Plant Physiol. (1993) 101: 1127–1130

Update on Cell Walls

Structural Cell Wall Proteins

Beat Keller*

Department of Plant Breeding, Swiss Federal Research Station for Agronomy, Reckenholzstrasse 191, CH-8046 Zurich, Switzerland

Biological structures as diverse as skin, hair, spider webs, silk cocoons, connective tissue, and some algal cell walls share a common property: they consist mainly of structural proteins. Some of these proteins have been studied in detail and have contributed greatly to our understanding of protein structure. Silk fibroin, for example, is the model protein for the β-pleated sheet secondary structure, collagen for a triple-stranded helix. The amino acids Gly and Pro (or Hyp) are frequently found to be major constituents of these structural proteins and, therefore, appear to be well suited as building blocks for structural proteins.

Not surprisingly, plant cell walls, the major structural part of the plant cell, also contain structural proteins. The primary amino acid sequences of many of these proteins have been deduced from cDNA and genomic sequences, but less is known about the precise localization and developmental expression pattern of these proteins. However, it has become clear that both the synthesis and the cross-linking of these wall proteins are under strict developmental control. In addition, their synthesis and cross-linking can be environmentally induced by a number of triggers. Despite our growing knowledge about these proteins, their biological function is still largely a matter of speculation. Three major classes of structural wall proteins have been recognized to date: extensins, PRPs, and GRPs. Other wall proteins that have been described might also have a structural role, but less is known about these proteins and this discussion will focus on the well-characterized wall proteins.

HRGPs OR EXTENSINS

Except for certain algae that have cell walls made almost exclusively of proteins (for a review, see Adair, 1988), cell walls in plants contain only relatively small amounts of protein. Primary walls contain more protein than secondary walls. The best-known structural wall proteins are the extensins, or HRGPs, which are characterized by the repeated pentapeptide sequence Ser-$(Hyp)_4$ (for reviews, see Cassab and Varner, 1988; Showalter and Varner, 1989). Their structure consists of an extended polyproline II helix. Most Pro's in these proteins are hydroxylated to give Hyp and are then *O*-glycosylated with Ara. Similarly, the Ser is often *O*-substituted with Gal. Extensins are generally also rich in Lys, making them basic proteins, possibly interacting with acidic pectic blocks in the cell wall. The abundant Tyr residues might be involved in isodityrosine cross-links that have been proposed to be responsible for the observed insolubilization of HRGPs in cell walls. In the monocot maize, similar proteins with slightly different motifs such as Ser-Hyp-Lys-Pro-Hyp (Kieliszewski et al., 1990) have been described, revealing an evolutionary related motif. Extensins with a Ser-$(Hyp)_4$ motif have not only been described in dicots and monocots but also in a gymnosperm (Fong et al., 1992).

The study of different extensins has demonstrated that a specific extensin is synthesized in only one or a few cell types in a plant. A soybean extensin was found in sclerenchyma and in hour-glass cells in the seedcoat (Cassab and Varner, 1987). A gene encoding a tobacco extensin was specifically expressed in one or two cell layers in emerging lateral roots (Keller and Lamb, 1989). These cell layers were localized at the tip of a newly formed lateral root that is mechanically breaking through the root cortex. Deposition of extensin might strengthen their walls. Genes encoding other tobacco extensin or extensin-like proteins show pistil-specific expression (De S. Goldmann et al., 1992). The cell-type-specific expression of extensin genes suggests that the proteins are functionally important parts of a particular cell wall.

The expression of extensins is not only developmentally regulated but also induced after pathogen attack. In bean infected with the fungal pathogen *Colletotrichum lindemuthianum*, in situ hybridization with a specific extensin cDNA was performed (Templeton et al., 1990). In an incompatible reaction, the gene was induced immediately after infection in epidermal and cortical cells adjacent to the infection site. In a compatible interaction, HRGP was induced slowly after infection and not only adjacent to the site of infection. These results suggest that HRGP is an important component of the localized hypersensitive resistance mechanism.

At the ultrastructural level, an extensin has been localized in carrot storage roots and was found to be uniformly distributed across the primary cell wall but was absent from the middle lamella (Stafstrom and Staehelin, 1988). There is also evidence that extensin cannot cross the middle lamella separating the walls of adjacent cells, suggesting that extensin is made by each cell itself (Stafstrom and Staehelin, 1988). In a different study at the ultrastructural level, increased deposition of HRGP was observed at the interface of pea root cells interacting with mycorrhizal fungi (Bonfante-Fasolo et al., 1991).

* Fax 41-1-377-7201.

Abbreviations: GRP, glycine-rich protein; HRGP, hydroxyproline-rich glycoprotein; PRP, proline-rich protein.

PRPs

PRPs can also contain some Hyp (H/PRP), but they do not have the Ser-$(Hyp)_4$ repeat of the extensins. The distinction between extensins and PRPs is somewhat artificial and has become less clear with the increasing number of proteins analyzed and primary sequences available (Kieliszewski et al., 1992a). Both groups of proteins should be considered as members of a large superfamily of related proteins. Many of the H/PRPs have a pentapeptide repeat of the form Pro-Pro-Val-X-Lys. Such proteins may contain structural oddities such as six consecutive His's (Sheng et al., 1991) or peptide palindromes (Kieliszewski et al., 1992b). H/PRPs have been found in both monocots and dicots. Their sequences and expression patterns are best characterized in soybean (Wyatt et al., 1992) and maize (Josè-Estanyol et al., 1992). Three soybean PRPs were localized immunologically in the light microscope (Marcus et al., 1991) and gene expression patterns were analyzed by in situ hybridization (Wyatt et al., 1992).

Similar to the extensins, all these proteins show a cell-type-specific expression pattern, being synthesized either in a number of different cell types (PRP2) or only in one cell type (PRP3) at a certain developmental stage. The expression patterns can change during development of an organ. Figure 1 outlines the cell-type-specific localization of several cell wall proteins in the elongating part of a young soybean hypocotyl. In maize embryos, two different H/PRPs had

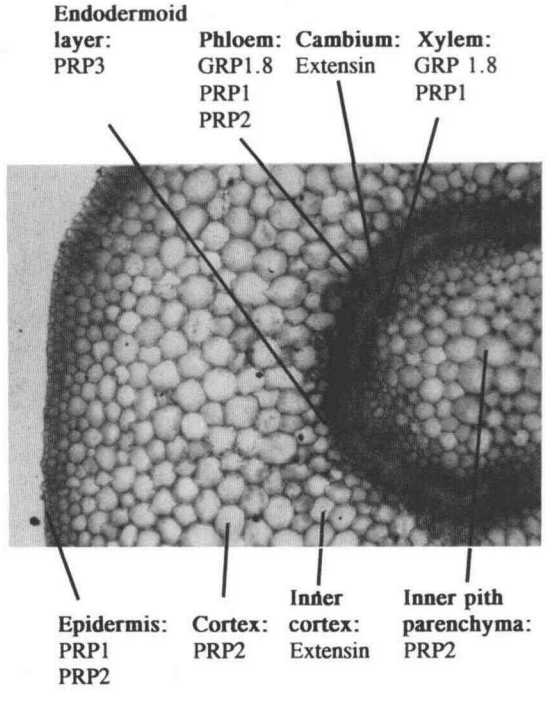

Figure 1. Cell-type-specific localization of cell wall proteins in the elongating part of young soybean hypocotyls. A cross-section of a 4-d-old hypocotyl is shown and the localization of five different cell wall proteins is indicated in the different tissues. Most cells contain at least one structural wall protein. Certain cell types, e.g. in the phloem and xylem, may contain more than one specific protein.

complementary expression patterns. One was synthesized in the scutellum and nonprovascular cells of the axis, whereas the other was made mainly in vascular cells (Josè-Estanyol et al., 1992).

It is interesting that in soybeans, a seed color genotype that is involved in the formation of anthocyanin pigments strongly influenced the accumulation of PRP1 in soybean seed coats (Lindstrom and Vodkin, 1991). Whereas the dominant *I* gene resulted in large amounts of PRP1 in seed coats, a mutant isogenic line with *i/i* genotype accumulated PRP1 to a much lower level. PRP2, a closely related protein that is synthesized later in the development of the seed coat, was not affected. The correlation between anthocyanin biosynthesis and the quantitative levels of a specific cell wall protein remains unexplained.

GRPs

GRPs represent the third main class of structural wall proteins. Although many proteins that are rich in Gly (containing between 50 and 70% Gly) have been described, only two proteins have actually been shown to be part of the cell wall: grp-1 from petunia (Condit et al., 1990) and GRP 1.8 from *Phaseolus vulgaris* (Keller et al., 1988; Ryser and Keller, 1992). The genes encoding these proteins are wound induced. Similar proteins that are likely to be cell wall proteins were found in *Arabidopsis*, rice, barley, tomato, and other plant species. They all have a similar arrangement of their primary sequence: there is a pairwise arrangement of the amino acids that can be written as $(Gly\text{-}X)_n$, where X is frequently Gly. Such a sequence suggests that GRPs have a β-pleated sheet secondary structure (Condit and Meagher, 1986).

The bean GRP 1.8 was localized in phloem fibers and early xylem elements of hypocotyls (Ye et al., 1991; Ryser and Keller, 1992) and appears to colocalize with PRP (Ye et al., 1991). Because PRPs have not yet been localized at the ultrastructural level, it is not clear if both types of proteins are found in the same cell wall. Theoretically, PRPs and GRPs might interact by formation of isodityrosine cross-links. A different cross-link could be formed by transglutaminases between the Gln's in GRP and the Lys's in PRP. Such ϵ-(γ-glutamyl)Lys cross-links are found between structural proteins of the mammalian epidermis (Mehrel et al., 1990).

The ultrastructural study of bean GRP 1.8 deposition has resulted in some unexpected findings concerning the localization and sites of synthesis of the protein (Ryser and Keller, 1992). Whereas mature xylem cells (defined by the lack of any detectable cytoplasm and the presence of strongly modified primary walls) contained a high amount of GRP 1.8 in modified primary walls, living xylem cells did not contain any immunoreactive GRP 1.8 in the Golgi apparatus or the primary cell wall. However, xylem parenchyma cells neighboring dead xylem cells showed strong labeling in the Golgi apparatus and cell corners, but not in the cell walls. These data suggest that GRP 1.8 may be synthesized by the xylem parenchyma only after the death of the xylem cells and is then exported to the modified primary wall of xylem. Such a mechanism would require protein transport through the wall of xylem parenchyma cells and then a trapping or insolubilization mechanism in the protoxylem cell walls. The molec-

ular details of such a mechanism are not clear, but the analysis of interactions of GRP 1.8 with other cell wall components should provide some of the answers.

PRPs AND RAPID CROSS-LINKING MECHANISMS

Soybean PRP2 was localized in cortical cells and in the vascular tissue of hypocotyls and in inner integuments of the seed coat (Wyatt et al., 1992). The same protein was found to be rapidly insolubilized in the wall after elicitor treatment of soybean cell cultures (Bradley et al., 1992). This oxidative cross-linking into the wall structure was mediated by H_2O_2, initiated within 2 min, and completed after 10 min. The same cross-linking was observed in wounded bean hypocotyls close to the wound sites. Insolubilization of PRP2 also occurred during normal development; in mature regions of hypocotyls, PRP was insoluble but immunologically detectable in the wall.

In addition, in stem-petiole junctions that are subjected to mechanical stress, PRP was insolubilized, whereas the same protein was still soluble in internodes. The developmental and stress-controlled insolubilization of PRP2 demonstrates the involvement of cell wall proteins both in development and in defense. The observed temporal and spatial separation of PRP2 mRNA synthesis and the site of cross-linking of the protein indicates that control of cross-linking, in addition to transcriptional control, represents an important mechanism of regulation of cell wall properties by proteins.

FUTURE RESEARCH DIRECTIONS AND CONCLUDING REMARKS

Many GRPs/PRPs are known only as amino acid sequences derived from cloned genes. They contain amino-terminal signal peptides but have not yet been shown to be cell wall proteins. Thus, localization, ultimately at the ultrastructural level, is necessary to know which proteins are present in the wall of a specific cell type and to know the precise localization within these walls. Such information will be essential to formulate a working hypothesis about the function of cell wall proteins during development and stress. There is good evidence that wall proteins can strengthen a cell wall. The very rapid cross-linking (Bradley et al., 1992) and the highly specific expression of an extensin during lateral root development (Keller and Lamb, 1989) suggest a mechanical strengthening of cell walls by proteins. An additional role was suggested for PRP2 from soybean that is localized in the middle lamella and the intercellular spaces of the cortex (Marcus et al., 1991). It was suggested that this protein acts to "cement" these cells.

The synthesis and active function of cell wall proteins can be regulated both at the transcriptional and the cross-linking level. Transcriptional control can be very complex, as was recently described for the bean GRP 1.8 (Keller and Baumgartner, 1991). It is likely that wall proteins provide some functional properties to the wall that carbohydrates and polyphenolic polymers cannot. Elasticity and strength might be mediated by a GRP, similar to the GRPs that make up the strong but flexible spider silk (Lewis, 1992). In analogy to a major mammalian epidermal skin protein in the highly cornified, insoluble envelope (55% Gly, Mehrel et al., 1990), GRPs might be involved in sealing off some walls. Immunolabeling of modified primary walls for GRP antigen gave a very dense reaction (Ryser and Keller, 1992), suggesting that there is enough protein for such a function. Proteins might also act as scaffolds for the deposition of other molecules, and the defined spacing of Tyr residues would control cross-linking and the "pore-size" of the wall. In addition, the genetically defined and precise length of such molecules might be important in a network of other macromolecules with no precise mechanism for length determination.

Little is known about the protein secondary structure of GRPs and PRPs as well as about their interaction with other wall polymers. Biochemical studies as well as the fate of mutated cell wall proteins in the wall will hopefully reveal more details about such interactions. Structural information and a better knowledge of the physical properties of isolated GRPs and PRPs will contribute to a working hypothesis on the biological function of such proteins. Future studies on cell wall proteins will certainly lead to new and surprising concepts of cell wall structure and the role of structural proteins.

ACKNOWLEDGMENT

I would like to thank Ueli Ryser and Jürg Schmid for critical reading of the manuscript.

Received October 19, 1992; accepted December 15, 1992.
Copyright Clearance Center: 0032-0889/93/101/1127/04.

LITERATURE CITED

Adair WS (1988) Organization and *in vitro* assembly of the *Chlamydomonas reinhartdtii* cell wall. *In* JE Varner, ed, Self-Assembling Architecture. Alan L Liss, New York, pp 25–41

Bonfante-Fasolo P, Tamagnone L, Peretto R, Esquerée-Tugayé MT, Mazau D, Mosiniak M, Vian B (1991) Immunocytochemical location of hydroxyproline rich glycoproteins at the interface between a mycorrhizal fungus and its host plants. Protoplasma **165:** 127–138

Bradley DJ, Kjellbom P, Lamb CJ (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

Cassab GI, Varner JE (1987) Immunocytolocalization of extensin in developing soybean seedcoat by immunogold-silver staining and by tissue printing on nitrocellulose paper. J Cell Biol **105:** 2581–2588

Cassab GI, Varner JE (1988) Cell wall proteins. Annu Rev Plant Physiol Plant Mol Biol **39:** 321–353

Condit CM, McLean BC, Meagher RB (1990) Characterization of the expression of the petunia glycine-rich protein-1 gene product. Plant Physiol **93:** 596–602

Condit CM, Meagher RB (1986) A gene encoding a novel glycine-rich structural protein of petunia. Nature **323:** 178–181

De S Goldman MH, Pezzotti M, Seurinck J, Mariani C (1992) Developmental expression of tobacco pistil-specific genes encoding novel extensin-like proteins. Plant Cell **4:** 1041–1051

Fong C, Kieliszewski MJ, de Zacks R, Leykam JF, Lamport DTA (1992) A gymnosperm extensin contains the serine-tetrahydroxyproline motif. Plant Physiol **99:** 548–552

Josè-Estanyol M, Ruiz-Avila L, Puigdomènech P (1992) A maize embryo-specific gene encodes a proline-rich and hydrophobic protein. Plant Cell **4:** 413–423

Keller B, Baumgartner C (1991) Vascular-specific expression of the bean GRP 1.8 gene is negatively regulated. Plant Cell **3:** 1051–1061

Keller B, Lamb CJ (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, Lamb CJ (1988) Glycine-rich cell wall proteins in bean: gene structure and association of the protein with the vascular system. EMBO J **7:** 3625–3633

Kieliszewski M, de Zacks R, Leykam JF, Lamport DTA (1992a) A repetitive proline-rich protein from the gymnosperm Douglas fir is a hydroxyproline-rich glycoprotein. Plant Physiol **98:** 919–926

Kieliszewski MJ, Kamyab A, Leykam JF, Lamport DTA (1992b) A histidine-rich extensin from *Zea mays* is an arabinogalactan protein. Plant Physiol **99:** 538–547

Kieliszewski MJ, Leykam JF, Lamport DTA (1990) Structure of the threonine-rich extensin from *Zea mays*. Plant Physiol **92:** 316–326

Lewis RV (1992) Spider silk: the unraveling of a mystery. Accounts Chem Res **25:** 392–398

Lindstrom JT, Vodkin LO (1991) A soybean cell wall protein is affected by seed color genotype. Plant Cell **3:** 561–571

Marcus A, Greenberg J, Averyhart-Fullard V (1991) Repetitive proline-rich proteins in the extracellular matrix of the plant cell. Physiol Plant **81:** 273–279

Mehrel T, Hohl D, Rothnagel JA, Longley MA, Bundman D, Cheng C, Lichti U, Bisher ME, Steven AC, Steinert PM, Yuspa SH, Roop DR (1990) Identification of a major keratinocyte cell envelope protein, loricrin. Cell **61:** 1103–1112

Ryser U, Keller B (1992) Ultrastructural localization of a bean glycine-rich protein in unlignified primary wall of protoxylem cells. Plant Cell **4:** 773–783

Sheng J, D'Ovidio R, Mehdy MC (1991) Negative and positive regulation of a novel proline-rich protein mRNA by fungal elicitor and wounding. Plant J **1:** 345–354

Showalter AM, Varner JE (1989) Plant hydroxyproline-rich glycoproteins. *In* PK Stumpf, EE Conn, eds, Biochemistry of Plants, Vol 15. Academic Press, New York, pp 485–520

Stafstrom JP, Staehelin LA (1988) Antibody localization of extensin in cell walls of carrot storage roots. Planta **174:** 321–332

Templeton MD, Dixon RA, Lamb CJ, Lawton MA (1990) Hydroxyproline-rich glycoprotein transcripts exhibit different spatial patterns of accumulation in compatible and incompatible interactions between *Phaseolus vulgaris* and *Colletotrichum lindemuthianum*. Plant Physiol **94:** 1265–1269

Wyatt RE, Nagao RT, Key JL (1992) Patterns of soybean proline-rich protein gene expression. Plant Cell **4:** 99–110

Ye Z-H, Song Y-R, Marcus A, Varner JE (1991) Comparative localization of three classes of cell wall proteins. Plant J **1:** 175–183