The Plant Extracellular Matrix: News from the Cell's Frontier

In reminiscing about his 40 years of research on the chemistry and the biology of the cell walls of flowering plants, Peter Albersheim (University of Georgia) revealed that, after experiencing a series of early technical problems, he came very close to abandoning his goal of deducing the chemical structure of the plant's primary cell wall. What a mistake that would have been! Just one year later his group's derivatization techniques and chromatographic separations revealed the fundamental linkage structures of several complex pectic polysaccharides, confirmed the structure of the major cross-linking glycan, xylogucan (XyG), and as a result, they proposed the first model of the plant's primary cell wall. At that time many researchers were deducing linkage structures of plant polysaccharides, but Albersheim's group was the first to assemble a working model of the interactions of the various polymers they found in the primary wall.

In the years that followed, an ever-growing world community of cell wall researchers developed more refined technologies to study the fine structure of the cell wall, its dynamic nature, and its diverse biological functions. With a discovery-packed 3 years since the first meeting in Santa Fe, the second Keystone Symposium on the Extracellular Matrix of Plants: Molecular, Cellular and Developmental Biology, comprising about 150 scientists, convened in Tamarron, Colorado, in March 1996.

Discoveries that were highlights of the meeting for us were the cloning of the catalytic subunit of plant cellulose synthase, the identification of boron diesters as polysaccharide cross-linkers, the insight into control of lignification gained from secondary product antisense and null mutants, the identification of oligoglycan receptors, the potential role of glycochaperones in secretion and wall assembly, the ultra-structure of cell plate assembly, the molecular analysis of expansins and structural wall proteins, and the nature of wall-mediated signaling events in development and pathogenesis. We have selected these and several other contributions for more thorough discussion in the context of the major themes that we see emerging from the meeting.

To Make a Polysaccharide, First Catch Your Synthase

The plant's primary cell wall is depicted as a network of cellulose and cross-linking glycans embedded in a gel matrix of pectic substances and reinforced with structural proteins and aromatic substances (McCann and Roberts, 1991; Carpita and Gibeaut, 1993). Cellulose and callose are synthesized at the plasma membrane of plant cells. Noncellulosic polysaccharides, the matrix polysaccharides, are synthesized in the Golgi apparatus, packaged in secretory vesicles, and exported to the surface, where they are integrated with cellulose microfibrils.

Despite many years of effort, not one gene encoding a plant cell wall polymer synthase had been unambiguously identified prior to this meeting. In the keynote address for the symposium, John Lowe (University of Michigan) described elegant expression and selection systems to clone mammalian fucosyl transferases, which add terminal fucose units with remarkable specificity to the O-2, O-3, and O-4 positions of the galactosyl residues of oligosaccharides of secreted glycoproteins. Lowe noted that each different linkage in a complex oligosaccharide usually requires a separate, specific glycosyl transferase, not modified versions of the same glycosyl transferase. The similarity among the different animal fucosyl transferases is surprisingly low, and so cloning of the broad spectrum of plant transferases by homology is anticipated to be difficult.

Nevertheless, sequence similarity played an important part in the identification of a novel higher plant clone for cellulose synthase, the cotton *CelA* gene, as reported by Deborah Delmer (Hebrew University). Initial efforts to clone plant cellulose synthase cDNAs using cross-hybridization to the existing clone for the cellulose synthase catalytic subunit, the *BcsA* gene from *Acetobacter* (Wong et al., 1990; Saxena et al., 1995) yielded ambiguous clones. However, many other bacterial glucosyl transferases that synthesize $(1\rightarrow4)$-β-D-glucosyl units in a polysaccharide chain have since been cloned and sequenced, and consensus UDP-glucose (UDP-Glc) binding domains emerged—domains expected to be in the plant glucosyl transferase catalytic site. Several such putative UDP-Glc binding domains are evident both in the *Acetobacter BscA* and the cotton *CelA-1* (Figure 1).

In collaboration with the cotton research group at Calgene Inc. (Davis, CA) headed by David Stalker and Julie Pear, Delmer and Yasushi Kawagoe (also at Hebrew University) sequenced clones randomly from a cDNA library constructed from cotton fibers harvested at the onset of secondary wall cellulose deposition. Their effort yielded two interesting clones. These encoded proteins with, in addition to the UDP-Glc binding consensus sequences, several domains that had greater than 50% similarity with those of the bacterial cellulose synthase genes (Figure 1). A peptide derived from a subcloned region of the cotton *CelA-1* gene spanning these domains was also shown to bind UDP-Glc, and when one of the putative UDP-Glc binding domains was deleted, no binding occurred.

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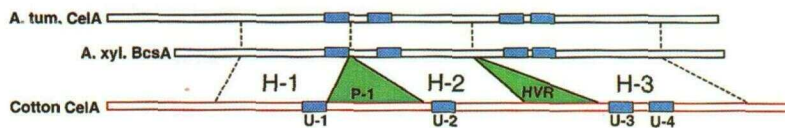
Figure 1. Comparison of Cellulose Synthase Genes from Bacteria and Plants.

The bacterial cellulose synthase genes *BcsA* from *Acetobacter xylinum* and *CelA* from *Agrobacterium tumifaciens* are aligned with the *CelA-1* gene from cotton. Three regions (H-1, H-2, and H-3) in the deduced amino acid sequence of the plant *CelA* gene product are similar to domains in the bacterial proteins. Within the conserved regions are four highly conserved subdomains (U-1, U-2, U-3, and U-4) previously suggested by Delmer and Amor (1995) and Saxena et al. (1995) to be critical for catalysis and/or binding of the substrate, UDP-Glc. The plant *CelA* gene product contains two internal insertions of sequence that are not found in the bacterial genes. One (P-1) is conserved among the plant CelA proteins, whereas the other (HVR) is hypervariable.

The cotton *CelA-1* gene is expressed strongly in the fibers at the onset of secondary wall formation, but there is little expression in roots, flowers, or seeds. DNA gel blot analysis suggests that four to eight genes may be present in cotton and four in rice. A considerable amount of the *CelA* sequence is unique to plants, and the functions of the different domains can only be surmised for now.

The observation of more than one UDP-Glc binding domain in the catalytic site of plant CelA and *Acetobacter* BscA may help to explain a topological problem in the synthesis of cellulose and other glycans (e.g., XyGs, glucomannans, and grass β-D-glucans) that contain the (1→4)-β-D-glucosyl linkages. The problem is that this linkage results in a 180° inversion in the orientation of each glucosyl unit with respect to its neighbor. Two or an even number of UDP-Glc transferases could add glucosyl units synchronously from opposite faces of the synthase to form cellulose in two-sugar cellobiose units that "rachet" through the synthase with rotation of neither synthase nor the growing chain (Figures 2A to 2C). Callose synthesis is a possible default activity of cellulose synthase, perhaps caused by loss of one of the transferases (Figures 2D and 2E). This loss may be triggered by wounding and pathogenesis.

The mixed linkage (1→3),(1→4)-β-D-glucans, which are found only in grasses, are synthesized by a β-D-glucan synthase

in the maize Golgi apparatus. An active Golgi-enriched fraction was obtained by flotation centrifugation, and a sequence-dependent glucanase was used to show that the synthase produced the expected products, cellotriosyl and cellotetraosyl unit structures, in vitro (Gibeaut and Carpita, 1994). However, a callose synthase activity was observed in the maize Golgi membranes in addition to the mixed-linkage glucan synthase. Nick Carpita (Purdue University) concluded that the β-D-glucan synthase may be derived from an ancestral cellulose synthase. The kind of linkages formed, the substrate concentrations for optimal activity, and the appearance of callose synthase in Golgi-enriched membrane fractions all pointed toward this interpretation. Carpita showed how the precise arrangement of (1→4) and (1→3) linkages in the cereal glucan may arise simply through the acquisition by a cellulose synthase of an additional transferase catalytic site (Figure 2F).

The components of callose synthase are being investigated by Bruce Wasserman (Rutgers University) and Paul Bolwell (University of London). A 160-kD synthase candidate was one of the few proteins that Wasserman found that was resistant to exoplasmic proteolysis and that also copurified with synthase activity on gradients and by product entrapment, a technique in which synthases are pelleted together with the dense callose they make. Bolwell found that elicitor treatment

of bean cell suspension cultures induces, along with callose synthase activity, a 70-kD putative regulatory protein with homology to protein disulfide isomerase, which is an ER chaperone, and a 55-kD protein of unknown function.

Although a candidate for the catalytic component of cellulose synthase has been identified, the various protein components that make up the rosettes, the six-membered hexagonal arrays that extrude cellulose microfibrils in some algae and all flowering plants (Brown et al., 1996), are also being sought. Candace Haiger (Texas Tech University) and Delmer's group showed recently that sucrose synthase (SuSy) is associated with the plasma membrane and may supply UDP-Glc directly to cellulose synthase (Amor et al., 1995). They have been examining immunolabeling patterns of membrane-associated SuSy in cotton fibers, and find that the pattern of localization parallels that of the deposition of cellulose, suggesting that SuSy may be part of the rosette complex.

The cellular slime mold, *Dictyostelium discoideum*, has two distinct modes of cellulose synthesis that occur in the separate developmental stages of the stalk tube and the stalk cell wall. Larry Blanton (Texas Tech University) and Haigler have shown by freeze-fracture EM that a linear array of particles in the plasma membrane is associated with synthesis of the tube cellulose. An analogous mechanism is found in *Acetobacter* (Brown et al., 1976). In contrast, cellulose synthesis in the cell wall is associated with patches of particles rather than linear arrays, and these patches have the appearance of rudimentary rosettes.

Nucleotide-sugars are the substrates for the synthesis of the vast majority, if not all, of the cell wall polysaccharides. Synthesis of the noncellulosic polysaccharides at the Golgi apparatus, especially those containing numerous kinds of sugars, requires the coordination of synthase activity with the uptake of nucleotide sugars from the cytosol. Ariel Orellana (University of Chile) established that UDP-
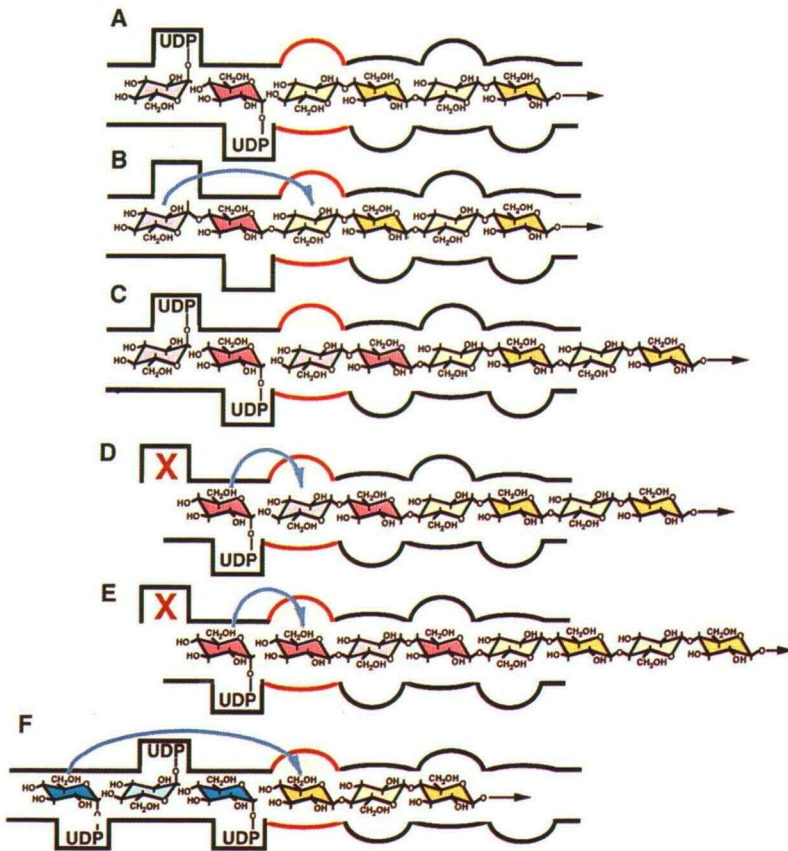
Figure 2. A Model for the Synthesis of Cellulose, Callose, and β-D-Glucans by a Single Synthase Complex.

The problem in forming a (1→4)-β-D-glucosyl linkage is the 180° inversion in the orientation of each glucosyl unit with respect to its neighbor, depicted here by the different shading. Instead of synthesis by monomeric additions, two or an even number of UDP-Glc transferases **(A)** could add glucosyl units from opposite faces of the synthase polypeptide to form cellobiosyl units **(B)**, which then add to a growing cellulose chain that rachets (arrows) directly through the synthase two units at a time **(C)**. Endoglycanases often have clefts to position up to six glucosyl residues of the chain for catalysis. Like glycanases, synthases may have multisite clefts or channels to position the glucan terminus for accepting an added sugar residue. This terminus (in red) always positions the terminal accepting glucose so that subsequent cellobiose units are added to its O-4 position (lightly shaded residues). However, if a UDP-Glc transferase site is lost (red X), then the default synthase still functions, but only one glucose residue participates in the racheting mechanism **(D)**. From the second cycle on, the terminal glucose (shaded darkly) will expose the O-3, not the O-4, and the (1→3)-β-D-glucan (i.e., callose) will be made instead of cellulose **(E)**. Acquisition of an additional transferase catalytic site by the grass β-D-synthase, i.e. an odd number of synchronously active transferases, would result in the racheting of three units per cycle and exposure of the O-3 of the terminal sugar **(F)**. The additional transferase explains the formation of the cellotriosyl-(1→3)-β-D-glucose units that make up most of the β-D-glucan molecule, and, hence, how the β-D-glucan synthase may have high sequence similarity to cellulose synthase but make a different glucan product with specific sequences of (1→3)- and (1→4)-β-D-glucosyl units. As with cellulose synthase, loss of the outer transferases results in callose synthase activity, as observed in the Golgi apparatus (Gibeaut and Carpita, 1994).

Glc is taken up into the lumen of the Golgi by an antiport mechanism coupled to the export of UMP. Inosine diphosphatase, a common marker for the Golgi apparatus, actually functions as a UDPase, converting the UDP that is produced by transfer of the sugar moiety from UDP-Glc to polysaccharide, to UMP. The topology of glucan synthesis was determined by protease protection assays, and Orellana found that the synthase faces the lumenal side of the Golgi membranes.

Many of the enzymes of the nucleotide-sugar synthesis and interconversion pathway are localized within membranes thought to be ER and Golgi. Orellana found that transport of other nucleotide sugars involved in the synthesis of XyG, namely, UDP-Xyl, UDP-Gal, and GDP-Fuc, also occurs from cytosol to the ER and Golgi lumen. Because several of Wolf-Dieter Reiter's (University of Connecticut) cell wall mutants of Arabidopsis may result from defective enzymes of the nucleotide-sugar interconversion pathways, these mutants may be useful for studies of the synthesis of complex polysaccharides within the Golgi lumen.

Heretofore it has been nearly impossible to synthesize polysaccharides in vitro that correspond to the fine structure of those made in vivo. At this meeting, unequivocal demonstration of the polymerization of GalA from UDP-GalA into (1→4)-α-D-galacturonan was shown by Debra Mohnen (University of Georgia). The appropriate linkage was demonstrated by enzyme hydrolysis of the radioactive reaction product to di- and trioligouronides, although the size of the product is not much greater than the 15-mer acceptor, indicating a limited processivity of the enzyme. The reaction product must be treated with alkali for better hydrolysis, an indication that the galacturonan formed in vitro is also esterified.

Both the mannan synthase and the galactosyl transferase required for synthesis of galactomannans have been solubilized successfully from fenugreek endosperm by Mary Edwards and Grant Reid (University of Stirling). The mannan

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synthase can form mannan chains independently of the addition of Gal, but the galactosyl transferase, of course, has an absolute requirement for an appropriate acceptor. The galactosyl transferase adds Gal units to the expected O-6 position of the oligomannan acceptors. Transferases such as these, which add a single sugar as a side group on the growing polymer backbone, are often stable after detergent extraction and this feature renders them amenable to conventional purification. Fucosyl transferases from pea epicotyls retain substantial activity after solubilization with nonionic detergents. Ken Keegstra's group (Michigan State University) found that the transferase adds Fuc from GDP-Fuc to a galacto-XyG acceptor. Paul Dupree (Cambridge University) is using tryptic digests of proteins resolved by two-dimensional electrophoresis to identify additional proteins in Arabidopsis associated specifically with ER and the Golgi apparatus.

New Tools Reveal the Fine Structure of Cell Wall Polysaccharides

Nucleic acid chemistry was revolutionized with the discovery and use of sequence-specific enzymes, the restriction endonucleases. Polysaccharide sequence-dependent glycanases likewise promise quick and reproducible resolution of oligosaccharides with repeating unit structures or polymer domains (for review of polysaccharide structure, see Carpita and Gibeaut, 1993). Grant Reid and his coworkers at Unilever have opened up the toolkit of XyG hydrolases from nasturtium seed, where XyG is a major storage carbohydrate. In their characterization of a nasturtium XyG endo-transglycosylase (XET), they have shown that XET recognizes a five-unit $(1\rightarrow4)$-β-D-glucan backbone with specific forms of substitution.

The sequence-dependent enzymes have also revealed new information about pectin structure. Alphons Voragen (Agricultural University of Wageningen) demonstrated how commercially available enzymes from

Novo Nordisk, which are used to generate differences in food textures and states by partial hydrolysis of component wall polymers, can be used to elucidate the fine structure of rhamnogalacturonan I (RG I). For example, the influence of side-group substitution greatly modifies the activity of the selective rhamnogalacturonases (RGases), some of which cleave the GalA-$(1\rightarrow2)$-Rha linkages and others the Rha-$(1\rightarrow4)$-GalA linkages. These enzymes reveal "smooth" regions of RG I, which have very little side group substitution and are digested by RGases, and "hairy" regions, which possess substantial decoration with arabinan and galactan oligomers and polymers and are not hydrolyzed as extensively.

Until this meeting, there were three recognized kinds of pectins (polysaccharides rich in galacturonic acid): homogalacturonan (HGA), RG I, and RG II. Andrew Mort (Oklahoma State University) and Voragen used sequence-dependent enzymes, followed by biochemical analyses of the resulting products, to independently reveal a new class of pectin, xylogalacturonan. Like RG II, xylogalacturonans have homogalacturonan backbones, but with subtending groups of nonreducing terminal Xyl units attached to the O-3 position of about half of the GalA units.

Mort also used sequence-dependent enzymes and low temperature-selective anhydrous HF solvolysis to identify interactions between pectins and other wall polymers. Treatment of polymer mixtures with endoglucanase yields XyG oligomers and an RG I that become chromatographically separable. Dearabinosylation renders hydroxyproline-rich glycoproteins (HRGPs) susceptible to trypsin digestion, and such treatment also releases RG I. The observation that enzyme treatment, but not alkali treatment or anhydrous HF solvolysis alone, is effective at releasing both resolvable oligosaccharides from the pectic fraction and from HRGPs indicates that the extent of covalent cross-linking of RG I to XyGs and HRGPs may be greater than that represented in current models of the wall.

To deduce the fine structure and taxonomic diversity of XyGs, William York (University of Georgia) used sequence-specific glycanases to give a spectrum of oligosaccharides that, when analyzed by NMR spectroscopy and HPLC-MS, provides a fingerprint of the fine structure of an entire polysaccharide. Sugar-linkage structure and subtle alterations in structure, such as the acetylation of the glucosyl backbone of the Solanacean arabinosylated XyGs, are determined in underivatized oligomers by Matrix Assisted Laser Desorption Ionization–Time of Flight MS.

York and his colleagues are constructing an NMR spectral database to release on the Internet for diagnostic use as they continue to identify correlations between spectral detail and chemical structure. The combination of techniques, called a structural reporter approach, reveals that in jojoba XyGs, unique L-Gal units are attached to D-Gal sidegroup substituents found in most XyG units. In a collaboration with Wolf-Dieter Reiter on the characterization of the XyG of the Arabidopsis mutant *mur1*, which lacks fucose in the shoot tissues (Reiter et al., 1993), York reported that a significant portion of the units contain an L-Gal unit in place of the fucose. The galactosylated XyG appears to retain much of the biological activity of the fucosylated XyG oligomers (Zablackis et al., 1996).

Sequence Information Reveals the "Domain Mosaic" Structure of Wall Proteins

Cell wall proteins have traditionally been classified as glycine-rich (GRPs), proline-rich (PRPs), arabinogalactan-rich (AGPs), or HRGPs (Showalter, 1993). The first AGPs sequenced contained characteristic Hyp-Ala repeats, and Allan Showalter (Ohio University) has obtained a full-length clone of a tomato AGP with these motifs. However, information presented at this meeting revealed that the designations proline-rich, hydroxyproline-rich, and

glycine-rich may be more applicable to sequence domains within the proteins than to the proteins themselves. For example, two new glycoproteins that have been characterized and cloned from the wall and extracellular fluid of pistils of *Nicotiana alata* by Adrienne Clarke's group (University of Melbourne) defy being categorized as classical AGPs, despite containing over 20% Hyp/Pro and carbohydrate moieties enriched in galactose and arabinose. One, a 120-kD glycoprotein, contains domains of Ser-Pro$_{2-7}$ and Pro$_4$Ala + Pro$_{2-3}$ motifs. Two of the domains have extensin-like O-linked sugar linkages (short Ara side chains), whereas a third domain has AGP-like O-linked sugar linkages (galactan chains).

A galactose-rich stigma glycoprotein (GaRSGP) contains a domain rich in Pro-X-Lys-Pro-Pro motifs, a characteristic of PRPs, and is covered in short galactan side chains. The 120-kD protein is found in the extracellular fluid of the the transmitting tissues of the style and eventually finds its way into the cytoplasm of the growing pollen tube, whereas the GaRSGP is found in the primary wall of the stigma.

The AGPs are identified by their carbohydrate structures and their ability to bind the Yariv reagent, a β-D-glucoside of phloroglucinol. Keith Roberts (John Innes Centre) reported a protein from carrot cell suspension cultures that also defies classification under the current system, despite reactivity with Yariv reagent. Unlike a classical AGP, it contains no Hyp. It is a mosaic of Pro-rich, Cys-rich, and His-rich peptide domains and has homology to a PRP (Sheng et al., 1991). Although its function is unclear, the protein binds to some pectins (but not HGA) and the His-rich domains may be involved in this binding. Two AGPs isolated from different carrot suspension cultures were shown to have the same protein core although differing in their immunoreactivity to carbohydrate-recognizing antibodies, a rare demonstration that a single peptide core may be modified by different carbohydrate moieties.

In a twist on the architect Louis Sullivan's "form follows function," Marcia Kieliszewski (Ohio University) derives "function follows form" to mean that protein primary structures can direct associations in the cell wall—the structure of proteins such as extensin, for example, may help to align matrix polysaccharides. Extensins are a mosaic of Ser-(Hyp)$_4$ domains and domains containing Tyr-X-Tyr-Lys motifs. The Ser-(Hyp)$_4$ repeats of the HRGP extensin are only one type of domain, and Kieliszewski has been studying other Pro-containing motifs in several wall proteins to determine amino acid sequences that target the Pro residues for hydroxylation and subsequent attachment of Ara units. Contiguity of Pro residues in the polypeptide is one possible hydroxylation signal, and HRGPs were treated with pronase to yield pentapeptides in which the sequence of Pro and Hyp can be determined by tandem MS. The position of the arabinose units and chains is not random, indicating that amino acids other than the contiguous Pro residues contribute to the consensus signal for hydroxylation and subsequent addition of sugars.

Developmental regulation of the cell wall PRPs has been elegantly dissected by Mary Tierney (University of Vermont). Transcription of SbPRP2 in the cotyledons, hypocotyl, and root tip is upregulated by auxin, and three separate auxin-responsive elements, identified by 70% homology to other auxin-induced promoter motifs, controlled expression in each of these locations. A light-responsive element was also present in cotyledons, and two wound-inducible elements were present in stems and leaves.

A New Wall Is Born: Formation of the Cell Plate and the Assembly of Polysaccharides

The earliest stages of wall assembly are evident during the formation of the cell plate that divides a mother cell into two daughters. With beautiful preservation of cell structure by high-pressure freezing/ freeze substitution, Andrew Staehelin (University of Colorado) has examined the fine structure and development of the Golgi apparatus (Zhang and Staehelin, 1992) and followed the course of cell plate formation during division of semisynchronous tobacco BY-2 cells (Samuels et al., 1995). The Staehelin group divides cell plate formation into four distinct stages (Figure 3). They showed, immunocytochemically, that callose synthesis predominates during the assembly and outward-directed fusion of cell plate vesicles via very thin, coated (non-clathrin) tubules. They suggest that callose may help to stabilize the vesicles as they fuse and flatten to form the daughter wall.

Betty Lemmon and Roy Brown (University of Southwestern Louisiana) describe the development of the cell wall in cereal endosperm cells as quite different from that of typical cell plate formation. The advancing syncytium and the cross walls of cereal endosperm develop independently, lagging behind nuclear divisions. Incipient cell walls, however, all share callose as an abundant initial component, with mixed-linkage glucans accumulating much later in development of the walls of cereal endosperm.

The local interactions of molecules give rise to larger order interactions in wall assembly, and these interactions can be studied using computer models and in vitro models of interacting polysaccharides. Molecular modeling of XyG is used by Sam Levy (University of Colorado) as an approach to understand how sidegroups affect formation of steric bonds to one face of a cellulose microfibril, or how arabinose units added to the glucan chain might interfere with the binding of XyG to cellulose. The predictions were successfully tested in binding studies using XyGs of equal size isolated from pea epicotyls and nasturtium seeds, and avicel, a microcrystalline cellulose.

Sarah Whitney (Unilever Research) has also used an in vitro reconstitution assay, mixing plant matrix polymers with nascent cellulose microfibrils secreted by a bac-

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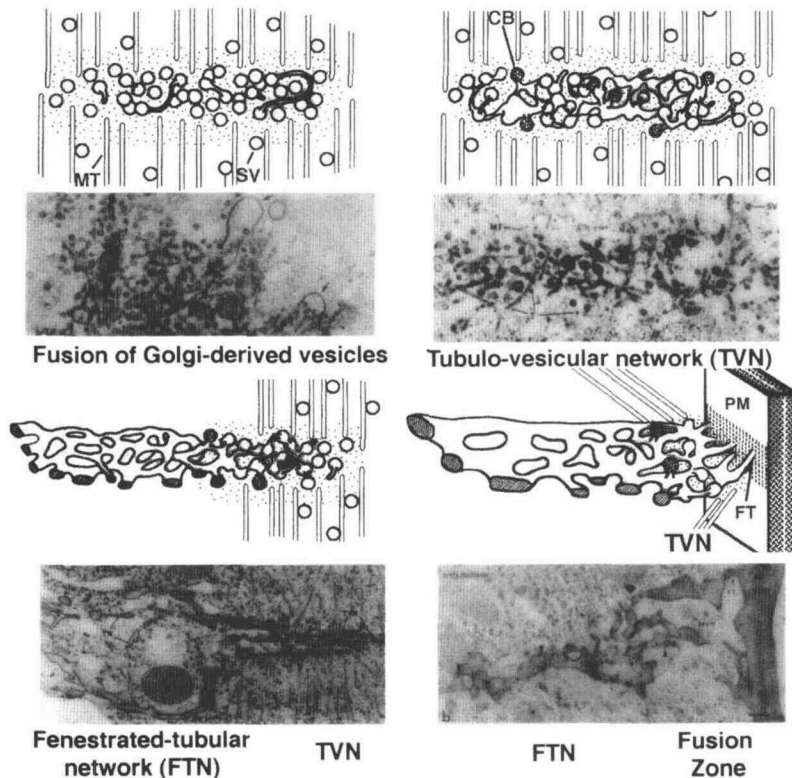
Figure 3. Model of Cell Plate Development in a Higher Plant Cell.

Diagrams of representative sections of high-pressure frozen, freeze-substituted dividing cells illustrating each stage of cell plate development. The fusion of Golgi-derived vesicles (SV) begins at the equatorial zone at the interface of juxtaposed microtubules (MT). The "tubulo-vesicular network" (TVN) is formed quickly. Vesicular boli are interconnected by thin tubules with clathrin-coated buds (CB). The "fenestrated-tubular network" (FTN) of smooth membranes filled with callose and other polysaccharides is left behind as the TVN and associated microtubules migrate centrifugally to the outer wall. As the new wall develops within the FTN, the fenestrations consolidate and the smooth membranes become a continuous sheet, with the fenestrations confined to strands of ER that traverse the presumptive sites of plasmodesmata formation. At the site of contact of the TVN and parent wall, fingerlike fusion tubes (FT) attach to the plasma membrane in the area once occupied by the preprophase band. The new wall partitions the cells, and cellulose content begins to approach that of the parent wall (adapted from Samuels et al., 1995, by copyright permission of the Rockefeller University Press).

terium in culture, to show that the process of wall assembly relies at least partially on self-assembly mechanisms. The nature of the cellulose spacing and cross-links formed in the network are visualized by EM, and assessed by solid-state/cross-angle polarization NMR. Whitney found that the nonfucosylated XyG from tamarind seeds is incorporated into a composite network with the generation of cross-bridges with lengths similar to those previously measured in native plant cell walls (McCann et al., 1990). The microfibrils in this network show a greater degree of lateral order than those in the cellulose-only pellicle normally produced by the bacteria. In contrast, cellulose and glucomannan composites are more compact, less hydrated structures than the XyG-cellulose ones. Both composites are weaker in tensile tests but more pliable than the cellulose-only pellicles. This reconstitution system will prove very powerful in defining the contribution of specific polymers to the macroscopic properties of cell wall strength and elasticity.

The cross-linked cellulose microfibrils in mature cell walls are thought to be embedded in a pectin gel. However, there is considerable uncertainty about the chemical nature of the linkages among the pectin components and between the pectins and cross-linking glycans coating the microfibrils. Thus, a significant observation made by several labs is that borate diesters may cross-link two RG II molecules (Figure 4). In contrast to other pectic polysaccharides that show variable backbone substitution patterns, RG II is a precisely defined molecule of about 4800 D that contains several linked Rha units and numerous unique sugars and sugar acids, linked in a precise sequence attached as oligomers onto the O-2 units of an HGA. That RG II molecules may exist as dimers linked by single borate diesters between two apioses, branched sugars unique to RG II, was a discovery made independently in the laboratories of Tadashi Ishii (Bioresources Ibaraki), David Loomis (Oregon State University), and Alan Darvill (University of Georgia). Each RG II monomer contains two apiose units, but only one is capable of forming the intermolecular linkage.

An extensive survey of the state of RG II in plants is lacking, but we know already that the pool will be a dynamic one. For example, the RG II of the primary walls of pea epicotyls is all dimerized, whereas in some red wines, a rich source of RG II, all of the molecules are in the monomeric form. The diesters can be formed in vitro in nonenzymatic reactions catalyzed by barium, lead, or strontium salts. Providing evidence for the importance of boron in maintaining wall integrity, Gladys Cassab (National University of Mexico) showed that boron starvation
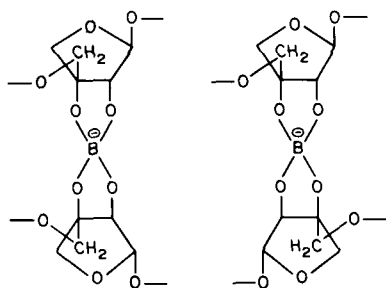
Figure 4. Borate Ester Cross-Links in Rhamnogalacturonan II.

RG II is one of four major pectic polysaccharides in flowering plants. RG II is a homogalacturonan, substituted with two oligomeric structures of defined sequence, with a molecular mass of about 4800 D. RG II is noted for its collection of rare sugars, in particular a branched hexofuranose, termed apiose. Each RG II molecule contains two apiose units, but only one is able to participate in a borate ester cross-link. The borate ester is likely to be located on the C-2 and C-3 of a 3-linked apiose residue. The diagram shows both of the possible diasteriomeric forms of the borate-apiose esters (figure provided by Alan Darvill).

leads to less incorporation of extensin into the walls of root nodules in *Phaseolus vulgaris.*

Part of the new frontier in the cell biology of the plant extracellular matrix is the regulation of interaction and assembly of wall polymers. Intermolecular interactions that occur during the insertion and exit of proteins from the ER require protein chaperones which keep proteins from misfolding and becoming aggregated or degraded. Plants have a functional complement of ER chaperones, such as calnexin, calreticulin, and reticuloplasmin (Edith Van Dooren, Leiden University). One of the themes to emerge from the meeting is the complexity of higher order interactions that result in the appropriate wall component getting to the right place at the right time. The glycochaperone workshop, building on this theme, introduced the idea that the conventional definition of molecular chaperones could be extended to include chaperones that

are either glycans themselves or use glycans to carry out their function (Lawrence Griffing, Texas A&M University).

Three presentations from nonplant research areas provided illustrations of glycochaperones. Calnexin and calreticulin, once thought to be resident proteins of the ER in eukaryotes, can now be identified as chaperones of glycoproteins destined for exit from the ER. As discussed by Daniel Hebert (Yale University), both retain glycoproteins in the ER until they have become properly folded. Calnexin (Hebert et al., 1995) and calreticulin bind to monoglucosylated N-linked glycans in different regions of glycoproteins that have been partially processed in the ER prior to transfer to the Golgi. A transglucosidase will reglucosylate proteins which are misfolded and which are to be retained in the ER. Delmer found a calnexin associated with the SuSy that eventually associates with cellulose synthase at the plasma membrane—an indication of the potential importance of such a system in plants.

As shown by Peter Scheiffele (EMBL Heidelberg), an N-glycan is a signal that allows the polarized secretion of a glycoprotein. In the mouse kidney epithelial cultures used as a model for polarized cells, an introduced N-linked glycan serves as an active signal for polarized secretion of an otherwise uniformly secreted nonglycosylated protein. The nature of the glycan-recognition protein, possibly a lectin (Fiedler and Simons, 1994), has yet to be determined, but the sorting event appears to occur in the later compartments of the Golgi.

Once outside the cell, oligosaccharides may play a role in assembling the wall. An example provided by Clifton Barry (NIH) is the role of arabinogalactan oligosaccharides in determining the nature of the assembly of the long-chain mycolic acids in the wall of mycobacteria.

Glycochaperones may also be involved in the assembly of the *Chlamydomonas* cell wall. Sabine Waffenschmidt (University of Köln) illustrated how glycochaperones may serve to prevent aggregation of

HRGPs prior to cell wall assembly. As Kieliszewski described, protein primary structures may direct intermolecular associations in the cell wall, and these interactions may contribute to wall integrity. The possibility was raised that AGPs or expansins could act as chaperones through their interaction with glycans en route to the wall or during wall assembly to prevent premature aggregation. Expansins (see below) have putative cellulose-binding domains (CBDs; Dan Cosgrove, Pennsylvania State University), and AGPs are known to bind oligosaccharides. Carpita showed that pulse labeling of the glycan moiety of newly synthesized AGPs exhibit higher turnover than the matrix polysaccharides that accumulate in the wall. The possibility that membrane-bound AGPs could act as recyclable chaperones which interact relatively nonspecifically with newly secreted oligosaccharides would be consistent with the labeling patterns seen and the known biochemistry of AGPs.

The Wall Is Remodeled during Growth and Development

Once assembled, cell wall architecture has to be extensible. Sustained cell growth depends on the coordination of loosening of the original structure with deposition of newly synthesized wall material. One of the most influential advances toward understanding auxin-induced and acid growth has been the discovery of expansins by Cosgrove. Expansins are small proteins that induce wall extension in vitro by disrupting noncovalent steric or hydrogen bonding between the matrix polysaccharides and cellulose (McQueen-Mason and Cosgrove, 1995). Expansins have no hydrolytic or transglycosylase activity and are the only wall-associated proteins capable of inducing extension in vitro. Growth rate, levels of expansin mRNA, and "acid creep" are strictly correlated along the growing cucumber hypocotyl. Exogenous expansins accelerate the rate of elongation of Arabidopsis

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hypocotyls. The expression of one of the cucumber expansin genes (S1) is upregulated by auxin and downregulated by light. Furthermore, immunogold labeling studies show that expansins are present in growing and nongrowing cell walls, with the implication that expansins are inactivated when growth stops. Their activity is also affected by changes in other wall components, as pectin methylesterase activity is correlated with a loss of sensitivity to added expansin.

Several members of the expansin gene family have been cloned and the expansin cDNAs have 60 to 87% sequence identity at the amino acid level. The expansin family in Arabidopsis numbers at least nine; three have been identified in young rice seedlings, and two are present in the growing hypocotyl of cucumber. The expansin genes encode proteins with a signal peptide, a Cys-rich region containing eight highly conserved cysteines, a basic domain, and a Trp-rich domain. The spacing of these domains is similar to that of the cellulose-binding domains of hydrolases from cellulytic bacteria.

Expansin activity explains how the cellulose–cross-linking glycan network might be loosened during growth, but apparently does not result in coordinated changes in the pectin network. Many other reversible cross-links are also present in the cell wall, and the identification of the RG II borate diesters has prompted studies to assess its involvement in dynamic pectin cross-linking. Loomis showed that borate is a pH-sensitive cross-link and about one-third of the esters become cyclic diesters rather than di-diesters at pHs corresponding to acid growth. However, Darvill reported that the pH of dimer formation was closer to pH 3.2 to 3.4 than to physiological acid-growth pH. The borate diesters are presumed to be formed in the cell wall, and the search is on for an esterase capable of hydrolyzing the borate diesters.

Many different proteins are present in cell walls at different locations and times during development. The deposition of specific wall components is necessary in particular cell types for specialization, and it is no surprise that the cell wall remodeling enzymes are encoded by large multigene families under developmental regulation. For example, the XETs, which have also been implicated in wall loosening and retethering, are encoded by a large gene family. Imad Saab (University of Illinois) found an XET gene strongly upregulated in roots during flooding of maize plants. The maize-root XET may be involved in development of flooding-induced aerenchyma, the large air spaces generated by cell wall digestion that serve as snorkels to deliver oxygen to the roots.

Five genes encoding endo-$(1\rightarrow4)$-β-D-glucanases have now been described in tomatoes by David Brummel (University of California, Davis). Three of these genes are associated with cell expansion, whereas two are induced during fruit development. One of these genes, tomCel7 is upregulated by auxin and is active during fruit expansion, but not ripening.

The polygalacturonase active during tomato fruit ripening has a catalytic domain that is sufficient and necessary for pectin degradation. A second polypeptide in the complex, a 38-kD "β-subunit," modifies pH optimum, thermal stability, and increases the binding of polygalacturonase to cell walls (Watson et al., 1994). Dean DellaPenna (University of Nevada–Reno) reported that plants with antisense of the β-subunit show a 60% increase in polyuronide solubilization during ripening. The mature protein has a repeated 14–amino acid consensus sequence rich in phenylalanines that are post-translationally modified such that they are not susceptible to Edman degradation. There are several homologous β-subunit genes in Arabidopsis and tomato, all containing the repeated 14–amino acid motif, similar precursor structure, and over 15% aromatic amino acid residues. DellaPenna described them as a new class of cell wall proteins, the AroGPs.

John Bedbrook (DNA Plant Technology Corporation) has used sense suppression to inactivate an endo-$(1\rightarrow4)$-β-D-glucanase in ripening pepper pericarp. The choice of enzyme downregulation was informed by the intimate correlation between its activity and fruit color, indicative of the developmental stage of ripening. The sense plants showed an alteration in the cross-linking of glycan, but not pectic, fractions in the cell wall, and reduced water loss over time, which offers the significant commercial advantage of increased shelf life. Such biotechnological applications will become ever more common as we understand more of the regulation of cell wall metabolism.

Even though XETs are able to permit wall extension without sacrifice of the tethering function of the cross-linking glycans, other wall proteins may function to cross-link wall polymers even during cell expansion to preserve wall integrity and mechanical strength. For example, Roberts found a peroxidase gene from elongating mung bean hypocotyls that is upregulated during elongation. Maureen McCann (John Innes Centre) and Roberts have also found that expression of a pectate lyase gene from Zinnia elegans is upregulated by auxin during elongation of mesophyll cells.

Cell growth and developmental mechanisms are also being dissected by molecular genetics. Arabidopsis cell wall mutants will become particularly useful in such studies. For example, Reiter showed that the Arabidopsis mur1 plant is unable to synthesize GDP-fucose de novo. As Lowe described, the lack of a single fucosyl residue on a surface carbohydrate can have profound effects on animal physiology. Curiously, the growth habit of mur1 plants appears to be almost unaffected despite lacking the critical fucosyl residue in its XyGs, but the tensile strength of the stem of mur1 plants is reduced to one-third that of the wild type. York reported years ago that the fucosyl group on the XyG oligosaccharide was essential to attenuate auxin-induced growth in vitro. He found that the fucosyl group in mur1 plants was replaced in part by L-galactose (Zablackis et al., 1996), and that the L-Gal residue was sufficient to inhibit auxin-induced growth. The decrease in tensile

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strength remains to be satisfactorily explained.

Several reports at this meeting illustrated how each wall of a plant cell becomes unique during differentiation. According to Darvill, RG I is a marker of cells that will not differentiate into hair cells in the epidermis of Arabidopsis roots, and in the in vitro Zinnia system McCann found that RG I is secreted in large quantities to the wall and the culture medium as the mesophyll cells are induced to differentiate into tracheary elements. Differential localization of wall components was also reported in the distribution of galactans in developing flax fibers by Tatyana Gorshkova (Kazan Institute of Biology), pectins and AGPs in lily pollen tubes by Betty Lord (University of California, Riverside), and fucosylated XyG, methylesterified HGA, and AGPs in Zinnia leaves by McCann. Katia Ruel (CNRS, Grenoble) used immunogold labeling to show the differential localization of guaiacyl and para-hydroxyphenyl propane homopolymer, and guaiacyl-syringyl copolymer lignins in different cell types and regions around cells in wheat straw and maize.

Zinnia tracheary element formation has emerged as one of the best studied examples of the inducible differentiation of a single cell type in culture (Chasan, 1994). Several new model systems have been discovered to study the cellular and biochemical aspects of the growth and differentiation of specific cell types. For example, Andrew Staehelin revealed a convenient root cap cell culture for study of the Golgi secretory pathway, and Alan Jones (University of North Carolina) characterized a line of maize cells in liquid culture that, upon addition of sucrose, spontaneously form the reticulate wall thickenings that resemble transfer cells.

Secondary Walls Are Elaborated with Lignin, but not Randomly

As primary walls cease growth, they can become impregnated with lignin to form stiff and incompressible secondary walls.

The commercial significance of lignification is vast because it contributes to losses in nutritive quality of forage grasses and affects efficient pulping of wood products. The structure, expression, and regulation of cinnamoyl CoA reductase (CCR) and cinnamoyl alcohol dehydrogenase (CAD) genes of Eucalyptus are the focus of the work by Alain Boudet (Paul-Sabatier University). By antisense technology, he and Claire Halpin (Zeneca Plant Science) evaluated lignin structure and extractability in transgenic poplar and tobacco that had CAD activities reduced to about 10% of normal. Total lignin content in the transgenic plants is nearly normal but more easily extracted. On the other hand, diminution of CCR by antisense constructions decreased lignin content by ~25 to 35%, increased soluble phenolics, and resulted in a higher proportion of collapsed xylem vessels. Because of the impact of lignin on normal development and defense against pathogens and predators, alternative strategies are being explored to modify lignin structure without compromising its biological function.

Antisense and cosuppression in transgenic plants have proven useful to assess CAD activity, but the amount of lignin is generally unaffected, or the results equivocal because of high residual activities. The red pigmentation of the vasculature of these transgenic plants looks similar to that of the brown mid-rib (bmr) mutants of the Poaceae as a result of the accumulation of the cinnamoyl aldehydes into the lignin (Cherney et al., 1991). Bruce Stone (La Trobe University) showed that the total lignin content is unaffected in grass bmr mutants but that it is less extractable by the acid-detergent procedure than in normal plants. The amount of etherified ferulic acid, probably involved in ester-ether bridges between lignin and polysaccharides, is lower than in normal plants.

A mutant loblolly pine, distinguished by red pigmentation in the xylem, was discovered by John MacKay and Ron Sederoff (North Carolina State University). Whereas reduced CAD activity in transgenic plants gives substantial variability in lignin

content, the loblolly pine mutant, on the other hand, is a near null CAD (only 1% normal activity) and contains significantly less lignin and increased coniferaldehyde and vanillin.

The classical view of lignin formation is that the cinnamoyl alcohols are glycosylated for transport to the cell wall where they are deglycosylated, oxidized by peroxidases, and form a randomly interconnected network via ester, ether, and phenyl linkages. Two aspects of that scheme are changing. First, the assembly of lignin is not as random as once thought, and second, the role of laccase and its interaction with peroxidase in lignin formation are being reevaluated.

In grasses, ferulic acids are attached to many of the arabinosyl units of the cross-linking glucuronoarabinoxylans (GAXs), and diferulates cross-link the GAXs to form a network. If the precursors of diferulate are formed by radical-generating mechanisms, John Ralph (University of Wisconsin) reasoned that many other diferulate ether and phenyl linkages should be possible. Ralph showed that many other couplings exist in the grasses, some in higher amounts than the classical 5-5' structure. Nevertheless, the structures formed are not random, some possibilities are missing, and the ferulates are found to react only with certain lignin monomers. This specificity enables Ralph to propose that the ferulate esters of GAX can serve as initiation sites for lignification in the grasses.

Lignins are classified by the preponderance of certain cinnamoyl alcohols. This specificity of intermolecular coupling was also illustrated by Norm Lewis (Washington State University). Lignins always form 8-β-O-4 structures, whereas lignans couple strictly 8-8'. Of the many permutations of possible couplings among the family of substrates and radicals, only a few predominate. Ostensibly, the selectivity of stereospecific couplings arises by the specificity of the peroxidases, laccases, and catechol oxidases that catalyze them. For example, coniferyl alcohol is directed toward lignin by H_2O_2 and peroxidase, or

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toward lignan dimers by laccase and O_2. Dimers formed in the latter reaction can be further converted to the lignin pathway by peroxidases. Conversion of coniferol to pinoresinol by the *Forsythia* laccase is accelerated and made optically pure by the action of a 78-kD protein. A cDNA for this laccase-associated protein has been sequenced, and it is not homologous with other known proteins.

Model systems of cells in liquid culture have also been important for the lignin researchers to identify the synthesis and regulatory features of lignin biosynthesis. Lewis found that loblolly pine cells are induced to form lignin precursors by addition of sucrose to the medium. Jeff Dean (University of Georgia) has evaluated the role of laccases during *Zinnia* lignification. Laccase is associated with the thickenings of in vitro tracheids, and in the *Zinnia* plant it is associated with phloem fibers, collenchyma, and xylem. From comparison of the gene sequences between the *Acer* and poplar laccases, an amino acid conversion of methionine to leucine within the Cu^{2+}-binding site may result in a redox potential change that could favor the oxidation of coniferol in poplar. Transformation of poplar with an antisense laccase construct results in an ∼10% loss of lignin.

Cell Fate Determination: The Medium Is the Message

One of the most exciting recent discoveries in cell wall biology has been the direct demonstration that extracellular molecules may influence cell fate in plants. This "autocrine mechanism," whereby secreted components act back on these cells and their neighbors, underpins the cooperativity of determination events in animal cells (Gurdon et al., 1993).

The brown alga *Fucus* is used by Colin Brownlee (Marine Biological Association, Plymouth, UK) to show that the cell wall has positive feedback influence on cell fate determination. The *Fucus* zygote divides asymmetrically into two distinct cell

types: a rhizoid and a thallus cell. However, thallus or rhizoid protoplasts are pluripotent and will regenerate complete embryos. Redifferentiation into thallus or rhizoid depends on contact with the cell wall, and prolonged contact of one protoplast type with the cell wall of the other type can switch its fate. Both rhizoid-inducing factors, perhaps sulfated oligosaccharides, and rhizoid-inhibiting factors are present in cell wall fractions. In plants, oligosaccharides can trigger a range of cell responses, and information may be stored in the precisely elaborated cell wall to reinforce the identity of the cell type.

As Brownlee described, the site at which the rhizoid will grow out from a zygote can be identified by higher local activity of inward-directed Ca^{2+} channels, and Ralph Quatrano (University of North Carolina) showed the subsequent appearance of an actin patch in the cell cortex. The site of rhizoid emergence is not fixed until about 8 hr after fertilization, at which time the actin patch becomes the target site for polarized deposition of sulfated polysaccharides, such as fucoidin. Treatment with cytochalasin to disrupt the actin network, removal of the cell wall, or the blocking of secretion with Brefeldin A each block the fixation of polarity. The nature of the structural proteins mediating the cytoskeletal and secretory response during axis fixation is unclear, but a fucoidin-binding protein in the wall at the site of axis fixation may function in this deposition. A candidate fucoidin-binding protein has been cloned from *Fucus*. The bacterially expressed protein is recognized by antibodies against the heparin-binding domain of human vitronectin, but the cDNA possesses little similarity to the vitronectin gene.

The plasma membrane is an interface of cytoskeleton and cell wall, and all three form the scaffolding for signal transduction. Elusin, which is a putative cell wall anchor protein with a transmembrane domain and a serine/threonine-kinase cytoplasmic domain, was discovered in Arabidopsis by Bruce Kohorn (Duke University). Kohorn speculated that elusin

is a candidate for mediating transducing signals from the wall to the cytoplasm.

Arabidopsis mutants also illustrate the importance of cooperativity between the cytoskeleton and cell wall for normal development. A temperature-sensitive mutant that shows radial swelling in the root cells was reported by Tony Arioli (Australian National University) to have a defective cortical cytoskeleton and greatly reduced content of cellulose. Regina McClinton (University of California, Berkeley) showed that the *fass* mutant contained a normal spindle apparatus but lacked normal cortical arrays of microtubules resulting in lack of proper cell elongation and shaping mechanisms.

A Matter of Life or Death at the Cell Wall

When foreign organisms interact with plant cells, the first step is often a wall–wall interaction. What follows is mutual recognition that ultimately results in a successful defensive response by the plants or a successful breaching of that defense by the invading organism, be it a pathogen or a beneficial symbiont. These signaling events can be separated into a spatial progression series indicating a more and more intimate association of plant and microbe. The first event is environmental signaling that may occur as the foreign organism positions itself to interact optimally with the plant.

A notable example of a plant response to a diffusible signal produced by a foreign organism is the induction of nodule formation by Nod factors, lipochitin oligosaccharides produced by *Rhizobia* sp. In developing the hypothesis that endogenous lipochitin oligosaccharides may play a role in plant organogenesis (de Jong et al., 1993), Herman Spaink (University of Leiden) summarized the evidence that the Nod factors induce organogenesis (i.e., nodule formation) in the absence of bacteria by triggering the cell cycle. Work in the early part of this century implicated a factor produced by the stele in the

induction of cell division, and Spaink suggested that the signal might be uridine, which can induce cortical cell division at picomolar concentrations (Smit et al., 1995).

Just as plants respond to the presence of the invader prior to invasion, so the invader can respond to the plant in order to identify the optimal site for initiating an infection. An intriguing example described by Bruno Moerschbacher (University of Münster) is the directional growth of a rust uredospore germ tube toward the stoma of a wheat leaf. The hypha differentiates into a swollen appressorium above the stoma, and a penetration peg emerges into an underlying mesophyll cell and develops an haustorium within the cell. The growth is guided by a combination of topographical and biochemical cues on the leaf surface. Appressorium formation can be induced in vitro in about one-third of the hyphae grown on silicon wafers with ridges of defined height (Hoch et al., 1987). Moerschbacher found that several cues, such as coating the silica wafers with epicuticular waxes containing trans-2-hexinol, were needed to induce appressorium formation in 95% of the hyphae. At the point of haustorium formation, a membrane-mediated response by the plant may include an oxidative burst at the plasma membrane and subsequent cross-linking of certain wall polymers. In resistant wheat plants, components of the haustorial cell wall trigger hypersensitive host-cell death which involves the intracellular biosynthesis of a syringyl lignin. Moerschbacher pointed out that the decision by a cell to die is not taken lightly — several cues by the invader must be delivered before the hypersensitive response is carried out.

The mediators of wall–wall interactions may include polysaccharides, polysaccharide-binding proteins, and hydrolytic enzymes and their inhibitors. Events restricted to the extracellular domain are often hydrolytic, overcoming the important physical barrier presented by the wall. Many of the plant proteins induced by pathogens or elicitors, the pathogenesis-related (PR) proteins, as well as many plant wall structural proteins are resistant to proteolytic cleavage.

In an overview of PR proteins, Bernard Fritig (CNRS, Strasbourg) noted that PR family 6 proteins are active inhibitors of microbial proteases, and that the plant is also capable of mounting a significant hydrolytic response to fungal invasion; of the 11 different families of PR proteins, five have amylase, glucanase, or chitinase-lysozyme activity. Fritig suggested that the complementarity of hydrolytic activity of PR proteins may result in synergistic effects allowing more rapid breakdown of fungal walls.

To restrict breakdown of their own walls, plants produce inhibitors of polysaccharide-degrading enzymes. A family of bean genes encoding polygalacturonase-inhibiting proteins (PGIPs) is being characterized by Guilia De Lorenzo and coworkers (University of Rome-La Sapienza). These proteins have Leu-rich repeats (LRR) typical of those involved in protein-protein recognition (Kobe and Deisenhofer, 1994), and are induced by carbohydrate elicitors. PGIP mRNA accumulates rapidly during the hypersensitive response in resistant (incompatible) interactions between Colletotrichum lindemuthianum and bean (Phaseolus vulgaris L.), but with a more delayed time course in susceptible interactions. Heterologous expression of bean PGIP in tobacco and tomato resulted in an altered sensitivity to inducing agents and an altered spectrum of inhibition to fungal polygalacturonases. This example demonstrates that the signal received by the plasma membrane and the subsequent processing of a response protein during its delivery to and through the wall are important modulators of the wall–wall interaction.

The perception of a microbe by a plant may occur through the binding at the plant plasma membrane of oligosaccharide or protein conjugates secreted directly by the microbe or produced in the plant cell wall by hydrolysis of native plant polymers. Evidence for the latter includes the findings of Ted Farmer (University of Lausanne)

that endogenous plant pectins, when hydrolyzed into component galacturonide fragments of 10 or more residues in length, induce phosphorylation of a 34-kD protein in plant plasma membrane preparations. A 22-kD hydrophilic protein, which sticks tightly to these membranes, was identified as a candidate for mediating the interaction between ligand and putative receptor. Several approaches toward analyzing the galacturonide–22-kD protein interaction were tested, but the nature and extent of the specific binding to purified galacturonides remain to be elucidated.

On the other hand, the nature of the specific binding of fungal wall components to the plant plasma membrane has a longer history, and pieces of the puzzle are slowly becoming identified. Michael Hahn (University of Georgia) solubilized and purified by ligand affinity chromatography the soybean plasma membrane receptors of Phytophthora β-glucoside, and Naoto Shibuya (Agrobiological Resources, Japan) purified a rice membrane receptor of N-acetylchitooligosaccharides. In soybean, a 70-kD and a 55-kD protein are found which retain activity as an aggregate, but which lose activity upon treatment with DTT. In rice, a 64-kD protein and a 44-kD protein elute from the affinity column. Photoaffinity labeling has also identified 70-kD subunits for both soybean and rice receptors, and the work in rice, using an ^{125}I-labeled photolabile aryl azide of N-acetylchitooctaose, shows that the 70-kD protein is enriched in plasma membrane preparations.

The transient appearance of reactive oxygen species (ROS) in the wall, the oxidative burst, and the nature of its linkage to initial elicitor recognition events were discussed by Chris Lamb (Salk Institute). The oxidative burst occurs very rapidly, within 2 to 5 min after reception of the elicitor, placing it proximal to the receptor in the signal transduction chain. NADPH oxidase has been suggested as a link in the chain because it is an important link in the analogous defense-related oxidative burst in phagocytes (Mehdy, 1994), and inhibitor studies do not rule it out (Auh and

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Murphy, 1995). However, Lamb's group found no changes in NADPH levels that could be correlated with the burst. Hence, the nature of the reductant used during the production of ROS in the wall has yet to be established. Wall alkalinization often accompanies or precedes ROS production, and Lamb's group has identified two peroxidases with pH optima greater than pH 7 that could participate in the production of ROS. The spatial organization of the reductant, the peroxidase, and other elements of the signal transduction pathway (e.g., the receptor) need to be resolved, and Lamb speculated that it may result from a kind of "smart" cross-linking, or directed assembly process, through interaction of peroxidases with other wall components.

Resolving the downstream signaling events of the receptors discussed at this meeting, which have been identified by virtue of their interaction with wall-derived ligands, may benefit from studies on another class of putative receptors, the receptor-like kinases (Braun and Walker, 1996), for which no ligands have been identified, but which provide a kinase handle on the downstream signal transduction events. The future challenge is to close the circle in the life history of these defense-related receptors.

Conclusions

Most notably, the conferees witnessed a substantial bridging of the gap in our understanding of the fine structure of cell wall molecules and the genes responsible for their existence. Advances in genetic engineering coupled with our improved understanding of molecular architecture of the wall are paving the way for designer crops that produce better foods and fibers. If cell wall researchers seem to be touched with such zeal about their subject these days, it is because they are beginning to see the fruits of slow and painstaking chemical analyses ripen into an understanding of underpinning biological

mechanisms of growth and development. It was clear to us in Tamarron that we have built our walls on good foundations.

ACKNOWLEDGMENTS

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