The Wall Becomes Surmountable

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Perhaps of all the cellular structures in plants, the cell wall has been the slowest to reveals its secrets. In no small part, this is because the classical tools of biochemistry have often proven to be too crude to effectively elucidate structures as biochemically complex and ornate as the plant cell wall. The techniques of molecular biology, however, have provided new approaches toward understanding the plant cell wall, and the remarkable progress that has been made in this regard in recent years was in great display at the Ninth International Cell Wall Meeting held this past September in Toulouse, France. This convention brought together hundreds of scientists from scores of countries to discuss both formally and informally the enormous strides that have been made in recent years in understanding plant cell wall function. Among the highlights of the meeting were many pioneering reports concerning efforts that are under way to use micro-array analysis to study the effects of specific physiological situations related to cell walls, although none of these studies have yet to come to full fruition. Studies of cell wall mutants were also prominent, as was the more widespread application of new techniques, such as Fourier transfer infrared resonance (FTIR) spectroscopy, for identifying such mutants. Enormous progress has also been made in recent years in understanding arabinogalactan-protein function. The discussion that follows is but a small taste of the bountiful feast of plant cell wall research that was presented.

SYNTHESIS OF CELL WALL POLYSACCHARIDES

The identification of the biosynthetic enzymes involved in cell wall biosynthesis remains one of the major unsolved problems of plant biology. Of the four major types polysaccharides of the plant cell wall, cellulose and callose are synthesized at the plasma membrane, whereas pectin and hemicellulose are synthesized in the Golgi apparatus.

Cellulose is a polymer of β-(1→4)-linked Glc residues. Despite its apparently simple structure, many aspects of cellulose biosynthesis remain unclear. The site of cellulose synthesis is believed to be the rosette structures occurring in the plasma membrane. The composition of the rosette complex changes during development, apparently because distinct cellulose synthase A (CesA) types are required at different stages. More than one gene product is required for the rosette to function properly, and several papers were presented concerning the structure and function of the presumed components of these rosettes.

Arabidopsis and maize (*Zea mays*) contain a family of at least 10 CesA-encoding genes. Although genetic evidence suggests that the CesA proteins function as glycosyltransferases during cellulose synthesis, direct evidence has been lacking. Deborah P. Delmer (University of California, Davis) reported, however, that the herbicide CGA 325'615 inhibits the synthesis of crystalline cellulose, and promotes the accumulation of a noncrystalline form in cotton (*Gossypium hirsutum*) fibers. She reported that cellulase-mediated digestion of this glucan releases CesA protein, demonstrating that CesA is intimately associated with chain elongation. The finding that cellulase treatment also released sitosterol-β-glucan (SG) led Delmer to propose that SG might serve as a primer for chain elongation. The ability of cotton fibers to make SG from UDP-Glc increases substantially at the onset of secondary cell wall synthesis. Moreover, membranes derived from fibers pretreated with the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile (DCB) showed a reduced capacity to synthesize SGs and sterol-cellodextrins, suggesting that DCB might inhibit cellulose synthesis by inhibiting synthesis of the primer. This idea was supported by their observation that the inhibition of cellulose synthesis by DCB in vivo can be partially overcome by supplying fibers with SG.

Several presentations originating from Simon Turner's laboratory (University of Manchester, UK) brought the audience up to date on the cellulose-deficient *irx* (*irregular xylem*) mutants of Arabidopsis. The *irx* mutants exhibit a phenotype in which the xylem vessels of the plants are so weak that they collapse inwards under the negative pressures associated with the upward transport of water. To date, four cellulose-deficient *irx* loci have been identified, three of which are members of the Arabidopsis CesA family. Progress has been made using immunogold labeling to determine the exact ultrastructural location of IRX1 and IRX3, both of which are believed to be CesA isoforms. *IRX5* apparently encodes for a member of the CesA family that is required for secondary cell wall synthesis. Evidence was presented that two or more distinct CesAs may be required within a single cellulose synthase complex.

Another interesting Arabidopsis cell wall mutant is *procruste* (*prc*). This mutant exhibits a severe cell elon-

gation defect in roots and dark-grown hypocotyls. The mutation affects a gene encoding a cellulose synthase catalytic subunit (CesA6), resulting in impaired cellulose synthesis. Soizic Rochange (Institut National de la Recherche Agronomique, Versailles, France) reported on the results of a screen for suppressor mutations of the *prc1-1* mutation. Three Arabidopsis mutant lines were identified in which hypocotyl and root elongation was less impaired than in *prc1-1* mutants. The extent of restoration appeared to be Suc dependent. Cellulose synthesis activities in these new mutants were intermediate between wildtype and the *prc* mutants, suggesting that the suppressor mutations affect a mechanism directly linked to cellulose synthesis.

Takao Itoh (Kyoto University) reported on his laboratory's success in demonstrating the existence of terminal complexes containing a catalytic subunit and/or related proteins of cellulose synthase. The catalytic region of cotton cellulose synthase was expressed in *Escherichia coli* and polyclonal antibodies were produced. Using an SDS-solubilized freezefracture replica labeling technique, the antibodies were specifically localized to plasma membrane complexes on the P-fracture face of rosette complexes.

Because cellulose composes the world's most abundant renewable biomass and important industrial products such as cotton fibers and wood, the metabolic regulation and environmental sensitivity of cellulose synthesis are important questions. For example, when cotton fibers are exposed to nighttime cool temperatures (15°C–18°C), their rate of cellulose synthesis for secondary wall deposition falls to 12% to 25% of the maximum rate at 28°C to 34°C. In temperate cotton-growing regions, this often results in harvest of a lower yield, lower quality fiber crop. Candace Haigler (Texas Technical University, Lubbock) has been studying the biochemical basis of this problem. Her laboratory's research suggests that Suc is the preferred substrate for secondary wall cellulose synthesis, and this implicates Suc phosphate synthase (SPS) as a possible enzyme involved in the metabolic control and environmental sensitivity of cellulose synthesis. SPS is a primary regulator of flux to Suc in leaves. Haigler reported that transgenic cotton plants that constitutively overexpressed SPS produced higher quality fibers with thicker secondary walls when the plants were grown under 34°C/ 5°C day/night cycling temperature. These data suggest that regulation of flux to Suc is both a point of cool temperature sensitivity in cotton fiber cellulose synthesis.

Callose is a general term for the 1,3-β-glucans that are widely distributed in higher plants. During normal growth and development, callose is deposited at the cell plate of dividing cells. Callose is also found associated with some specialized cell walls, and with sieve plates and plasmodesmata. Callose deposition also occurs between the plasma membrane and the cell wall in response to various types of stresses. Although 1,3-β-glucan synthases have proven to be recalcitrant to study by conventional biochemistry, Geoffrey V. Fincher (University of Adelaide, Australia) described recent successes that his laboratory has had in characterizing these enzymes by alternative molecular biological techniques. A barley (*Hordeum vulgare*) cDNA encoding a homolog of the *FKS* gene of *Saccharomyces cerevisae* was cloned and heterologously expressed in *E. coli*. Polyclonal antibodies raised against fragments of the barley cDNA-encoded protein bound to a 220-kD protein that shows 1,3-β-glucan synthase activity in gel.

Hemicelluloses are crosslinked with cellulose microfibrils in the cell wall of most plant cells, forming a cellulose/hemicellulose framework that functions as the mechanical underpinning of the cell wall. Natasha V. Raikhel (Michigan State University, East Lansing) brought the audience up to date on her and Ken Keegstra's (Michigan State University) laboratories' collaborative efforts to identify enzymes involved in the synthesis of xyloglucan, the most common hemicellulose in plants. Xyloglucan is synthesized in the Golgi apparatus and is composed of a linear β-1,4-glucan backbone with side chains of Xyl, Gal, Fuc, and Ara residues. A gene encoding for the glycosyltransferase that adds the terminal Fuc to xyloglucan (*AtFT1*) has been previously identified in Arabidopisis, but Raikhel pointed out that there are no available assays for the remaining enzymes expected to be involved in the synthesis of xyloglucan. However, their group recently has developed a biochemical assay to analyze xylosyltransferase activity. Raikhel outlined a bioinformatics-based approach that has led toward the identification of a putative alpha-xylosyltransferase gene family that is involved in xyloglucan biosynthesis. By screening mRNA populations from cotton fibers at different developmental stages, the group has identified several good candidates. Two of these genes are predicted to be Golgi type II membrane proteins and glycosyltransferases.

Novel approaches toward the study of hemicellulose biosynthesis were also the topic of Laurence Bindschedler's (Royal Holloway University of London) talk. His laboratory has established a cell suspension culture of tobacco (*Nicotiana tabacum*) transformed with the *Agrobacterium tumefaciens Tcyt* gene that leads to high endogenous levels of cytokinin. In response to optimal concentrations of auxin and Suc, this cell line shows increased cell wall aggregation, elongated cells, and a 5-fold increase in wall thickness. Recovery of wall material was 50% greater in the *Tcyt* culture. Several enzymes (UDP-Glu dehydrogenase, xylan synthase, and an 80-kD soluble decarboxylase) involved in hemicellulose synthesis in this system were also described.

UDP-glucoronic acid, which provides about half of the biomass of the cell walls of Arabidopsis, is the dominant nucleotide sugar for hemicelluloses and

pectin. Raimund Tenhaken (University of Kaiserlautern, Germany) reported on the occurrence of three newly discovered isoforms of UDP-Glu dehydrogenase in Arabidopsis. Using reporter genes, they found a developmentally regulated expression of these isoforms that suggests that the genes are expressed in cells only when UDP-Glc-derived precursors are needed for the synthesis of new matrix polymers. In young seedlings, an alternative pathway operates in which inositol is oxidized directly to Glc. They also showed, contrary to previous reports in the literature, that UDP-Glc is the only substrate accepted by the enzyme. Moreover, they discovered that UDP-Glc dehydrogenase is strongly inhibited by UDP-Xyl, indicating a feedback loop for the regulation of sugar nucleotide precursors.

Antibodies provide highly specific tools with which to study the dynamics of plant cell walls. Michael G. Hahn's (University of Georgia, Athens) laboratory has generated several monoclonal antibodies that recognize specific carbohydrate structures (epitopes) in plant cell wall polysaccharides. These antibodies were used to study the distribution of selected carbohydrate structures found in pectic and hemicellulosic polysaccharides in tissues of wild-type and mutant Arabidopsis plants. Their studies demonstrate that polysaccharide structures differ among walls of different cell types, and differ even among walls of a single cell. They also showed that the insertion of specific polysaccharide structures into plant cell walls is under tight developmental control. Furthermore, they provided evidence that plant cells and tissues utilize different sets of enzymes for the biosynthesis of wall components at different points in growth and development.

Galactomannans are polysaccharides with a (1→4)-β-D-mannan backbone that is variably (1→6)-α-D-galactosyl-substituted according to the species. Galactomannans compose most of the endosperm cell walls of many seeds where they serve as post-germinative food reserves. Those from legume seeds are especially important in the water relations of seed germination, and are widely exploited in the dairy industry. Golgi membrane-bound galactomannan galactosyltransferases (GMGTs) are important in the regulation of Gal substitution in the pathway of galactomannan biosynthesis. Grant Reid (University of Sterling, UK) reported on his laboratory's characterization of a GMGT from fenugreek (*Trigonella foenum-graecum*). This enzyme and related ones were overexpressed in *Pichia pastoris* and shown to have GMGT activity. The ability to transgenically express GMGTs of different types has allowed for the production of novel galactomannan varieties that may have commercial application.

Man is a cell wall constituent found in a wide variety of non-cellulosic polysaccharides, several of which are of agronomic importance. In Arabidopsis, Man constitutes 4% to 9% of the neutral sugars present in the leaf tissue, but the biological functions of these Man-containing polysacchandes are as yet unknown. Non-cellulosic polysacchandes, including mannans, are synthesized in the Golgi apparatus. Synthesis occurs by the action of glycosyltransferase enzymes that use nucleotide sugars as substrates. Mannosyltransferase requires Man to be supplied as GDP-Man. The synthesis of GDP-Man and other sugar nucleotides occurs in the cytosol. However, the catalytic site of mannosyltransferase is likely to be luminal. Therefore, a GDP-Man sugar nucleotide transporter could be required in the Golgi membrane. Transporters of various nucleotide sugars are known to reside in the Golgi membrane in animal and yeast cells, and biochemical evidence has supported their presence in plants, but no plant transporter has been molecularly characterized until now. Timothy Baldwin (University of Cambridge, UK) reported upon the identification of a gene encoding a putative Arabidopsis GDP-Man transporter (*GONST1* for Golgi nucleotide sugar transporter) that shows extensive homology to *Vrg4*, a GDP-Man transporter from *S. cerevisiae* and to *Lpg2*, a GDP-Man transporter from *Leishmania* sp. His laboratory has shown by transient gene expression in onion (*Allium cepa*) epidermal cells that the GONST1 gene product is localized in the Golgi apparatus. In addition, a *GONST1* promoter::GUS reporter gene construct showed that the gene is essentially ubiquitously expressed. *GONST1* can complement *vrg4⁻* yeast, indicating that its product can transport GDP-Man in vivo. Moreover, in vitro transport assays of isolated yeast membranes containing GONST1 have increased transport of GDP-Man. Their demonstration that GONST1 is a Golgi-localized GDP-Man transporter supports the model that such transporters are required for Golgi glycosylation reactions, and open up the possibility of performing experiments to alter cell wall synthesis by altering transport of sugar nucleotides across the Golgi membrane.

Pectins, such as homogalacturonan and rhamnogalacturonans (RGs) I and II, are major components of primary cell walls. The pectic polysaccharides are structurally the most complex of the matrix polymers of plant cell walls and, as a consequence, the functions of these polymers are far from clear. Insights into the molecular biology and biochemistry of pectin have been slow to develop. No pectin biosynthetic enzyme has been purified to homogeneity and none of the enzymes involved in pectin biosynthesis have been cloned and sequenced.

Homogalacturonan is involved in a range of cell wall activities that influence cell adhesion, cell expansion, wall porosity and defense. An important aspect of this modification is the modulation of the degree and pattern of methylesterification of homogalacturonan domains. The degree and pattern of methylesterification, for example, can influence such

properties as calcium binding, gelling properties, and susceptibility to enzymatic cleavage during development and pathogenesis. The extent and pattern of methylesterification are regulated primarily by the action of wall-based pectin methylesterases that remove specific methylesters in diverse patterns. William G.T. Willats (University of Leeds, UK) reported on his laboratory's use of very well-characterized antibody probes, specific for certain methylester distribution patterns, to study the occurrence of populations of homogalacturonan types within cell wall microdomains. Grégory Mouille (Institut National de la Recherche Agronomique) also employed antipectin antibodies in a whole-mount staining procedure of Arabidopsis roots. The anti-1,4-β-galactan antibody LM5 showed an easily distinguishable staining pattern. After screening the roots of 20,000 TDNA insertion lines for altered staining patterns, 16 pectin mutant lines were isolated.

Keiko Sugimoto (John Innes Centre, Norwich, UK) reported on his exploitation of two Arabidopsis mutants, *hyp6* and *e31.10*, to explore the role of pectin polysaccharides in plant growth and development. Both mutants were initially identified in a screening for short hypocotyls from ethyl methanesulfonate-mutagenized populations. Further screening with FTIR microspectroscopy showed that both *hyp6* and *e3l.10* are altered in pectin compositions. From gas chromatography-mass spectrometry analysis of cell wall neutral sugars, they found that *hyp6* and *e31.10* are deficient in Man content and rhamnose content, respectively. Both *hyp6* and *e3l1.10* exhibit various growth defects, including strongly reduced hypocotyl elongation, immature trichome development, and suppressed root hair formation. This suggests that HYP6 and E31.10 are essential for both diffuse and tip growth. Scanning electron microscopic observations revealed that the hypocotyl epidermal cells of both mutants have severely decreased growth anisotropy and some cells undergo abnormal radial swelling. Transverse sections of hypocotyls showed these mutants have incomplete cell walls, indicating that HYP6 and E31.10 are required for proper cell wall formation.

RG-I is an abundant pectic polymer that is highly variable in structure and occurrence. J. Paul Knox (University of Leeds) made monoclonal antibodies to arabinan (LM6)1 and galactan (LM5)2 epitopes that are common components of side chains attached to rhamnose residues of RG-1. These two antibodies promise to provide important insights into the occurrence and function of these RGI-related structures in plant cell walls.

RG-II is only a minor component of the plant cell wall, but has a major effect on cell wall porosity. Borate esters form cross-links to RG-II molecules, generating a macromolecular pectic network. The formation of these cross-links results in a pectin structure with a reduced size exclusion limit. Low pH (1.5–3.0) buffers and neutral solutions of calcium chelators are known to convert the borate ester cross-linked RG-II dimer (dRG-II-B) to the RG-II monomer. These treatments also solubilize wall-bound pectin and cause an increase in the size exclusion limit of pectin-rich cell walls. The low pH and chelator-mediated effects are prevented or diminished by the addition of boric acid (1 or 10 mM). Axel Fleischer (Humboldt University, Berlin) reported that the B-dependent increase in size exclusion limit caused by acid buffers is tissue dependent and may depend on the physiological state of the cells. For example, the walls of rapidly growing suspension-cultured *Chenopodium album* cells are more sensitive to the RG-II-dissociating treatments than cells in the stationary phase. This suggests that pectin in the walls of stationary cells contain additional (secondary?) cross-links that render the pectin network less sensitive to hydrolysis of dRG-11-B. Walls isolated from a cell culture of the L-Fuc-deficient *mur-1* mutant of Arabidopsis were found to be more sensitive to low pH treatment than the walls of wild-type cells. Walls of the *mur-1* mutant and WT cells differed in the pH dependence of boric acid release, pectin solubilization, and size exclusion limit increase. These differences in wall properties are consistent with the observation that *mur-1* dRG-II-B, which contains L-Gal rather than L-Fuc residues, is less stable in vitro at low pH than wild-type dRG-II-B.

Lignins are complex phenolic polymers of plant cell walls that are linked to wood fibers. They provide hydrophobicity to xylem vessels, mechanical support to plant stems, and aid in plant defense against microorganisms. Although lignins are essential for normal plant growth and development, they are commercially undesirable because they decrease forage digestibility, and must be removed from cellulose by chemical treatments during pulping. Almost all of the genes of the lignin biosynthesis pathway have now been cloned and genetically manipulated. Lignification has three steps: (a) biosynthesis of the monolignols (e.g. *p*-hydroxycinnamyl, coniferyl, and sinapyl alcohols), (b) transport and secretion of monolignols, and (c) polymerization of the monolignols by dehydrogenation. Several contributions concerned various transgenic strategies for altering the lignification process. Zara Harrison (Institute of Food Research, Norwich, UK) reported on her group's transgenic expression in tobacco of a bacterial gene encoding the enzyme 4-hydroxycinnamoyl-coenzyme A (CoA) hydratase/lyase, which has been shown to convert *p*-coumaryl CoA, caffeoyl CoA, and feruloyl CoA to the corresponding benzaldehydes. Plants expressing the *HCHL* gene exhibited a severely altered phenotype, including some features (stunted growth, interveinal chlorosis, curling of leaf margins, and red/orange coloration) characteristic of plants with altered phenylpropanoid metabolism. A reduction in cell wall-bound

phenolics was shown to occur in the leaves and lignified tissues, and levels of intracellular soluble phenolics, such as chlorogenic acid and rutin, were reduced by 90% or more.

CELL WALL PROTEINS

Although the cell wall is largely composed of carbohydrate, structural proteins also form a network that contributes to cell wall architecture and to plant resistance to pathogen attack. Enzymes that modify cell wall structure are also important in regulating growth and development and response to the environment.

Pro-rich cell wall proteins (PRPs) are widely distributed in plants and are believed to function by modeling the architecture of the primary cell wall-surrounding specific cell types. In legumes, a subset of PRPs is specifically expressed during symbiotic root nodule formation. Jim B. Cooper (University of California, Santa Barbara) reported that PRPs are deposited as a sheet surrounding the intercellular junctions regions of cortical and vascular parenchyma tissues, and in the pit regions of tracheary elements. The pattern of PRP deposition in xylem walls is a mirror image of the pattern of lignin deposition. The inhibition of phenylpropanoid biosynthesis eliminates the normal pattern of PRP deposition, indicating that lignin deposition is required for the spatial restriction of PRPs in xylem cell walls.

Two classes of multiple-domain PRPs have been identified in Arabidopsis based on their primary sequence and domain organization. The expression of one class is limited to guard cells and the early stages of lateral root development. A second class of AT-PRPs (ATPRP1 and ATPRP3) has been localized to sites of root hair initiation. Mary L. Tierney (University of Vermont, Burlington) described experiments in which polyclonal antibodies raised against the PRP domain of ATPRP3 were used to show that ATPRP1 and ATPRP3 are localized to the cell wall during seedling growth. Immunohistochemical analysis of ATPRP3, using an epitope-tagged version of the protein expressed in transgenic plants, showed that AtPRP3 is localized to the base of the root hair and the growing tip. This pattern indicates a role for ATPRP3 in tailoring the structure of the newly formed root hair cell wall. To further characterize AtPRP3's function in root hair growth, Tierney's group generated *AtPRP3* antisense lines. They have also identified an *AtPRP3*/T-DNA insertion mutant. Seedlings homozygous for the T-DNA knockout mutation show a root hair branching phenotype, supporting a functional role for AtPRP3 in the determination and/or maintenance of root hair structure. Segregation of root hair branching is 1:1 (wild type: mutant) in F_2 seedlings generated from a backcross, suggesting that a single functional copy of *AtPRP3* may not be sufficient for proper root hair growth.

Arabinogalactan proteins (AGPs) are proteoglycans of the plant cell extracellular matrix that have been implicated in a variety of processes in plant growth and development, but their precise functions are only now beginning to emerge. Most AGPs are anchored to the plasma membrane by a glycosylphosphatidyl-inositol (GPI) anchor. Allan Showalter (Ohio University, Athens) found that LeAGP-1, a GPI-anchored AGP from tomato (*Lycopersicon esculentum*) is immunolocalized to the surface of protoplasts and detectable in plasma membrane preparations. LeAGP-1 was immunolocalized to developing metaxylem of stem and petiole, the cell walls and intercellular spaces of stylar-transmitting cells, and pollen tube tips. LeAGP-1 was also associated with cell wall thickening and lignification of particular cell types, specifically in secondary cell wall thickenings of maturing metaxylem and secondary tracheary elements in roots and stems, and in thickened walls of phloem sieve tube elements.

Arjon van Hengel (John Innes Centre) reported that one AGP (AtAGP30) is not anchored to the plasma membrane. Analyses of the expression pattern revealed that the gene transcript is only detectable in the root tip of the seedling, and that stress conditions and hormone applications alter *AtAGP30* expression. The overexpression of *AtAGP30* leads to normal root development, but poor shoot development. A knockout mutant revealed a decrease in sensitivity to Yariv reagent. Analysis of cell walls revealed that walls from *Atagp30* mutants are chemically distinct from wild types, and that mutant root tips contain lower amounts of arabinogalactan and protein. It is interesting that *Atagp30* mutants germinated faster on medium containing abscisic acid than did wild type.

Carolyn Schultz (University of Melbourne) identified four different classes of *AGP* genes. One of these classes encodes AG peptides with protein backbones of between 10 and 13 amino acid residues. Most of these AG peptides, with the exception of AtAGP16, are predicted to be anchored to the plasma membrane by a GPI anchor. To determine whether AtAGP16 is processed before the addition of a GPI anchor, Schultz's laboratory purified AGPs from Arabidopsis tissues for deglycosylation. Protein backbones were then analyzed by matrix assisted-laser disorption ionizing-time of flight mass spectrometry and/or sequencing by Edman degradation. These experiments confirmed the posttranslational processing of the AG peptides and helped determine the cleavage site (ω) residue for the addition of the GPI anchor for each AG peptide. Gene-specific probes for several of the AG-peptide genes are being used in in situ hybridization experiments to determine if genes are expressed in multiple cell types within a tissue or whether they are restricted to a single cell type. Her laboratory is also studying the expression of the genes using the publicly available Arabidopsis micorarray data. This has enabled them

to investigate the expression of these genes in the greater than 100 different developmental, mutant, and environmental conditions tested so far.

Kim Johnson (University of Melbourne) reported on a new class of Arabidopsis proteins that have β-Ig-H3/fasciclin-like domains in addition to AGP-like domains. Similar to their animal counterparts, the fasciclin-like AGPs of plants may act as cell adhesion molecules and function in cell-to-cell communication.

Azeddine Driouch (Université de Rouen, France) provided evidence that alterations in AGP may underlie the altered phenotype of the *reb1-1* (root epidermal bulger) mutant of Arabidopsis. This mutant is characterized by reduced root elongation rates and by the bulging of trichoblasts. It is interesting that a similar phenotype can be obtained by growing Arabidopsis seedlings in the presence of (β-D-Glc3) Yariv reagent, which is known to bind to AGPs. In addition, *reb1-1* mutants contain only 30% as much AGPs as do the wild type. In the root elongation zone, antibodies against AGP epitopes scarcely stain the trichoblasts of the mutant, but strongly stain the trichoblasts of wild-type plants. Moreover, the cell walls of the trichoblasts of the mutants are only one-half as thick as the cell walls of the wild-type. Georg Seifert (John Innes Centre) reported that *reb1* is identical to a previously described *rhd1* mutant, and determined that it is a member of the UDP-Glc 4-epimerase family. He presented evidence that *RHD1/REB1* may act in planta in the biosynthesis of D-Gal in roots. This suggests the involvement of cell wall galactans and arabinogalactans in the control of cell volume and cell elongation.

Clare Steele-King (University of Leeds) identified a *Yariv super-sensitive* (*yss*) mutant that has a pH-dependent phenotype. When *yss* mutants are grown in the presence of Yariv reagent, their inhibition of growth is even more dramatic than that seen in wild-type seedlings exposed to Yariv reagent. Immunochemical studies indicate that *yss* has an altered AGP composition. Yariv reagent also increases the staining of both *yss* and wild-type seedlings by a monoclonal antibody that recognizes pectin galactan, suggesting an interaction between pectin and AGP.

Yolanda Gaspar (University of Melbourne) reported on her identification of a number of Arabidopsis mutants with T-DNA insertions in or around the coding region of the classical AGP proteins backbone using a PCR based approach. One of these mutants was identified independently based on its resistance to *A. tumefaciens rat1* (resistant to *A. tumefaciens* transformation).

Hyp-rich glycoproteins, such as extensin, are small peptide repeats that are extensively *O*-glycosylated at their Hyp residues. The Hyp contiguity hypothesis is based on correlations between Hyp oligoarabinosylation and blocks of contiguous Hyp residues, and predicts Hyp galactosylation and subsequent arabi-

nogalactan polysaccharide addition to clustered non-contiguous Hyp. One of the highlights of the meeting was Marcia Kieliszewski's (Ohio University) presentation in which she reported results that confirmed that amino acid sequences can be used to predict the glycosylation profiles of cell wall proteins such as AGPs and extensins. She described how using a series of simple Hyp-rich glycoproteins, her laboratory was able to identify the first *O*-glycosylation code of plants and provided several examples illustrating its use for predicting glycoprotein structure from a gene.

Expansins are a large multigenic class of proteins that are thought to aid in cell wall extension during growth by disrupting hydrogen bonds at the interfaces between individual cell wall polymers. Previous studies have implicated expansins in diverse processes, including cell growth and polarity, root hair formation and growth, organogenesis and fruit ripening. Research from Hans G. Edelmann's laboratory (University of Bonn) provided new insights into the subcellular and tissue-specific localization of expansins in maize. The authors noted great diversity in the labeling patterns depending upon the type of expansin antibody used. One type labeled cell plates in the meristem and pitfields in the elongation region, whereas another recognized longitudinal cell walls throughout growing root apices and tips of young root hairs. A third type produced strong cytoplasmic labeling that accumulates predominantly with the bulging areas associated with emergent root hairs. These findings indicate that different expansins are found at unique subcellular locations, suggesting that they may have different physiological functions.

Endoxyloglucan transferases (XETs) are a class of enzymes that cut and rejoin xyloglucan chains. For expansion to occur in plant cells, cellulose microfibrils need to move past one another. It is believed that wall loosening results when xyloglucan, the major hemicellulose in the cell wall and capable of tethering adjacent microfibrils, is modified. During this process, XETs play a central role in the construction and modification of cell wall architecture. XETs are encoded by a large multigene family termed xyloglucan-related proteins and are classified into three or four subfamilies with respect to their deduced amino acid sequences. The completion of the Arabidopsis genome project allowed Kazuhiko Nishitani (Tohoku University, Japan) and his coworkers to characterize this family in detail. They identified 33 open reading frames that putatively encode for XETs. Each member of this family exhibits a distinct expression profile in terms of both organ specificity and responsiveness to hormonal signals. In situ hybridization and promoter::GFP gene constructs of select members of these 33 open reading frames revealed strict and specific cell-type expression patterns within respective organs. These facts

imply that each member of this gene family is individually committed to a certain specific process that is regulated by different hormones in a specific tissue at a specific point in development.

Ryusuke Yokoyama (Tohoku University) presented data concerning the secretion pathways of XET in the tobacco Bright-Yellow 2 cell system. In the cell enlargement process, which occurs mainly during the interphase of the cell cycle, the protein was extensively secreted into the apoplast via the ER-Golgi apparatus network. However, as the cell progressed to mitosis, the apoplast-directed transport was diminished, and the protein was then exclusively located in the phragmoplast and eventually transported to the cell plate. These results indicate a role for XET in both the construction of the cell plate and the cell wall, and also suggest that the existence of a cell-cycle dependent switching mechanism that precisely controls the direction of the delivery of secretory vesicles containing the same protein.

Kris Vissenberg (University of Antwerp, Belgium) developed a fluorescent technique where active XET incorporates fluorescent substrates into the cell wall. In Arabidopsis roots, high XET activity was confined to the elongation zone and is up-regulated at the site of future root hair emergence. Closer examination revealed that the fluorescence in the cell wall exhibited a fibrillar pattern, reminiscent of the pattern of cortical microtubules and of cellulose microfibrils in the cell wall. Interference with cellulose deposition greatly reduced the fluorescence and the fibrillar pattern, whereas application of oryzalin resulted in the loss of the parallellism in the fibrillar pattern. Latrunculin B on the other hand decreased the fluorescence, but failed to abolish the fibrillar pattern. From these results, the authors concluded that XET probably has different roles in the cell wall. Besides a potential role in cell wall loosening, XETs are important in the buildup and restructuring of xyloglucans in the cell wall. It is most likely that expansin is the primary cell wall-loosening factor, but needs the concerted action of active XETs and endoglucanases to accomplish its task.

MECHANISMS OF CELL GROWTH

Both Peter Schopfer (University of Freiburg, Germany) and Stephen C. Fry (University of Edinburgh, UK) presented papers relating to the role of hydroxyl radicals in cell wall loosening and cell growth. Wall-localized reactions may lead to the production in the apoplast of ($\cdot$OH) hydroxyl radicals, a highly reactive species that can cause nonenzymic scission of polysaccharides. Such scission may loosen the wall and thus promote cell expansion or fruit softening. To provide a method for detecting the proposed action of endogenous $\cdot$OH radicals in vivo, Fry's laboratory devised a method for "fingerprinting" the products formed when plant cell wall polysaccharides are at-

tacked by $\cdot$OH. Treatment of wall polysaccharides with $\cdot$OH radicals prompted oxidative scission and simultaneously increased the number of NaB^3H_4-reacting groups present. The latter are proposed to be glycosulose and glycosulosuronate residues that are NaB^3H_4-reducible back to the original sugar residue and to their more unusual epimers. Driselase- or acid-catalyzed hydrolysis of the $\cdot$OH-treated polymers gave characteristic "fingerprints" of radiolabeled products seen after chromatography and electrophoresis. Products from tamarind xyloglucan included [^{3}H]isoprimeverose, [^{3}H]Gal, [^{3}H]Glc, [^{3}H]Ara, [^{3}H]Rib, and [^{3}H]Man. Those from citrus pectin included [^{3}H]Gal, [^{3}H]GalA, and at least seven unidentified acidic products, probably including those derived from [^{3}H]TalA and [^{3}H]GulA. The patterns of ^{3}H-products are useful fingerprints by which polysaccharides that have been $\cdot$OH attacked during wall loosening in vivo may be recognized. Applied to maize coleoptile cell walls, the method showed no evidence for $\cdot$OH radical attack on polysaccharides during auxin-induced wall loosening. However, applied to the walls of ripening pear (*Pyrus communis*) fruit, the method demonstrated a steady increase in NaB^3H_4-reducible groups during the softening process. In contrast to Fry's findings, Schopfer presented evidence that oxygen radical chemistry may also play a role in polymer breakdown during auxin-induced extension growth. Backbone cleavage of cell wall polysaccharides can be accomplished in vitro by $\cdot$OH produced from hydrogen peroxide in a Fenton reaction or in a reaction catalyzed by peroxidase supplied with O_2 and NADH. They presented evidence that coleoptile growth of maize seedlings is accompanied by the release of reactive oxygen intermediates in the cell wall. Auxin promotes release of $O_2^{\cdot-}$ and subsequent generation of $\cdot$OH when inducing elongation growth. Experimental generation of $\cdot$OH in the wall causes an increase in wall extensibility in vitro and replaces auxin in inducing growth. Auxin-induced growth can be inhibited by scavengers of $O_2^{\cdot-}$, hydrogen peroxide, or $\cdot$OH, or inhibitors interfering with the formation of these molecules in the cell wall. These results support the idea that $\cdot$OH produced by peroxidase acts as a wall-loosening agent during auxin-induced growth.

METHODS AND MODELS: HIGH-VOLTAGE ELECTRON TOMOGRAPHY

Until recently, the analysis of cellular structures at the electron microscopical level was limited by both specimen preparation methods and by image analysis problems. For example, when cells are preserved by chemical fixatives, the preservation of different types of cellular structures in their native state is limited both by the slow rate of cross-linking reactions and by the selective nature of the cross-links

that are formed. On the other hand, the deciphering of three-dimensional cellular structures is limited by the thickness of the serial thin sections used for the reconstructions. L. Andrew Staehelin's (University of Colorado, Boulder) research laboratory has overcome both of these limitations by combining cryofixation/freeze-substitution methods in conjunction with dual-axis high-voltage electron tomography of serial thick sections (0.25–0.4 pm). This new methodology produces tomographic slices that look like electron micrographs but are only 2.3 nm thick versus 60 to 80 nm for normal thin sections. Overall, the three-dimensional resolution in these reconstructed specimens is about 6 nm, which enables them to see and identify large molecules such as clathrin triskelions, dynamin spirals, and kinesin motor proteins within the thick sections. By tracing the outlines of cellular structures in the individual 2.3-nm slices they can also produce high resolution, three-dimensional models of membrane compartments and cytoskeletal systems.

NMR studies, mostly from the laboratory of Mike Jarvis (Glasgow University, UK), are providing new details of the submolecular organization of cell walls. For example, at least four polymorphs of cellulose exist, namely cellulose I to IV. The native form, cellulose I, and the mercerized or regenerated form, cellulose II, are the two most common polymorphs, cellulose II being the most stable crystalline form. Native cellulose contains two allomorphs, Iα and Iβ, the proportions of which are dependent on the organism in which it is synthesized. There is evidence that the cellulose microfibrils of higher plants have a crystalline core of cellulose Iα, Iβ or both. Microfibrils from the textile celluloses cotton, flax (*Linum usitatissimum*) and ramie (*Boehmeria nivea*) are larger than those from other higher plants, and contain only 20% to 30% of surface chains rather than the normal figure of 60% to 70%. Taking linear combinations of the solid-state ^{13}C NMR spectra of flax and celery (*Apium graveolens*) cellulose, Jarvis and his coworkers extracted the complete ^{13}C spectrum of the surface chains, which differed considerably from the spectra of the Iα and Iβ forms. They also extracted the spectrum of the surface chains in an independent way by ^{13}C spin relaxation editing, taking advantage of their greater mobility than the chains in the crystalline core of the microfibrils. A good correspondence was observed between the spectra obtained by these two approaches. They showed that the surface chains had a mixture of two conformations at C-6, both different from the *trans*-gauche conformation found in crystalline cellulose Iα and Iβ. Neither of the C-6 conformations in the surface chains permits the formation of the intramolecular hydrogen bond from O–2 that stabilizes the chain conformation in crystalline celluloses. This implies that H bonding to other polysaccharides or water is possible for both O6-H and O2-H in the surface chains. The spin relaxation editing experiments also provided information on the degree to which each C atom in the surface chains was freed from the rigid network of H bonds that hold the core chains together. More direct information on H bonding was obtained through an [1]H-^{13}C heteronuclear correlation experiment. This allowed the ^{1}H NMR spectrum of the hydroxyl protons in cellulose to be extracted. Because the ^{1}H spectra of hydroxyl protons depend principally on the strength of H bonding, this experiment provides a novel way to elucidate H bonding patterns in the solid state.

FTIR spectroscopy is becoming more commonplace as a means to identify mutations that affect plant cell wall polysaccharide components and wall architecture. Nick Carpita's laboratory (Purdue University, West Lafayette, IN), for example, has identified a broad range of genes involved in the biogenesis and dynamic alteration of plant cell wall architecture during growth and development. Among the mutations his group has been able to identify so far are detects in formation of nucleotide-sugar substrates, in polysaccharide synthases that make polymer backbones, in glycosyl-transferases that add side groups, in secretion and targeting, in cytoskeletal orientation that directs wall architecture, in assembly of wall polysaccharide components, in wall dynamics during growth, and in wall disassembly and recycling of hydrolyzed monosacchandes. The FTIR "forward" screen can be used to detect specific alterations in wall structure and architecture caused by chemical mutagens or by the insertion of DNA "tags," regardless of the genetic basis of the alteration. In this respect, the screen is powerfully selective at the end-product level. They are also using reverse genetic approaches to identify cell wall biogenesis-related genes in Arabidopsis and maize. It is anticipated that this approach will provide a broad set of cell wall mutants that will serve to further the development of libraries of FTIR "spectrotypes" that will be useful for determining gene function. They have devised a systematic protocol, which employs biochemical, spectroscopic, and imaging methods, to categorize defects in wall structure and architecture into one of six stages of wall biogenesis or disassembly. A major practical goal is to generate plants with genetically defined variation in composition and architecture to permit assessment of modifications on wall properties and plant development. Several of these genes, as well as several of the plants with genetically defined alterations, may be of economic importance. Examples include the modification of pectin-cross-linking or cell-cell adhesion to increase shelf life of fruits and vegetables, the enhancement of dietary fiber contents of cereals, the improvement of yield and quality of fibers, and the relative allocation of carbon to wall biomass for use as biofuels.

FTIR spectroscopy was also used by Mike Jarvis (Glasgow University) to obtain ordinary and polarized spectra in the hydroxyl-stretching region of flax

cellulose I and cellulose II samples. At the same time, spectra obtained on surface-deuterated samples in the polarized IR beam enabled them to distinguish the behavior of internal chains from the behavior of surface chains. It is possible, by comparison of the experimental spectra with modeled FTIR spectra, to gain information about the distribution of orientations of the intrachain and interchain hydrogen bonds. This information is relevant to the H-bonding arrangements that attach surface cellulose to interior cellulose.

The mechanical properties of cell walls are relevant for understanding how cells and tissues grow and function. Their stiffness properties generally include elastic and viscous components, and are non-linear with strain. David M. Bruce and his coworkers (Silsoe Research Institute, Bedford, UK) have taken a modeling approach toward understanding the mechanics of plant tissue and cell walls, based on describing the wall as a structure rather than a material. Potato tuber tissue was employed as a model system. These fluid-filled cells with high adhesion, have been shown to be incompressible up to 22% tissue strain over time scales of up to 15 s. Given an imposed deformation in one direction, the constancy of volume of each cell allows its dimensional changes in the other directions to be calculated. Within the tissue, the planar wall faces have been found to remain planar during deformation, but their shape has been observed to change as a function of the orientation of the walls relative to applied strain. Therefore, within the plane of a wall, the strain must be a function of direction. Key assumptions of their model are that cellulose microfibrils, the tensile elements in the wall, resist only tension and offer insignificant resistance to re-orientation or shortening. As a result of deformation imposed on the tissue, microfibrils, whatever their initial orientation in the wall, will become re-oriented toward alignment with wall tension. Their results suggest that microfibrils initially aligned with wall tension fail at a calculated strain of some 8%. This allows wall extension that brings other microfibrils more into alignment, and hence into play to resist wall extension. Using measured values of cell wall size and shape, and microfibril dimensions from electron micrographs, they deduced the shape of a stress-strain curve for a microfibril up to incipient failure of the tissue. Microfibril modulus (a maximum of 130 MPa) and strength (7.5 GPa) align well with values for cellulose in the literature.

Laurence Melton (University of Auckland, New Zealand) discussed the value of celery cell walls as a model for cell wall architecture. The parenchyma cell walls of celery were investigated using solid-state ^{13}C NMR, x-ray diffraction, and atomic force microscopy. Analyses indicated that the walls consist mainly of cellulose (42 mol %) and pectic polysaccharides (51 mol %), and contain a remarkably low level of xyloglucan (2 mol %) and xylan (2 mol %); hence, this is a good model system for studying the physical relations of cellulose microfibrils and pectic polysaccharides. Solid-state ^{13}C NMR results indicate the walls exist in three domains: rigid (cellulose microfibrils), semirigid (galacturonan), and mobile (arabinan and galactan). There was no evidence for non-cellulosic polysaccharides adhering to the surface of the cellulose microfibrils. Their results support a cell wall model in which cross-linking of cellulose microfibrils by individual non-cellulosic polysaccharides, such as xyloglucan, is neither expected nor necessary.