPPPPVYK	4	TCT CCA CCA CCA CCR TCA CCA TCA CCT CCT CCA TAC TAT TAT AAA	Corbin et al. (1987)
Maize THRGP	T0S0000VYK	1	ACC CCC AGC CCG CCG CCA TAC TAC	Kieliszewski et al. (1990); Stiefel et al. (1988, 1990)
Tomato extensin P1	S0000TOVYK	ND	NA	Lampert (1977); Smith et al. (1986)
Carrot extensin gene	SPPPPTVYKYK	7	TCT CCA CCA CCA ACA CCG GTT TAC AAG TAC AAP	Chen and Varner (1985b)
Tobacco extensin cDNA	SPPPPTVYK	6	TCN CCA CCA CCA ACT CCC GTT TAC AAG	J. Memelink, personal communication
Petunia extensin cDNA	SPPPPTVYK	1	TCC CCA CCA CCA ACC CCA GTT TAC AAG	C. Hironaka, personal communication
Maize THRGP	SPKPPPTP--KPTPPTVYK	6	TCC CCC AAG CCT CCG AGC CCC --- AAG CCG ACC CCG CCG TAC ACC CCT	Stiefel et al. (1988, 1990)
Maize THRGP	SPKPPPTP--KPTPPTVYK	5	AGC CCC AAG CCA CCG ACA CCT --- AAG CCG ACC CCG CCG TAC ACT CCA	Stiefel et al. (1988, 1990)
Maize THRGP	SOAKP0IP--KPT00TYTO	ND	NA	Kieliszewski et al. (1990)
Tomato extensin P3	S0000S0S0000VYK	ND	NA	Lampert (1977); Epstein and Lampert (1984)
Tomato extensin cDNA	SPPPPSPPPPVYK	3	TC3 CCA CCA CCA TCS CCA TCT CCT CCR CCA TAC TAC TAC AAP	Showalter et al. (1991)
Petunia extensin cDNA	SPPPPSPPPPVYK	3	TCA CCA CCA CCA TCA CCA TCA CCC CCA CCT CCC TAC TAC TAC AAP	C. Hironaka, personal communication
Bean extensin cDNA	SPPPPSPPPPVYK	6	TCA CCT CCR CCA CCA TC3 CCA CCA CCR CCG TAC TAC TAC AAP	Corbin et al. (1987)
Bean extensin cDNA	SPPPPSPPPPVYK	4	TCT CCA CCA CCA CCR TCA CCA TCA CCT CCT CCA TAC TAT TAT AAA	Corbin et al. (1987)
Maize THRGP	T0S0000VYK	1	ACC CCC AGC CCG CCG CCA TAC TAC	Kieliszewski et al. (1990); Stiefel et al. (1988, 1990)
Tomato extensin P1	S0000TOVYK	ND	NA	Lampert (1977); Smith et al. (1986)
Carrot extensin gene	SPPPPTVYKYK	7	TCT CCA CCA CCA ACA CCG GTT TAC AAG TAC AAP	Chen and Varner (1985b)
Tobacco extensin cDNA	SPPPPTVYK	6	TCN CCA CCA CCA ACT CCC GTT TAC AAG	J. Memelink, personal communication
Petunia extensin cDNA	SPPPPTVYK	1	TCC CCA CCA CCA ACC CCA GTT TAC AAG	C. Hironaka, personal communication
Tomato extensin P2	S0000VYKYK	ND	NA	Lampert (1977); Smith et al. (1986)
Soybean PRP cDNA	PPVYK	19	CCA CCA --- --- GTW TAC AAG	Averyhart-Fullard et al. (1988)
Soybean PRP gene	PPVYK	29	CCA CCA --- --- GTW TAC AAG	Hong et al. (1987)
Soybean PRP	POVYK	ND	NA	Lindstrom and Vodkin (1991)
Sugar beet extensin	SOOVHEYP00TOVYK	ND	NA	Li et al. (1990)
Soybean nodulin PRP cDNA	PP-HEKPPP	12	CCA CCT CAT GAG AAP CCA CCA CCA	Franssen et al. (1987)
Soybean PRP cDNA	PPVEK	16	CCV CCA GTT GAP AAP	Datta et al. (1989)
Soybean nodulin PRP cDNA	PPHEK	16	CCA CCT CAT GAG AAP	Franssen et al. (1987)
Tomato extensin P3	S0000S0S0000VYK	ND	NA	Lampert (1977); Epstein and Lampert (1984)
Maize HHRGP	A000HFPS00	ND	NA	Kieliszewski et al. (1992b)
Maize HHRGP	A0000	ND	NA	Kieliszewski et al. (1992b)
Maize HHRGP	A000	ND	NA	Kieliszewski et al. (1992b)
Maize HHRGP	A00APAP	ND	NA	Kieliszewski et al. (1992b)
Lolium AGP	S00APAP	ND	NA	Gleeson et al. (1989)

^a Note that eight sets of comparisons are presented, which examine (1) advanced dicot extensins versus a primitive dicot extensin, (2) advanced dicot extensins versus a monocot "extensin," (3) advanced dicot extensins versus a monocot "extensin," (4) advanced dicot extensins versus PRPs, (5) a primitive dicot extensin versus a nodulin PRP, (6) a PRP versus a nodulin PRP, (7) an advanced dicot extensin versus a monocot "extensin," and (8) a monocot "extensin" versus an AGP.

^b Note that conserved protein sequences for a given set are underlined, and that gaps (indicated by "-") were inserted in some sequences to maximize alignment of the sequences. Also, note that hydroxyproline (O) is indicated only in the case of amino acid studies; amino acid sequences deduced from DNA sequences cannot distinguish proline from hydroxyproline.

^c Special nucleotide symbols used include: P = A, G, C, T; R = A, T; S = C, G; W = G, T; 3 = A, C, T; N = A, C, G, T.

^d ND = not determined.

^e NA = not applicable (protein study).

an HRGP with (Pro)₃ and (Ser-Pro)_n motifs (Woessner and Goodenough, 1989), whereas the vegetative cells contain HRGPs with (Pro)₃, Pro-X-Pro, Pro-X-X-Pro, and Leu-Pro sequence repeats (Adair and Apt, 1990). Additionally, the plus and minus agglutinins of *Chlamydomonas*, which bring gametes of opposite mating types together during sexual reproduction, are HRGPs that are similar in nature to the vegetative HRGPs. The sequence Leu-Leu-Hyp-Hyp was found to be present in both sexual agglutinins and in a vegetative HRGP (Adair and Snell, 1990). In *Volvox*, a novel sulfated, extracellular HRGP, consisting of a globular and a rod-shaped domain, is expressed specifically during embryonic inversion. This glycoprotein was recently cloned, and its rod-shaped domain was found to consist of numerous Ser-Hyp₃₋₇ repeat units (Ertl et al., 1992).

The evolutionary relationships of the above extensins and the apparent extensin homologs, and hence of the plants that contain them, are now being examined by DNA and protein sequence analysis (Kieliszewski et al., 1990; Showalter and Rumeau, 1990). For example, as shown in Table 2, some intriguing similarities that may indicate interesting evolutionary relationships are seen between (1) the primitive dicot extensin of sugar beet and other dicot extensins; (2) a submotif of one maize extensin repeat, Ser-Hyp-Lys-Pro-Hyp-Thr-Pro-Lys, and the tomato extensin P1 repeat, Ser-Hyp₄-Thr-Hyp-Val-Tyr-Lys (note that amino acid sequence identity can be achieved with a Lys to Pro conversion and a Val-Tyr deletion); and (3) another maize THRGP repeat, Thr-Hyp-Ser-Hyp₄-Tyr, and a motif found in tomato extensin P3, Ser-Hyp-Ser-Hyp₄-Tyr (which is a portion of the larger tomato extensin repeat Ser-Hyp₄-Ser-Hyp-Ser-Hyp₄-Tyr₃-Lys). Additional data, however, will have to be obtained before precise evolutionary relationships are elucidated and tenuous links solidified.

Regulated Expression

Various conditions and treatments, as listed in Table 3, generally increase the expression of extensin (reviewed in Showalter and Rumeau, 1990). These studies were performed largely

with advanced herbaceous dicots, although maize THRGP expression is regulated during development and by wounding (Stiefel et al., 1988; Ludevid et al., 1990; Fritz et al., 1991; Ruiz-Avila et al., 1991), and *Chlamydomonas* and *Volvox* HRGP expression is developmentally regulated (Woessner and Goodenough, 1989; Adair and Snell, 1990; Ertl et al., 1992). Gene activation is probably involved with such changes; however, only in beans (*Phaseolus vulgaris*) has transcriptional regulation in response to wounding, fungal infection, fungal elicitor, and glutathione been verified by nuclear run-off studies (Lawton and Lamb, 1987; Wingate et al., 1988). Two groups have now reported the existence of nuclear *trans*-acting factors that interact with specific *cis*-acting elements of the carrot extensin gene promoter in a wound-specific and ethylene-specific fashion (Holdsworth and Laties, 1989a, 1989b; Granell et al., 1992). Similarly, Wycoff et al. (1991) have carried out promoter deletion experiments with a bean extensin gene promoter fused to the β -glucuronidase (GUS) reporter gene and demonstrated the existence of *cis*-acting regions involved in developmental and wound-regulated gene expression.

Some of the most exciting recent work with regard to the extensins, as well as some of the other cell wall proteins, has occurred in the area of cell and tissue localization. Tissue print RNA and protein blots have allowed extensin mRNA and protein to be localized in several plants and tissues. These simple yet elegant tissue print studies have complemented more sophisticated and labor-intensive studies performed using *in situ* hybridization and immunocytochemical localization. Table 4 shows that taken as a whole, these studies reveal that extensin gene expression and localization can apparently vary from plant to plant and among cell and tissue types, presumably in accord with the different functions of different cell types and tissues. Extensin is commonly associated with phloem tissue and cambium cells, but it can be associated with other tissues as well. In addition, transgenic plants carrying extensin promoters to drive GUS expression have been used to illustrate both tissue-specific and gene-specific expression for extensin (see Table 4).

Although tissue printing is a simple and powerful technique, it is limited by the efficiency of the transfer of protein or mRNA

Table 3. Conditions Regulating the Expression of the Five Major Classes of Structural Plant Cell Wall Proteins

Protein Class	Condition(s)
Extensins (dicot)	Wounding, fungal infection, viral infection, fungal elicitors, endogenous elicitors, ethylene, red light, heat shock, gravity, glutathione, cell culturing, development
"Extensins" (monocot)	Development, wounding
"Extensins" (<i>Chlamydomonas</i>)	Development
"Extensins" (<i>Volvox</i>)	Development
GRPs (dicot)	Development, viral infection, salicylic acid, abscisic acid, drought stress, wounding
GRPs (monocot)	Development, water stress, abscisic acid, mercuric chloride, wounding
PRPs	Wounding, endogenous elicitors, fungal elicitor, ethylene, cell culturing, light, red light, development
PRPs (nodulins)	Development
Solanaceous lectins	Wounding, viral infection
AGPs	Development, wounding

Table 4. Tissue Localization of Extensins, GRPs, and PRPs

Protein Class	Plant System	Tissue Localization	Reference
Extensins	Soybean stems and petioles	Cambium cells, in a few layers of cortex cells surrounding primary phloem, and in some parenchyma cells around the primary xylem; abundant in hypocotyl apical regions	Ye and Varner (1991)
Extensins	Bean stems and petioles	Cambium cells and in a few layers of cortex cells surrounding primary phloem	Ye and Varner (1991)
Extensins	Tomato stems and petioles	Outer and inner phloem	Ye et al. (1991)
Extensins	Petunia stems	Outer phloem	Ye et al. (1991)
Extensins	Tobacco stems	Outer phloem	Ye et al. (1991)
Extensins	Carrot roots	Phloem parenchyma walls	Stafstrom and Staehelin (1988)
Extensins	Soybean roots	Two to three layers of cortex cells around vascular bundles and in protoxylem; abundant in root apical regions	Ye and Varner (1991)
Extensins	Tomato root	Minor components of cortical and parenchyma cell walls	Benhamou et al. (1990)
Extensins	Tobacco with bean extensin-GUS transgene	Subset of pericycle and endodermal cells involved with lateral root initiation	Keller and Lamb (1989)
Extensins	Rape with rape extensin-GUS transgene	Phloem of rape roots	Shirsat et al. (1991)
Extensins	Soybean seeds	Sclerenchyma tissues (palisade epidermal and hourglass cells) of seed coats	Cassab and Varner (1987)
Extensins (i.e., THRGP)	Maize	Predominantly to sites of early vascular differentiation in embryos, leaves, and roots; these sites include xylem elements and surrounding sclerenchyma in leaves and metaxylem and protoxylem in roots	Stiefel et al. (1990)
GRPs	Soybean stems and petioles	Primary xylem, primary phloem, and in newly differentiated secondary xylem	Ye and Varner (1991)
GRPs	Bean stems	Protoxylem tracheary elements of the vascular system	Keller et al. (1989b)
GRPs	Bean stems	Protoxylem, primary xylem and phloem, and newly differentiated secondary xylem	Ye and Varner (1991)
GRPs	Bean stems	Protoxylem, cell corners of protoxylem and metaxylem elements, and phloem	Flyser and Keller (1992)
GRPs	Tomato stems	Xylem vessel elements and fibers; some in outer and inner phloem fibers	Ye et al. (1991)
GRPs	Petunia stems	Vascular tissue (phloem or cambium) and to a layer of cells at the epidermis	Condit et al. (1990)
GRPs	Petunia stems	Xylem vessel elements and fibers; some in outer phloem fibers	Ye et al. (1991)
GRPs	Tobacco stems	Xylem vessel elements and fibers	Ye et al. (1991)
GRPs	Tobacco with bean GRP 1.8-GUS transgene	Protoxylem tracheary elements of the vascular system	Keller et al. (1989a); Keller and Baumgartner (1991)
GRPs	Soybean roots	Primary xylem	Ye and Varner (1991)
GRPs	Bean seeds	Tracheary elements of the vascular tissue of seed coats	Keller et al. (1989b)
GRPs	Maize embryo	Scutellar epidermal cells surrounding embryo axis; epidermal cells of leaves	Gómez et al. (1988)
PRPs	Soybean stems	Xylem vessel elements of young stems and in both phloem fibers and xylem vessel elements and fibers of older stems	Ye et al. (1991)
PRPs (SbPRP1)	Soybean stems	Phloem, xylem, and epidermis	Wyatt et al. (1992)
PRPs (SbPRP2)	Soybean stems	Epidermis, cortical cells, phloem, and pith parenchyma	Wyatt et al. (1992)
PRPs (SbPRP3)	Soybean stems	Endodermis	Wyatt et al. (1992)
PRPs	Tomato stems	Xylem vessel elements and fibers; some in outer and inner phloem fibers	Ye et al. (1991)
PRPs	Petunia stems	Xylem vessel elements and fibers; some in outer phloem fibers	Ye et al. (1991)
PRPs	Tobacco stems	Xylem vessel elements and fibers	Ye et al. (1991)
PRPs	Potato stems	Xylem vessel elements and fibers	Ye et al. (1991)
PRPs	Soybean roots	Corner walls of the cortex and in the protoxylem	Ye et al. (1991)
PRPs (SbPRP1)	Soybean seeds	Group of sclerid cells of the seed coat near the hilum	Wyatt et al. (1992)
PRPs (SbPRP2)	Soybean seeds	Primarily to the aleurone layer of the seed coat	Wyatt et al. (1992)
PRPs	Maize embryo	Scutellum and in nonvascular cells from the embryo axis	José-Estanyol et al. (1992)
PRPs (ENOD2)	Pea nodules	Nodule parenchyma (i.e., inner cortex)	van de Wiel et al. (1990)
PRPs (ENOD2)	Soybean nodules	Nodule parenchyma (i.e., inner cortex)	van de Wiel et al. (1990)

out of the cut cells of the tissue to the filter membrane. Because the bulk of extensin becomes extensively cross-linked in the wall and insoluble, as discussed below, it does not transfer during the tissue printing process. Additionally, tissue printing of wounded tissue results in the detection of less than expected amounts of extensin, extensin mRNA, and GRP mRNA (A. Butt and A. Showalter, unpublished results). This may be due to degradation, wound healing, or insolubilization. Another problem that must be considered for the extensins in designing and analyzing tissue print RNA or protein blots, in situ hybridizations, and immunocytochemical localizations is the possibility that nucleic acid and antibody probes will cross-react with other extensin or extensin-related sequences.

Intermolecular Interactions and Functions

The rapid insolubilization of extensins once they are secreted into the wall not only serves as an impediment to extensin research on the protein level, but also represents a major unsolved mystery in terms of how insolubility occurs and, related to this, with what molecules extensins interact. To date, there are several clues but no direct evidence. Extensins may be cross-linked by intermolecular diphenylether linkages between tyrosines, although such isodityrosine cross-links have only been found intramolecularly (Epstein and Lampert, 1984). Other cross-links cannot as yet be ruled out. One study has shown that extensin monomers (i.e., soluble extensin) form a type of extensin oligomer in the presence of a crude cell wall enzyme preparation (Everdeen et al., 1988). Other recent work has shown that the extensin (and PRP) insolubilization is enhanced within just a few minutes of wounding, elicitor treatment, or glutathione treatment (Bradley, 1992). This insolubilization process is hypothesized to be mediated by the release of hydrogen peroxide and catalyzed by a wall peroxidase. This response is thought to be an ultrarapid defense response that serves to further strengthen the cell wall.

Extensin may also be covalently cross-linked to some wall carbohydrate(s). This was suggested years ago (Keegstra et al., 1973); however, X. Qi and A. Mort (personal communication) have only recently found biochemical evidence supporting the existence of an extensin-pectin cross-link.

It is also likely that extensin interacts ionically with pectins. The positively charged lysine and protonated histidine residues of extensin are candidates for ionic interactions with the negatively charged uronic acids of pectins. Such interactions could be regulated by changes in cell wall pH and Ca^{2+} and thus alter the physiochemical properties of the wall. Lysine residues may also form Schiff base linkages with polysaccharides, and these linkages could be reversibly altered by changes in the cell wall pH.

Although the structural and regulatory properties provide clues to the functions of the extensins, direct functional evidence is lacking. Thus, extensins have been proposed to be structural proteins that may also function in development, wound healing, and plant defense. In the case of wounding and plant defense, increased extensin deposition and

increased extensin cross-linking should lead to a more impenetrable cell wall barrier, thus impeding pathogen infection. Indirect evidence for this idea comes from the observation that cell walls of elicitor-treated bean cell cultures, which undergo ultrarapid extensin (and PRP) cross-linking, are tougher than cell walls of untreated cells, as determined by their resistance to digestion by protoplasting enzymes (L. Brisson and C. Lamb, personal communication). Extensin may also act as a kind of cell wall "fly paper" capable of immobilizing certain plant pathogens. This agglutination response was observed in the case of a few extensins and plant pathogens; it probably results from positively charged extensin molecules interacting ionically with negatively charged surfaces of certain plant pathogens (Leach et al., 1982; Mellon and Helgeson, 1982; van Holst and Varner, 1984). Finally, Akashi and Shibaoka (1991) have presented evidence consistent with their hypothesis that extensin interacts with transmembrane protein(s) and thereby stabilizes cortical microtubules.

GLYCINE-RICH PROTEINS

Structure

GRPs represent a relatively newly discovered class of plant proteins that are characterized by their repetitive primary structure, which contains up to 70% glycine arranged in short amino acid repeat units. The first GRP gene was isolated by Condit and Meagher (1986), who used Epstein-Barr virus DNA as a probe to try to isolate oncogenes from petunia. The protein encoded by this gene contains 67% glycine arranged predominantly in Gly-X repeat units, where X is most frequently Gly but can also be Ala or Ser. The observation that this GRP gene encoded a signal peptide sequence, together with protein data showing that a cell wall protein fraction of pumpkin seed coat contained 47% glycine (Varner and Cassab, 1986), suggested that GRPs are cell wall proteins. Keller et al. (1988) subsequently isolated a bean genomic clone containing two linked GRP genes that predicted proteins containing 63% and 58% glycine in which the glycines are arranged predominantly in repeating Gly-X units, similar to those predicted by the petunia GRP gene. Several other groups have also isolated and characterized GRP cDNAs or genes from tomato (Godoy et al., 1990; Showalter et al., 1991), Arabidopsis (de Oliveira et al., 1990; Quigley et al., 1991), petunia (Linthorst et al., 1990), tobacco (van Kan et al., 1988; Obokata et al., 1991), carrot (Sturm, 1992), and *Chenopodium rubrum* (Kaldenhoff and Richter, 1989). Most of these GRP clones predict one common protein feature, the presence of an amino terminal signal peptide. The idea that these GRPs are localized in the cell wall has subsequently been verified by immunolocalization studies with antibodies against the petunia GRP and one of the bean GRPs (Keller et al., 1988, 1989b; Condit et al., 1990). Because some of the dicot GRP clones apparently lack signal peptides and at least one of them also encodes an RNA binding

sequence (Sturm, 1992), it is likely that a subset of GRPs is located in the cytoplasm and not in the cell wall.

GRPs are not limited to dicotyledonous plants or, apparently, to the cell wall. Genes encoding GRPs have been isolated from maize (Gómez et al., 1988) and rice (Mundy and Chua, 1988; Lei and Wu, 1991), and GRP cDNAs have been isolated from maize (Didierjean et al., 1992), sorghum (Crétin and Puigdomènech, 1990), and barley (Rohde et al., 1990). The maize GRP gene encodes a protein that is 37% glycine and consists largely of Gly-Gly-Tyr-Gly-Gly and Arg-Arg-Glu amino acid repeats. One rice GRP gene encodes a protein that contains only 25% glycine and has no recognizable repeat units (Mundy and Chua, 1988). Interestingly, neither the rice GRP nor the maize GRP includes a signal peptide sequence, making their localization in the cell wall unlikely. In fact, antibodies produced against the rice GRP demonstrated a cytosolic location for this protein. By contrast, another rice GRP was found to encode a signal peptide and a mature protein containing 67% glycine (Lei and Wu, 1991). This rice GRP is clearly homologous to the petunia GRP and to one of the bean GRPs, GRP 1.8, and is thought to reside in the cell wall, not only because it contains a signal peptide but also because the bean GRP 1.8 antibody cross-reacts with an antigen contained in a rice cell wall fraction. The barley GRP cDNA also encodes a putative signal peptide and specifies a unique GRP that has some sequence homology to vertebrate cytokeratins. The sorghum GRP cDNAs appear to be very similar to the maize GRP and do not encode signal peptides. Of as yet unknown functional significance is the observation that the GRPs encoded by both the maize and sorghum GRP clones contain a common RNA binding sequence (Mortenson and Dreyfuss, 1989).

Regulated Expression

As shown in Table 3, dicot GRPs, like extensin, are expressed in response to a variety of developmental and stress conditions (Condit and Meagher, 1987; Keller et al., 1988; van Kan et al., 1988; Condit et al., 1990; de Oliveira et al., 1990; Linthorst et al., 1990; Showalter et al., 1991, 1992). Monocot GRPs are likewise expressed in response to a similar set of conditions (Gómez et al., 1988; Mundy and Chua, 1988; Lei and Wu, 1991; Didierjean et al., 1992).

Dicot GRPs are commonly localized to vascular bundles, particularly to xylem elements, and are clearly associated with cells that are going to be lignified (Table 4). Moreover, these GRPs are usually colocalized with the PRPs (Table 4). In contrast, Gómez et al. (1988) localized the maize GRP mRNA to scutellar epidermal cells surrounding the embryo and to epidermal cells of embryonic leaves.

Intermolecular Interactions and Functions

The picture that has emerged is that there are at least two broad classes of GRPs. One class is found in the cell wall and is developmentally regulated, whereas the second class is found

somewhere in the cytoplasm and is regulated by a variety of stress conditions, including abscisic acid and drought stress. Both GRP classes are represented in dicots and in monocots. The cell wall GRPs are most likely structural proteins that presumably have important functions with respect to plant vascular systems and wound healing. Secondary structure predictions for these cell wall GRPs indicate that they exist as β -pleated sheets composed of varying numbers of antiparallel strands as dictated by the particular GRP sequence; such a structure could provide elasticity as well as tensile strength during vascular development. The association of these GRPs with cells destined to become lignified is intriguing, and it has been suggested that they may serve as nucleation sites for such an event, possibly by the catalytic action that their tyrosine residues could have on the oxidative polymerization chain reaction of lignin synthesis (Keller et al., 1989b). The non-cell wall GRPs may play some role in drought tolerance as well as in wound healing. In addition, the presence of a consensus RNA binding site in at least some of these GRPs may indicate still another function.

One of the bean GRPs, GRP 1.8, is apparently insolubilized in the wall (Keller et al., 1989b); whether this will be the case for the other cell wall GRPs is unknown. Also unknown is what could cause this insolubilization. One possibility is that intermolecular isodityrosine linkages could be formed with other GRP molecules or wall proteins. Based upon recent tissue print data, an extensin-GRP cross-link is unlikely given that these wall proteins are not found in abundance in the same cell types. Perhaps more likely is their interaction with PRPs, given their colocalization, or their interaction with lignin, given that GRPs are deposited in tissues destined to be lignified (Ye et al., 1991). With respect to the latter point, it should be noted that tyrosine residues are capable of forming covalent bonds with dehydrogenated coniferyl alcohol in a reaction catalyzed by peroxidase *in vitro* (Whitmore, 1978).

Extensin clones have been used by Keller et al. (1988), Showalter et al. (1991), and Lei and Wu (1991) to select accidentally and serendipitously GRP clones from bean genomic, tomato cDNA, and rice genomic libraries, respectively, due to nucleotide sequence homology between these two protein classes. This homology is not unexpected and arises from the sequence complementarity between stretches of CCX, which encodes proline, and stretches of GGX, which encodes glycine. In other words, the noncoding strand of an extensin gene shares sequence homology to the coding strand of a GRP gene and vice versa. To date, no extensin gene has been found to be transcribed in the opposite orientation to give rise to a GRP mRNA or vice versa. It should also be noted that there are no sequence motifs corresponding to Gly₄-Asp/Glu in the GRPs (these would be encoded by the complementary sequence giving rise to Ser-Hyp₄ repeats of dicot extensins); instead, glycine residues occur in clusters of no apparent pattern (with the exception of the Gly-X motif observed in several GRPs, where X can be any of several possible amino acids including Gly). Similarly, no maize THRGP sequence motif can be encoded by the maize GRP gene. Perhaps a gene encoding an HRGP containing long stretches of hydroxyproline, such

as that found in *Volvox* but not yet cloned (Mitchell, 1980), will prove to be the most homologous sequence to the GRP genes; only additional study will tell.

PROLINE-RICH PROTEINS

Structure

PRPs represent another relatively newly identified class of plant cell wall proteins of which at least some, and perhaps all, members contain hydroxyproline. There are at least two broad subclasses of PRPs: those that are components of normal plant cell walls (Chen and Varner, 1985a; Hong et al., 1987, 1990; Averyhart-Fullard et al., 1988; Tierney et al., 1988; Datta et al., 1989; Datta and Marcus, 1990; Kleis-San Francisco and Tierney, 1990; Lindstrom and Vodkin, 1991; Sheng et al., 1991) and those that are *plant nodulins* (i.e., proteins produced in response to infection by nitrogen-fixing bacteria) and constitute part of the nodule cell wall (Franssen et al., 1987, 1989; Dickstein et al., 1988; Scheres et al., 1990; van de Wiel et al., 1990; Govers et al., 1991). The distinction between these two classes may not be clearcut because two pea nodulin PRPs, ENOD12A and ENOD12B, are also expressed in stems and flowers (Scheres et al., 1990; Govers et al., 1991).

All of the PRPs are characterized by the repeating occurrence of Pro-Pro repeats that are contained within a variety of other larger repeat units. For example, many of the normal cell wall PRPs and some nodulin PRPs, such as soybean, alfalfa, and pea ENOD2, are characterized by the presence of the repeating pentapeptide sequence Pro-Pro-X-Y-Lys, where X and Y can be valine, tyrosine, histidine, and glutamic acid. In some cases, the repetitive unit extends an additional proline residue beyond the other two proline residues. Those PRPs that have been isolated and characterized lack carbohydrate or are only lightly glycosylated (Datta et al., 1989) and contain approximately equimolar quantities of proline and hydroxyproline (Averyhart-Fullard et al., 1988; Datta et al., 1989; Kleis-San Francisco and Tierney, 1990). In addition, amino acid sequence analysis has shown that hydroxyproline occurs exclusively in the second position of a soybean cell wall PRP pentapeptide repeat (i.e., Pro-Hyp-X-Y-Lys) (Lindstrom and Vodkin, 1991) and in the second and third positions as well as only in the third position of two hexapeptide repeats found in a gymnosperm PRP (i.e., Pro-Hyp-Hyp-Val-Tyr-Lys and Pro-Pro-Hyp-Val-Val-Lys, respectively) (Kieliszewski et al., 1992a). It will be interesting to observe the hydroxylation patterns of the nodulin PRPs and determine whether they are consistent with the above motifs and other rules postulated for proline hydroxylation.

Josè-Estanyol et al. (1992) recently described the cloning and characterization of the first monocot PRP gene. This maize PRP consists of an N-terminal proline-rich domain with numerous Pro-Pro-Tyr-Val and Pro-Pro-Thr-Pro-Arg-Pro-Ser repeats and a C-terminal proline-poor domain that is hydrophobic and contains several Cys residues. This characteristic domain

arrangement is similarly exhibited by two dicot PRP sequences, one from bean (Sheng et al., 1991) and the other from tomato (Salts et al., 1991).

Regulated Expression

Regulatory studies indicate that PRPs are involved in various aspects of development, ranging from germination to the early stages of nodulation (reviewed in Showalter and Rumeau, 1990; Hong et al., 1989; Scheres et al., 1990; Josè-Estanyol et al., 1992). In addition, it has been reported that wounding, endogenous elicitors, fungal elicitor, ethylene, cell culturing, and light can affect PRP gene expression (Tierney et al., 1988; Marcus et al., 1991; Sheng et al., 1991).

Members of the PRP gene family exhibit tissue- and cell-specific patterns of expression (Table 4). As already mentioned, PRPs commonly show a localization pattern similar to that of most dicot GRPs, although some PRP expression occurs in the same cell types as extensins. Recently, Wyatt et al. (1992) used *in situ* hybridization with gene-specific probes to reveal different tissue-specific expression patterns for three members of the soybean PRP gene family in 4-day-old soybean hypocotyls (Table 4). Indeed, such gene-specific/protein-specific studies are important as they allow for the dissection of the gene/protein family without the complication of cross-hybridization or cross-reactivity to related family members. In the case of PRP nodulins and the maize PRP, other distinct patterns of expression are seen.

Intermolecular Interactions and Functions

It is likely that the PRPs, like the extensins and GRPs, are insolubilized in the wall over time. Indeed, data already exist that support this idea (Datta et al., 1989; Kleis-San Francisco and Tierney, 1990). Moreover, this insolubilization process can occur rapidly in response to stress and may be mediated by the release of hydrogen peroxide and catalyzed by a wall peroxidase (Bradley et al., 1992). The molecular interactions that cause insolubilization, however, are unknown. The relatively high tyrosine content of the PRPs raises the possibility of isodityrosine cross-links between PRP molecules and/or between PRPs and GRPs or extensins, given the above tissue localization data. As with the GRPs, the association of some PRPs with lignification raises the possibility that they are involved in the lignification process (Ye et al., 1991). In addition, given that the PRPs tend to be basic proteins because of their high lysine content and that salt extraction can solubilize some, but not all, soybean PRPs from the wall, it is conceivable that PRPs interact ionically with other wall components such as the acidic pectins, as is also likely for the extensins.

The PRPs may have important roles in normal development and in nodule formation, but their precise functions are unknown. In recent work, peptidyl proline hydroxylase inhibitors were applied to soybean cells in culture, with the result that

salt-extractable PRPs were reduced and cell growth was arrested (Schmidt et al., 1991). A similar study performed several years ago showed that tobacco cells treated with prolyl hydroxylase inhibitors led to the production of monster cells (i.e., cells that continued to grow but did not divide; J. Cooper, personal communication). Clearly, such experiments are interesting and their correlations intriguing; however, two complications should be kept in mind, namely that the inhibitors are not totally specific and may affect general cell metabolism, and that all hydroxyproline-containing proteins will be affected, making it impossible to ascribe a function to any one particular HRGP.

In the case of the PRP nodulins, roles in nodule morphogenesis and in the bacterial infection process are possible. Based on their expression patterns, the ENOD2 class of PRP nodulins from soybean, alfalfa, and pea is thought to be involved in nodule morphogenesis. They may function specifically in the erection of a cell wall oxygen barrier for the oxygen-sensitive nodules (van de Wiel et al., 1990). In contrast, the ENOD12 class of PRP nodulins is hypothesized to have as yet undefined roles in the bacterial infection process and in developmental processes occurring in other plant tissues as well (Scheres et al., 1990).

There is an obvious bit of sequence identity between the PRPs and the extensins (Table 2). This is best demonstrated by comparing the Pro-Pro-Val-Tyr-Lys repeat common to many PRPs with the Ser-Hyp₄-Val-Tyr-Lys repeats of tomato P2 extensin and the Ser-Hyp₄-Thr-Pro-Val-Tyr-Lys repeats of carrot, tobacco, and petunia extensins. Similarity can also be seen between the N-75 (or ENOD2) nodulin sequence Pro-Pro-His-Glu-Lys-Pro-Pro-Pro and a portion of the sugar beet extensin sequence Hyp-Hyp-Val-His-Glu-Tyr-Pro-Hyp-Hyp. Certain PRP sequences also show limited sequence identity to the N-terminal domain of γ -zein, which contains Pro-Pro-Pro-Val-His-Leu-Pro-Pro repeats, and to the animal salivary proline-rich proteins (Showalter and Rumeau, 1990).

SOLANACEOUS LECTINS

Structure

Lectins are carbohydrate binding proteins or glycoproteins of diverse origin. The solanaceous lectins represent a unique class of plant lectins that can be distinguished from other lectins by their restricted occurrence in solanaceous plants, their ability to agglutinate oligomers of *N*-acetylglucosamine, their predominantly extracellular location, and their unusual amino acid and carbohydrate composition in which hydroxyproline and arabinose are major constituents (reviewed in Allen, 1983; Showalter and Varner, 1989).

Potato tuber lectin (PTL) is the most well-studied member of the solanaceous lectins. It is a glycoprotein with a monomeric molecular weight of 50 kD and consists of 50% carbohydrate and 50% protein by weight (Allen and Neuberger, 1973; Allen et al., 1978; Matsumoto et al., 1983). The carbohydrate moiety

consists mainly of arabinose, which is linked to hydroxyproline. These linkages generally take the form of tri- and tetra-arabinosides; in addition, and to a lesser extent, galactose is linked to serine (Allen et al., 1978; Muray and Northcote, 1978; Ashford et al., 1982). The protein moiety is especially rich in four amino acids, hydroxyproline, serine, glycine, and cysteine. In its native form, it is extensively linked by disulfide bridges so that no free sulfhydryl groups are present. Exhaustive pronase digestion of PTL following reductive alkylation releases a 33-kD glycopeptide that is exceptionally rich in serine and hydroxyproline but not in glycine or cysteine (Allen et al., 1978). These data indicate that PTL consists of at least two distinct protein domains; one is rich in serine and hydroxyproline and contains the carbohydrate moiety, and the other(s) is rich in glycine and cysteine. An analogous experiment with the *Datura stramonium* lectin led to an essentially identical conclusion (Desai et al., 1981). The ability of PTL to bind *N*-acetylglucosamine oligomers is known to be associated with the glycine-cysteine-rich domain(s) (Allen et al., 1978; Ashford et al., 1981), and it is likely that this chitin binding domain will be homologous to that found in other chitin binding proteins such as wheat germ agglutinin, barley lectin, rice lectin, stinging nettle lectin, hevein, potato win1 and 2, poplar win8, bean chitinase, potato chitinase, and tobacco chitinase (reviewed in Chrispeels and Raikhel, 1991). In the case of many of these chitin binding protein genes, the chitin binding domain is fused to an unrelated protein domain.

The serine-hydroxyproline-rich glycopeptide domain of PTL and the other solanaceous lectins bears a striking biochemical resemblance to the extensins. The similarity between these two classes of HRGPs is reflected in their extracellular location, their abundance of serine, hydroxyproline, and arabinose, and in the presence of identical carbohydrate-protein linkages, namely hydroxyproline attached predominantly to tri- and tetra-arabinosides and serine attached to single galactose residues. These similarities probably reflect an interesting evolutionary relationship between these two classes of HRGPs that remains to be elucidated. Indeed, it is likely that the repeating Ser-Hyp₄ pentapeptide sequences that are characteristic of most dicot extensins will also characterize the serine-hydroxyproline-rich glycopeptide domain of PTL and the other solanaceous lectins.

Regulated Expression

It has been reported that PTL accumulates in potato tubers in response to wounding (Casalongué and Pont Lezica, 1985) and viral infection (Scheggia et al., 1988). It is also reported that PTL agglutinates avirulent, but not virulent, strains of *Pseudomonas solanacearum* (Sequeira and Graham, 1977) and that a hydroxyproline-rich lectin, curiously enough from bean plants, accumulates in bean suspension cultures in response to fungal elicitor (Bolwell, 1987).

Tissue print immunoblots have shown that PTL is deposited in a developmental fashion, with initial deposition occurring

in the cortex and pith regions of the tuber and subsequent distribution occurring uniformly in the whole tuber (Pont-Lezica et al., 1991). Additional tissue immunoblots revealed that the potato lectin is found in both the outer and inner phloem of other potato tissues (R. Pont-Lezica, personal communication). Unfortunately, because the potato lectin antibodies cross-react with extensin, it is impossible to know the contribution being made by each protein in these immunoblots.

Intermolecular Interactions and Functions

Because the solanaceous lectins appear to be localized in the cell wall and are apparently similar to many dicot extensins, these lectins conceivably have the potential to interact in much the same way as extensins. However, the tyrosine and lysine content is considerably less than that of most dicot extensins, making isodityrosine cross-links and ionic interactions with negatively charged wall components unlikely. The glycine-cysteine-rich domain would be available to interact with wall components as well, but it is perhaps more likely that this domain would be used for binding pathogens that have *N*-acetylglucosamine oligomers on their surfaces.

Although the unequivocal physiological role(s) of the solanaceous lectins (and other plant lectins as well) is unknown, it is thought that their ability to bind sugars and their predominantly extracellular location are somehow involved with their function. Consequently, one of the most frequently suggested roles for these lectins involves various forms of cell-cell interaction. Some previously proposed roles for the solanaceous lectins include sugar transport, stabilization of seed storage proteins, and control of cell division. The above regulatory data also indicate that these lectins may be involved with wound healing and plant defense. In the case of the latter, pathogen immobilization via binding of *N*-acetylglucosamine surface oligomers may be important.

ARABINO GALACTAN PROTEINS

Structure

AGPs are HRGPs that are generally very soluble and highly glycosylated (reviewed in Fincher et al., 1983; Showalter and Varner, 1989). AGPs are widely distributed in plants and typically comprise only 2 to 10% protein by weight. Their molecular weights are extremely heterogeneous, presumably reflecting different extents of glycosylation. AGPs are readily solubilized during tissue extraction with low ionic strength aqueous solutions. Most AGPs are also soluble in saturated ammonium sulfate. These solubility properties have greatly facilitated their isolation and characterization. The protein moiety of AGPs is typically rich in hydroxyproline, serine, alanine, threonine, and glycine and is resistant to proteolysis in its native state, a property that is presumably conferred by extensive glycosylation.

AGPs have isoelectric points in the range of pH 2 to 5. The N-terminal sequences of four different AGPs, three from carrot and one from ryegrass, have been determined; all four sequences contain Ala-Hyp repeats (Gleeson et al., 1985). More recently, sequences from five tryptic peptides from deglycosylated ryegrass AGP have also been determined; some of these sequences also contain Ala-Hyp repeats (Gleeson et al., 1989). Pending further investigation, such repeats may become a diagnostic characteristic of AGPs, because they are not known to occur in any of the other cell wall proteins, with the possible exception of the maize HHRGP (Table 2) (Kieliszewski et al., 1992b).

Carbohydrates account for most of the weight of AGPs, and, as their name implies, AGPs contain D-galactose and L-arabinose as the major carbohydrate constituents. Structural analysis has shown that carbohydrate moiety of some AGPs consists of polysaccharide chains with (1→3) linked β-D-galactopyranose residues branched with (1→6) linked β-D-galactopyranose side chains, which are in turn branched with arabinofuranose and other, less abundant monosaccharides (Fincher et al., 1983). Moreover, such moieties appear to be attached regularly to the protein core via β-D-galactopyranose-hydroxyproline linkages (Fincher et al., 1983; Bacic et al., 1987). It is worth noting that carbohydrate moieties similar, if not identical, to those present in AGPs also exist in the cell wall unattached to protein; these are the arabinogalactans, which have been reviewed elsewhere (Clarke et al., 1979).

Conclusive cellular localization of many AGPs is difficult because of their extreme solubility. It is clear, however, that AGPs are often found as constituents of the extracellular milieu. Cells in suspension culture from various tissue sources secrete AGPs into the culture medium; also, certain specialized cells, such as the stelar canal cells and some secretory cells that produce gummy exudates, secrete especially large quantities of AGPs into the cell wall compartment (Clarke et al., 1979). In addition, some AGPs are associated with the plasma membrane (Pennell et al., 1989; Norman et al., 1990). Yariv's antigen, a β-D-glucosyl artificial carbohydrate antigen, specifically precipitates most AGPs as a red-orange complex, greatly aiding the cellular and tissue localization studies, even though the interaction between Yariv's antigen and AGPs is not well understood (Fincher et al., 1983). This interaction also serves to illustrate the point that most AGPs are "β-lectins," with a broad binding specificity directed toward β-D-glycopyranosyl linkages.

Electrophoretic separation of AGPs in the presence of Yariv's antigen shows that AGPs are expressed in a tissue-specific manner and that a given plant tissue can contain more than one kind of AGP (van Holst and Clark, 1986). Whether these AGPs differ with respect to their protein moieties, carbohydrate moieties, or both is not yet known. In this context, Norman et al. (1990) have shown that a set of tobacco plasma membrane AGPs are produced by differential glycosylation of a 50-kD core protein.

Circular dichrometry shows that ~30% of the protein moiety of an AGP is in a polyproline II helix (van Holst and Fincher, 1984). Little is known about the conformation of the rest of the

protein or the carbohydrate chains. One model, based on the above information and the observation that some AGPs have low viscosities, predicts that carbohydrate moieties with ovoid or spheroidal shapes are attached at several positions on a core protein (Fincher et al., 1983).

Regulated Expression and Functions

No function(s) for AGPs has been established unequivocally. Based on their predominantly extracellular location and their various biochemical and physical properties, AGPs have been proposed to act as glues, lubricants, and humectants (Fincher et al., 1983). Their general abundance in the middle lamellae of the wall and in the styles of angiosperms and in the medulla of root nodules (Cassab, 1986) makes them likely candidates for functioning in cell-cell recognition. There is also some indication that AGPs accumulate in response to wounding (Fincher et al., 1983). Consequently, a role for AGPs in wound healing or plant defense is a possibility that requires further investigation.

Evidence for developmental regulation of plasma membrane AGPs in flowers, embryos, and roots has recently been obtained (Pennell and Roberts, 1990; Knox et al., 1991; Pennell et al., 1991). Thus, a role for these AGPs in floral histogenesis and differentiation is possible. Such a role may involve cell-cell interactions in which these plasma membrane AGPs act as cell adhesion molecules analogous to mammalian substrate adhesion molecules and capable of binding as yet undefined cell wall ligands (Pennell et al., 1991). Herman and Lamb (1992) have shown that plasma membrane AGPs are not only localized to tobacco leaf plasma membranes but also to intravacuolar multivesicular bodies. This observation has led them to propose that plasma membrane AGPs are involved with an endocytotic pathway for vacuolar-mediated disposal of the periplasmic matrix.

FUTURE DIRECTIONS AND QUESTIONS

Relatively straightforward structural and regulatory studies have now been done on numerous examples of the five major cell wall proteins. Such characterization work will surely continue and new family members will be isolated with perhaps a few surprises along the way. Regulatory studies of the wall protein genes will undoubtedly go the route of so many other genes, and *cis*- and *trans*-acting factors will be identified by now fairly routine molecular biology technology. Cloning of the as yet uncloned solanaceous lectins and AGPs should soon follow. Furthermore, it is almost inevitable that new wall proteins and their genes will be identified and characterized.

More difficult questions of function and molecular interactions are ahead, however. To ascertain the function of these proteins, several approaches are possible. First, knowing where the proteins are located in the plant (i.e., in which organs and

tissues) will be an important step in ascribing a function to these proteins. Such studies alone will not provide the functional answer, but they will provide critical clues, and any functional assignment will ultimately have to be consistent with the results of the localization studies. The most direct and unambiguous way to address function is to produce cell wall protein mutants and examine phenotypic alterations of mutant plants. Mutants could be produced with antisense RNA technology, with the aid of ribozymes, or by mutagenesis followed by screening with antibodies to the various wall proteins. Some of this work has begun in the labs of James Cooper, Andrew Staehelin, and Keith Roberts, but no clearcut picture has emerged. Indeed, these are exciting but difficult experiments because many questions concerning them can be raised. For what function does one look? Is one looking at the right place and time? Is one looking under the correct conditions to see an altered phenotype? Will other members of the gene family or other wall proteins compensate for the loss of one wall protein?

As for determining how the cell wall proteins interact in the wall, several approaches are possible: labeling the cells and characterizing proteolysis products from the cell wall, adding labeled synthetic peptides to cell cultures and testing for insolubilization and subsequent characterization of the cross-links, producing tagged chimeric proteins (i.e., part marker protein, part cell wall peptide motif) in transgenic plants and examining insolubilization and cross-links, purifying cell wall enzyme preparations capable of cross-linking purified cell wall protein monomers *in vivo*, and examining cell wall protein connections in plants via solid state nuclear magnetic resonance. Clearly, these are for the most part difficult biochemical experiments, and their interpretations may be equally difficult, particularly with respect to possible functional implications.

Our current state of knowledge has allowed for some evolutionary speculation, but more sequences are needed, particularly from more primitive plants, to see these evolutionary connections (or artifacts) more clearly. Is it really possible that all of the HRGPs, and even the GRPs, represent products of one giant supergene family? Indeed, some intriguing evolutionary studies lie ahead.

Finally, it would be of great use to select a model system for plant cell wall research. Arabidopsis is one logical choice, particularly because it is so amenable to mutagenesis studies to address functional questions, but others are clearly possible. Examining all of the plant cell wall components, including all of the major structural wall proteins, together in one system would allow for a more integrated approach to elucidate cell wall structure, intermolecular interactions, and ultimately cell wall function.

ACKNOWLEDGMENTS

The preparation of this review was made possible by an Academic Challenge Grant from the state of Ohio and by United States Department

of Agriculture Grant No. 90-37140-5499. I especially thank Joseph Varner, Marcia Kieliszewski, and Rebecca Chasan for their constructive comments and suggestions on earlier drafts of this manuscript.

Received January 27, 1992; accepted November 4, 1992.

REFERENCES

- Adair, W.S., and Apt, K.E.** (1990). Cell wall regeneration in *Chlamydomonas*: Accumulation of mRNAs encoding cell wall hydroxyproline-rich glycoproteins. *Proc. Natl. Acad. Sci. USA* **87**, 7355–7359.
- Adair, W.S., and Snell, W.J.** (1990). The *Chlamydomonas reinhardtii* cell wall: Structure, biochemistry, and molecular biology. In *Organization and Assembly of Plant and Animal Extracellular Matrix*, W. S. Adair and R.P. Mecham, eds (New York: Academic Press), pp. 15–84.
- Adams, C.A., Nelson, W.S., Nunberg, A.N., and Thomas T.L.** (1992). A wound-inducible member of the hydroxyproline-rich glycoprotein gene family in sunflower. *Plant Physiol.* **99**, 775–776.
- Akashi, T., and Shibaoka, H.** (1991). Involvement of transmembrane proteins in the association of cortical microtubules with the plasma membrane in tobacco BY-2 cells. *J. Cell Sci.* **98**, 169–174.
- Allen, A.K.** (1983). Potato lectin—a glycoprotein with two domains. In *Chemical Taxonomy, Molecular Biology, and Function of Plant Lectins*, I.J. Goldstein and M.E. Etzler, eds (New York: Alan R. Liss), pp. 71–85.
- Allen, A.K., and Neuberger, A.** (1973). The purification and properties of the lectin from potato tubers, a hydroxyproline-containing glycoprotein. *Biochem. J.* **135**, 307–314.
- Allen, A.K., Desai, N.N., Neuberger, A., and Creeth, J.M.** (1978). Properties of potato lectin and the nature of its glycoprotein linkages. *Biochem. J.* **171**, 665–674.
- Ashford, D., Menon, R., Allen, A.K., and Neuberger, A.** (1981). Studies on the chemical modification of potato (*Solanum tuberosum*) lectin and its effect on haemagglutinating activity. *Biochem. J.* **199**, 399–408.
- Ashford, D., Desai, N.N., Allen, A.K., Neuberger, A., O'Neill, M.A., and Selvendran, R.R.** (1982). Structural studies of the carbohydrate moieties of lectins from potato (*Solanum tuberosum*) tubers and thorn-apple (*Datura stramonium*) seeds. *Biochem. J.* **201**, 199–208.
- Averyhart-Fullard, V., Datta, K., and Marcus, A.** (1988). A hydroxyproline-rich protein in the soybean cell wall. *Proc. Natl. Acad. Sci. USA* **85**, 1082–1085.
- Bacic, A., Churms, S.C., Stephen, A.M., Cohen, P.B., and Fincher, G.B.** (1987). Fine structure of the arabinogalactan-protein from *Lolium multiflorum*. *Carbohydr. Res.* **162**, 85–94.
- Bao, W., O'Malley, D.M., and Sederoff, R.R.** (1992). Wood contains a cell-wall structural protein. *Proc. Natl. Acad. Sci. USA* **89**, 6604–6608.
- Benhamou, N., Mazau, D., and Esquerré-Tugayé, M.-T.** (1990). Immunocytochemical localization of hydroxyproline-rich glycoproteins in tomato root cells infected by *Fusarium oxysporum* f. sp. *radicis-lycopersici*: Study of a compatible interaction. *Mol. Plant Pathol.* **80**, 163–173.
- Biggs, K.J., and Fry, S.C.** (1990). Solubilization of covalently bound extensin from *Capsicum* cell walls. *Plant Physiol.* **92**, 197–204.
- Bolwell, G.P.** (1987). Elicitor induction of the synthesis of a novel lectin-like arabinosylated hydroxyproline-rich glycoprotein in suspension cultures of *Phaseolus vulgaris* L. *Planta* **172**, 184–191.
- Bradley, D.J., Kjellbom, P., and Lamb, C.J.** (1992). Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. *Cell* **70**, 21–30.
- Caelles, C., Delseny, M., and Puigdomènech, P.** (1992). The hydroxyproline-rich glycoprotein gene from *Oryza sativa*. *Plant Mol. Biol.* **18**, 617–619.
- Casalongué, C., and Pont Lezica, R.** (1985). Potato lectin: A cell-wall glycoprotein. *Plant Cell Physiol.* **26**, 1533–1539.
- Cassab, G.I.** (1986). Arabinogalactan proteins during the development of soybean root nodules. *Planta* **168**, 441–446.
- Cassab, G.I., and Varner, J.E.** (1987). Immunocytochemical localization of extensin in developing soybean seedcoat by immunogold-silver staining and by tissue printing on nitrocellulose paper. *J. Cell Biol.* **105**, 2581–2588.
- Cassab, G.I., and Varner, J.E.** (1988). Cell wall proteins. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **39**, 321–353.
- Cassab, G.I., Nieto-Sotelo, J., Cooper, J.B., van Holst, G.-J., and Varner, J.E.** (1985). A developmentally regulated hydroxyproline-rich glycoprotein from the cell walls of soybean seed coats. *Plant Physiol.* **77**, 532–535.
- Chen J., and Varner J.E.** (1985a). Isolation and characterization of cDNA clones for carrot extensin and a proline-rich 33-kDa protein. *Proc. Natl. Acad. Sci. USA* **82**, 4399–4403.
- Chen J., and Varner J.E.** (1985b). An extracellular matrix protein in plants: Characterization of a genomic clone for carrot extensin. *EMBO J.* **4**, 2145–2151.
- Chrispeels, M.J., and Raikhel, N.V.** (1991). Lectins, lectin genes, and their role in plant defense. *Plant Cell* **3**, 1–9.
- Clarke, A.E., Anderson, R.L., and Stone, B.A.** (1979). Form and function of arabinogalactans and arabinogalactan-proteins. *Phytochemistry* **18**, 521–540.
- Condit, C.M., and Keller, B.** (1990). The glycine-rich cell wall proteins of higher plants. In *Organization and Assembly of Plant and Animal Extracellular Matrix*, W.S. Adair and R.P. Mecham, eds (New York: Academic Press), pp. 119–135.
- Condit, C.M., and Meagher, R.B.** (1986). A gene encoding a novel glycine-rich structural protein of petunia. *Nature* **323**, 178–181.
- Condit, C.M., and Meagher, R.B.** (1987). Expression of a gene encoding a glycine-rich protein in petunia. *Mol. Cell. Biol.* **7**, 4273–4279.
- Condit, C.M., McClean, B.G., and Meagher, R.B.** (1990). Characterization of the expression of the petunia glycine-rich protein-1 gene product. *Plant Physiol.* **93**, 596–602.
- Corbin, D.R., Sauer, N., and Lamb, C.J.** (1987). Differential regulation of a hydroxyproline-rich glycoprotein gene family in wounded and infected plants. *Mol. Cell. Biol.* **7**, 4337–4344.
- Crétin, C., and Puigdomènech, P.** (1990). Glycine-rich RNA-binding proteins from *Sorghum vulgare*. *Plant Mol. Biol.* **15**, 783–785.
- Datta, K., and Marcus, A.** (1990). Nucleotide sequence of a gene encoding soybean repetitive proline-rich protein 3. *Plant Mol. Biol.* **14**, 285–286.
- Datta, K., Schmidt, A., and Marcus, A.** (1989). Characterization of two soybean repetitive proline-rich proteins and a cognate cDNA from germinated axes. *Plant Cell* **1**, 945–952.
- De Loose, M., Tère, G.G., Villarreal, R., Genetello, C., van Montagu, M., Depicker, A., and Inzé, D.** (1991). The extensin signal peptide

- allows secretion of a heterologous protein from protoplasts. *Gene* **99**, 95–100.
- de Oliveira, D.E., Seurinck, J., Inzé, D., van Montagu, M., and Botterman, J.** (1990). Differential expression of five *Arabidopsis* genes encoding glycine-rich proteins. *Plant Cell* **2**, 427–436.
- Desai, N.N., Allen, A.K., and Neuberger, A.** (1981). Some properties of the lectin from *Datura stramonium* (thorn-apple) and the nature of its glycoprotein linkages. *Biochem. J.* **197**, 345–353.
- Dickstein, R., Bisseling, T., Reinhold, V.N., and Ausubel, F.M.** (1988). Expression of nodule-specific genes in alfalfa root nodules blocked at an early stage of development. *Genes Dev.* **2**, 677–687.
- Didierjean, L., Frendo, P., and Burkard, G.** (1992). Stress responses in maize: Sequence analysis of cDNAs encoding glycine-rich proteins. *Plant Mol. Biol.* **18**, 847–849.
- Epstein L., and Lampport, D.T.A.** (1984). An intramolecular linkage involving isodityrosine in extensin. *Phytochemistry* **23**, 1241–1246.
- Ertl, H., Hallmann, A., Wenzl, S., and Sumper, M.** (1992). A novel extensin that may organize extracellular matrix biogenesis in *Volvox carteri*. *EMBO J.* **11**, 2055–2062.
- Esquerré-Tugayé, M.T., Mazau, D., Pélissier, B., Roby, D., Rumeau, D., and Toppan, A.** (1985). Induction by elicitors and ethylene of proteins associated to the defense of plants. In *Cellular and Molecular Biology of Plant Stress*, J.L. Key and T. Kosuge, eds (New York: Alan R. Liss), pp. 459–473.
- Evans, I.M., Gatehouse, L.N., Gatehouse, J.A., Yarwood, J.N., Boulter, D., and Croy, R.R.D.** (1990). The extensin gene family in oilseed rape (*Brassica napus* L.): Characterisation of sequences of representative members of the family. *Mol. Gen. Genet.* **223**, 273–287.
- Everdeen, D.S., Kiefer, S., Willard, J.J., Muldoon, E.P., Dey, P.M., Li, X.-B., and Lampport, D.T.A.** (1988). Enzymic cross-linkage of monomeric extensin precursors *in vitro*. *Plant Physiol.* **87**, 616–621.
- Fincher, G.B., Stone, B.A., and Clarke, A.E.** (1983). Arabinogalactan-proteins: Structure, biosynthesis, and function. *Annu. Rev. Plant Physiol.* **34**, 47–70.
- Fong, C., Kieliszewski, M.J., de Zacks, R., Leykam, J.F., and Lampport, D.T.A.** (1992). A gymnosperm extensin contains the serine-tetrahydroxyproline motif. *Plant Physiol.* **99**, 548–552.
- Franssen, H.J., Nap, J.-P., Gloudemans, T., Stiekema, W., van Dam, H., Govers, F., Louwerse, J., van Kammen, A., and Bisseling, T.** (1987). Characterization of cDNA for nodulin-75 of soybean: A gene product involved in early stages of root nodule development. *Proc. Natl. Acad. Sci. USA* **84**, 4495–4499.
- Franssen, H.J., Thompson, D.V., Idler, K., Kormelink, R., van Kammen, A., and Bisseling, T.** (1989). Nucleotide sequence of two soybean ENOD2 early nodulin genes encoding Ngm-75. *Plant Mol. Biol.* **14**, 103–106.
- Fritz, S.E., Hood, K.R., and Hood, E.E.** (1991). Localization of soluble and insoluble fractions of hydroxyproline-rich glycoproteins during maize kernel development. *J. Cell Sci.* **98**, 545–550.
- Gatehouse, L.N., Evans, I.M., Gatehouse, J.A., and Croy, R.R.D.** (1990). Characterisation of a rape (*Brassica napus* L.) extensin gene encoding a polypeptide relatively rich in tyrosine. *Plant Sci.* **71**, 223–231.
- Gleeson, P.A., Stone, B.A., and Fincher, G.B.** (1985). Cloning the cDNA for the arabinogalactan-protein from *Lolium multiflorum* (ryegrass). *AGP News* **5**, 30–36.
- Gleeson, P.A., McNamara, M., Wettenhall, E.H., Stone, B.A., and Fincher, G.B.** (1989). Characterization of the hydroxyproline-rich protein core of an arabinogalactan-protein secreted from suspension-cultured *Lolium multiflorum* (Italian ryegrass) endosperm cells. *Biochem. J.* **264**, 857–862.
- Godoy, J.A., Pardo, J.M., and Pintor-Toro, J.A.** (1990). A tomato cDNA inducible by salt stress and abscisic acid: Nucleotide sequence and expression pattern. *Plant Mol. Biol.* **15**, 695–705.
- Gómez, J., Sánchez-Martínez, D., Stiefel, V., Rigau, J., Puigdomènech, P., and Pagès, M.** (1988). A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine-rich protein. *Nature* **334**, 262–264.
- Govers, F., Harmsen, H., Heidstra, R., Michielsen, P., Prins, M., van Kammen, A., and Bisseling, T.** (1991). Characterization of the pea ENOD12B gene and expression analysis of the two ENOD12 genes in nodule, stem and flower. *Mol. Gen. Genet.* **228**, 160–166.
- Granell, A., Peretó, J.G., Schindler, U., and Cashmore, A.R.** (1992). Nuclear factors binding to the extensin promoter exhibit differential activity in carrot protoplasts and cells. *Plant Mol. Biol.* **18**, 739–748.
- Herman, E.M., and Lamb, C.J.** (1992). Arabinogalactan-rich glycoproteins are localized on the cell surface and in intravacuolar multivesicular bodies. *Plant Physiol.* **98**, 264–272.
- Holdsworth, M.J., and Laties, G.G.** (1989a). Site-specific binding of a nuclear factor to the carrot extensin gene is influenced by both ethylene and wounding. *Planta* **179**, 17–23.
- Holdsworth, M.J., and Laties, G.G.** (1989b). Identification of a wound-induced inhibitor of a nuclear factor that binds the carrot extensin gene. *Planta* **180**, 74–81.
- Hong, J.C., Nagao, R.T., and Key, J.L.** (1987). Characterization and sequence analysis of a developmentally regulated putative cell wall protein gene isolated from soybean. *J. Biol. Chem.* **262**, 8367–8376.
- Hong, J.C., Nagao, R.T., and Key, J.L.** (1989). Developmentally regulated expression of soybean proline-rich cell wall protein genes. *Plant Cell* **1**, 937–943.
- Hong, J.C., Nagao, R.T., and Key, J.L.** (1990). Characterization of a proline-rich cell wall protein gene family of soybean. *J. Biol. Chem.* **265**, 2470–2475.
- Hood, E.E., Shen, Q.X., and Varner, J.E.** (1988). A developmentally regulated hydroxyproline-rich glycoprotein in maize pericarp cell walls. *Plant Physiol.* **87**, 138–142.
- José-Estanyol, M., Ruiz-Avila, L., and Puigdomènech, P.** (1992). A maize embryo-specific gene encodes a proline-rich and hydrophobic protein. *Plant Cell* **4**, 413–423.
- Kaldenhoff, R., and Richter, G.** (1989). Sequence of a cDNA for a novel light-induced glycine-rich protein. *Nucl. Acids Res.* **17**, 2853.
- Kawasaki, S.** (1991). Extensin secreted into the culture medium of tobacco cells. II. Extensin subfamilies of similar composition. *Plant Cell Physiol.* **32**, 721–728.
- Keegstra, K., Talmadge, K.W., Bauer, W.D., and Albersheim, P.** (1973). The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiol.* **51**, 188–196.
- Keller, B., and Baumgartner, C.** (1991). Vascular-specific expression of the bean GRP 1.8 gene is negatively regulated. *Plant Cell* **3**, 1051–1061.
- Keller, B., and Lamb, C.J.** (1989). Specific expression of a novel cell wall hydroxyproline-rich glycoprotein gene in lateral root initiation. *Genes Dev.* **3**, 1639–1646.

- Keller, B., Sauer, N., and Lamb, C.J. (1988). Glycine-rich cell wall proteins in bean: Gene structure and association of the protein with the vascular system. *EMBO J.* **7**, 3625–3633.
- Keller, B., Schmid, J., and Lamb, C.J. (1989a). Vascular expression of a bean cell wall glycine-rich protein- β -glucuronidase gene fusion in transgenic tobacco. *EMBO J.* **8**, 1309–1314.
- Keller, B., Templeton, M.D., and Lamb, C.J. (1989b). Specific localization of a plant cell wall glycine-rich protein in protoxylem cells of the vascular system. *Proc. Natl. Acad. Sci. USA* **86**, 1529–1533.
- Kieliszewski, M., and Lampport, D.T.A. (1987). Purification and partial characterization of a hydroxyproline-rich glycoprotein in a graminaceous monocot, *Zea mays*. *Plant Physiol.* **85**, 823–827.
- Kieliszewski, M., and Lampport, D.T.A. (1988). Tying the knots in the extensin network. In *Self-Assembling Architecture*, J.E. Varner, ed (New York: Alan R. Liss), pp. 61–76.
- Kieliszewski, M.J., Leykam, J.F., and Lampport, D.T.A. (1990). Structure of the threonine-rich extensin from *Zea mays*. *Plant Physiol.* **92**, 316–326.
- Kieliszewski, M.J., de Zacks, R., Leykam, J.F., and Lampport, D.T.A. (1992a). A repetitive proline-rich protein from the gymnosperm Douglas fir is a hydroxyproline-rich glycoprotein. *Plant Physiol.* **98**, 919–926.
- Kieliszewski, M.J., Kamyab, A., Leykam, J.F., and Lampport, D.T.A. (1992b). A histidine-rich extensin from *Zea mays* is an arabinogalactan protein. *Plant Physiol.* **99**, 538–547.
- Kleis-San Francisco, S.M., and Tierney, M.L. (1990). Isolation and characterization of a proline-rich cell wall protein from soybean seedling. *Plant Physiol.* **94**, 1897–1902.
- Knox, J.P., Linstead, P.J., Peart, J., Cooper, C., and Roberts, K. (1991). Developmentally regulated epitopes of cell surface arabinogalactan proteins and their relation to root tissue pattern formation. *Plant J.* **1**, 317–326.
- Lampport, D.T.A. (1977). Structure, biosynthesis and significance of cell wall glycoproteins. *Recent Adv. Phytochem.* **11**, 79–115.
- Lawton, M.A., and Lamb, C.J. (1987). Transcriptional activation of plant defense genes by fungal elicitor, wounding, and infection. *Mol. Cell. Biol.* **7**, 335–341.
- Leach, J.E., Cantrell, M.A., and Sequeira, L. (1982). A hydroxyproline-rich bacterial agglutinin from potato: Extraction, purification, and characterization. *Plant Physiol.* **70**, 1353–1358.
- Lei, M., and Wu, R. (1991). A novel glycine-rich cell wall protein gene in rice. *Plant Mol. Biol.* **16**, 187–198.
- Li, X.-B., Kieliszewski, M., and Lampport, D.T.A. (1990). A chenopod extensin lacks repetitive tetrahydroxyproline blocks. *Plant Physiol.* **92**, 327–333.
- Lindstrom, J.T., and Vodkin, L.O. (1991). A soybean cell wall protein is affected by seed color genotype. *Plant Cell* **3**, 561–571.
- Linthorst, H.J.M., van Loon, L.C., Memelink, J., and Bol, J.F. (1990). Characterization of cDNA clones for a virus-inducible, glycine-rich protein from petunia. *Plant Mol. Biol.* **15**, 521–523.
- Ludevid, M.D., Ruiz-Avila, L., Vallés, M.P., Stiefel, V., Torrent, M., Torné, J.M., and Puigdomènech, P. (1990). Expression of genes for cell-wall proteins in dividing and wounded tissues of *Zea mays* L. *Planta* **180**, 524–529.
- Marcus, A., Greenberg, J., and Averyhart-Fullard, V. (1991). Repetitive proline-rich proteins in the extracellular matrix of the plant cell. *Physiol. Plant.* **81**, 273–279.
- Matsumoto, I., Jimbo, A., Mizuno, Y., Seno, N., and Jeanloz, R.W. (1983). Purification and characterization of potato lectin. *J. Biol. Chem.* **258**, 2886–2891.
- Mellon, J.E., and Helgeson, J.P. (1982). Interaction of a hydroxyproline-rich glycoprotein from tobacco callus with potential pathogens. *Plant Physiol.* **70**, 401–405.
- Mitchell, B.A. (1980). Evidence for polyhydroxyproline in the extracellular matrix of *Volvox*. M.S. Thesis (East Lansing, MI: Michigan State University).
- Mortenson, E., and Dreyfuss, G. (1989). RNP in maize protein. *Nature* **337**, 312.
- Mundy, J., and Chua, N.-H. (1988). Abscisic acid and water-stress induce the expression of a novel rice gene. *EMBO J.* **7**, 2279–2286.
- Murray, R.H.A., and Northcote, D.H. (1978). Oligoarabinosides of hydroxyproline isolated from potato lectin. *Phytochemistry* **17**, 623–629.
- Norman, P.M., Kjellbom, P., Bradley, D.J., Hahn, M.G., and Lamb, C.J. (1990). Immunoaffinity purification and biochemical characterization of plasma membrane arabinogalactan-rich glycoproteins of *Nicotiana glutinosa*. *Planta* **181**, 365–373.
- Obokata, J., Ohme, M., and Hayashida, N. (1991). Nucleotide sequence of a cDNA clone encoding a putative glycine-rich protein of 19.7 kDa in *Nicotiana sylvestris*. *Plant Mol. Biol.* **19**, 953–955.
- Pennell, R.I., and Roberts, K. (1990). Sexual development in the pea is presaged by altered expression of arabinogalactan protein. *Nature* **334**, 547–549.
- Pennell, R.I., Knox, J.P., Scofield, G.N., Selvendran, R.R., and Roberts, K. (1989). A family of abundant plasma membrane-associated glycoproteins related to the arabinogalactan proteins is unique to flowering plants. *J. Cell Biol.* **108**, 1967–1977.
- Pennell, R.I., Janniche, L., Kjellbom, P., Scofield, G.N., Peart, J.M., and Roberts, K. (1991). Developmental regulation of a plasma membrane arabinogalactan protein epitope in oilseed rape flowers. *Plant Cell* **3**, 1317–1326.
- Pont-Lezica, R.F., Taylor, R., and Varner, J.E. (1991). *Solanum tuberosum* agglutinin accumulation during tuber development. *J. Plant Physiol.* **137**, 453–458.
- Quigley, F., Villiot, M.-L., and Mache, R. (1991). Nucleotide sequence and expression of a novel glycine-rich protein gene from *Arabidopsis thaliana*. *Plant Mol. Biol.* **17**, 949–952.
- Raz, R., Crétin, C., Puigdomènech, P., and Martínez-Izquiero, J.A. (1991). The sequence of a hydroxyproline-rich glycoprotein gene from *Sorghum vulgare*. *Plant Mol. Biol.* **16**, 365–367.
- Rohde, W., Rosch, K., Kröger, K., and Salamini, F. (1990). Nucleotide sequence of a *Hordeum vulgare* gene encoding a glycine-rich protein with homology to vertebrate cytokeratins. *Plant Mol. Biol.* **14**, 1057–1059.
- Ruiz-Avila, L., Ludevid, M.D., and Puigdomènech, P. (1991). Differential expression of a hydroxyproline-rich cell-wall protein gene in embryonic tissues of *Zea mays* L. *Planta* **184**, 130–136.
- Ryser, U., and Keller, B. (1992). Ultrastructural localization of a bean glycine-rich protein in unglified primary walls of protoxylem cells. *Plant Cell* **4**, 773–783.
- Salts, Y., Wachs, R., Grussem, W., and Barg, R. (1991). Sequence coding for a novel proline-rich protein preferentially expressed in young tomato fruit. *Plant Mol. Biol.* **17**, 149–150.

- Sauer, N., Corbin, D.R., Keller, B., and Lamb, C.J. (1990). Cloning and characterization of a wound-specific hydroxyproline-rich glycoprotein in *Phaseolus vulgaris*. *Plant Cell Environ.* **13**, 257–266.
- Scheggia, C., Prisco, A.E., Dey, P.M., Daleo, G.R., and Pont Lezica, R. (1988). Alteration of lectin pattern in potato tuber by virus X. *Plant Sci.* **58**, 9–14.
- Scheres, B., van de Wiel, C., Zalensky, A., Horvath, B., Spaik, H., van Eck, H., Zwartkruis, F., Wolters, A.-M., Gloudemans, T., van Kammen, A., and Bisseling, T. (1990). The ENOD12 gene product is involved in the infection process during the pea-*Rhizobium* interaction. *Cell* **60**, 281–294.
- Schmidt, A., Datta, K., and Marcus, A. (1991). Peptidyl proline hydroxylation and the growth of a soybean cell culture. *Plant Physiol.* **96**, 656–659.
- Sequeira, L., and Graham, T.L. (1977). Agglutination of avirulent strains of *Pseudomonas solanacearum* by potato lectin. *Physiol. Plant Pathol.* **11**, 43–54.
- Sheng, J., D'Ovidio, R., and Mehdy, M.C. (1991). Negative and positive regulation of a novel proline-rich protein mRNA by fungal elicitor and wounding. *Plant J.* **1**, 345–354.
- Shirsat, A.H., Wilford, N., Evans, I.M., Gatehouse, L.N., and Croy, R.R.D. (1991). Expression of a *Brassica napus* extensin gene in the vascular system of transgenic tobacco and rape plants. *Plant Mol. Biol.* **17**, 701–709.
- Showalter, A.M., and Varner, J.E. (1987). Molecular details of plant cell wall hydroxyproline-rich glycoprotein expression during wounding and infection. In *Molecular Strategies for Crop Protection*, C.J. Arntzen and C. Ryan, eds (New York: Alan R Liss), pp. 375–392.
- Showalter, A.M., and Varner, J.E. (1989). Plant hydroxyproline-rich glycoproteins. In *The Biochemistry of Plants*, Vol. 15, P.K. Stumpf and E.E. Conn, eds (New York: Academic Press), pp. 485–520.
- Showalter, A.M., and Rumeau, D. (1990). Molecular biology of the plant cell wall hydroxyproline-rich glycoproteins. In *Organization and Assembly of Plant and Animal Extracellular Matrix*, W.S. Adair and R.P. Mecham, eds (New York: Academic Press), pp. 247–281.
- Showalter, A.M., Butt, A.D., and Kim, S. (1992). Molecular details of tomato extensin and glycine-rich protein gene expression. *Plant Mol. Biol.* **19**, 205–215.
- Showalter, A.M., Bell, J.N., Cramer, C.L., Bailey, J.A., Varner, J.E., and Lamb, C.J. (1985). Accumulation of hydroxyproline-rich glycoprotein mRNAs in response to fungal elicitor and infection. *Proc. Natl. Acad. Sci. USA* **82**, 6551–6555.
- Showalter, A.M., Zhou, J., Rumeau, D., Worst, S.G., and Varner, J.E. (1991). Tomato extensin and extensin-like cDNAs: Structure and expression in response to wounding. *Plant Mol. Biol.* **16**, 547–565.
- Smith, J.J., Muldoon, E.P., and Lampport, D.T.A. (1984). Isolation of extensin precursors by direct elution of intact tomato cell suspension cultures. *Phytochemistry* **23**, 1233–1239.
- Smith, J.J., Muldoon, E.P., Willard, J.J., and Lampport, D.T.A. (1986). Tomato extensin precursors P1 and P2 are highly periodic structures. *Phytochemistry* **25**, 1021–1030.
- Stafstrom, J.P., and Staehelin, L.A. (1986). Cross-linking patterns in salt-extractable extensin from carrot cell walls. *Plant Physiol.* **81**, 234–241.
- Stafstrom, J.P., and Staehelin, L.A. (1988). Antibody localization of extensin in cell walls of carrot storage roots. *Planta* **174**, 321–332.
- Stiefel, V., Pérez-Grau, L., Albericio, F., Giral, E., Ruiz-Avila, L., Ludevid, M.D., and Puigdomènech, P. (1988). Molecular cloning of cDNAs encoding a putative cell wall protein from *Zea mays* and immunological identification of related polypeptides. *Plant Mol. Biol.* **11**, 483–493.
- Stiefel, V., Ruiz-Avila, L., Raz, R., Vallés, M.P., Gómez, J., Pagés, M., Martínez-Izquierdo, J.A., Ludevid, M.D., Langdale, J.A., Nelson, T., and Puigdomènech, P. (1990). Expression of a maize cell wall hydroxyproline-rich glycoprotein gene in early leaf and root vascular differentiation. *Plant Cell* **2**, 785–793.
- Stuart, D.A., and Varner, J.E. (1980). Purification and characterization of a salt-extractable hydroxyproline-rich glycoprotein from aerated carrot discs. *Plant Physiol.* **66**, 787–792.
- Sturm, A. (1992). A wound-inducible glycine-rich protein from *Daucus carota* with homology to single-stranded nucleic acid-binding proteins. *Plant Physiol.* **99**, 1689–1692.
- Tierney, M.L., Wiechert, J., and Pluymers, D. (1988). Analysis of the expression of extensin and p33-related cell wall proteins in carrot and soybean. *Mol. Gen. Genet.* **211**, 393–399.
- van de Wiel, C., Scheres, B., Franssen, H., van Lierop, M.-J., van Lammeren, A., van Kammen, A., and Bisseling, T. (1990). The early nodulin transcript ENOD2 is located in the nodule parenchyma (inner cortex) of pea and soybean root nodules. *EMBO J.* **9**, 1–7.
- van Holst, G.-J., and Clarke, A.E. (1986). Organ-specific arabinogalactan-proteins of *Lycopersicon peruvianum* (Mill) demonstrated by crossed electrophoresis. *Plant Physiol.* **80**, 786–789.
- van Holst, G.-J., and Fincher, G.B. (1984). Polyproline II conformation in the protein component of arabinogalactan-protein from *Lolium multiflorum*. *Plant Physiol.* **75**, 1163–1164.
- van Holst, G.-J., and Varner, J.E. (1984). Reinforced polyproline II conformation in a hydroxyproline-rich cell wall glycoprotein from carrot root. *Plant Physiol.* **74**, 247–251.
- van Kan, J.A.L., Cornelissen, B.J.C., and Bol, J.F. (1988). A virus-inducible tobacco gene encoding a glycine-rich protein shares putative regulatory elements with the ribulose biphosphate carboxylase small subunit gene. *Mol. Plant-Microbe Interact.* **1**, 107–112.
- Varner, J.E., and Cassab, G.I. (1986). A new protein in petunia. *Nature* **323**, 110.
- Varner, J.E., and Lin, L.-S. (1989). Plant cell wall architecture. *Cell* **56**, 231–239.
- Whitmore, F.W. (1978). Lignin-protein complex catalyzed by peroxidase. *Plant Sci. Lett.* **13**, 241–245.
- Wingate, V.P.M., Lawton, M.A., and Lamb, C.J. (1988). Glutathione causes a massive and selective induction of plant defense genes. *Plant Physiol.* **87**, 206–210.
- Woessner, J.P., and Goodenough, U.W. (1989). Molecular characterization of a zygote wall protein: An extensin-like molecule in *Chlamydomonas reinhardtii*. *Plant Cell* **1**, 901–911.
- Wyatt, R.E., Nagao, R.T., and Key, J.L. (1992). Patterns of soybean proline-rich protein gene expression. *Plant Cell* **4**, 99–110.
- Wycoff, K.L., Lamb, C.J., and Dixon, R.A. (1991). Interaction of nuclear DNA-binding factors with an HRGP promoter from French bean (*Phaseolus vulgaris* L.). *Plant Physiol.* **96** (suppl.), 5 (abstr.).
- Ye, Z.-H., and Varner, J.E. (1991). Tissue-specific expression of cell wall proteins in developing soybean tissues. *Plant Cell* **3**, 23–37.
- Ye, Z.-H., Song, Y.-R., Marcus, A., and Varner, J.E. (1991). Comparative localization of three classes of cell wall proteins. *Plant J.* **1**, 175–183.
- Zhou, J., Rumeau, D., and Showalter, A.M. (1992). Isolation and characterization of two wound-regulated tomato extensin genes. *Plant Mol. Biol.* **20**, 5–17.