COMPOSITION AND ARCHITECTURE OF THE CELL WALLS OF
GRASSES AND THE MECHANISMS OF SYNTHESIS OF CELL WALL
POLYSACCHARIDES

Final Report

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Introduction

A 13-year continuously supported program was completed this year. The program was devoted toward complete understanding of the polysaccharide structure and architecture of the primary cell walls grasses and cereals, and the biosynthesis of the mixed-linkage (1→3),(1→4)β-D-glucan (hereafter, called β-glucan), a cellulose interacting polymer that is synthesized uniquely by grass species and close relatives. With these studies as focal point, the support from DOE was instrumental in the development of new analytical means that enabled us to characterize carbohydrate structure, to reveal new features of cell wall dynamics during cell growth, and to apply these techniques in other model organisms. The support by DOE in these basic studies was acknowledged on numerous occasions in review articles covering current knowledge of cell wall structure, architecture, dynamics, biosynthesis, and in discovery of genes related to cell wall biogenesis. This final report summarizes this work in several sub-sections representative of the spectrum of studies undertaken.

The principal focus of the work has always been devoted to the biosynthetic mechanisms of the synthesis *in vitro* of β-glucans. They are cell wall polysaccharides unique to cereals and constitute almost 70% of the wall mass in the grains of some cereals, such as oats and barley. In contrast, maize and rice are notably β-glucan-poor. The β-glucans are the causal agents in the ability of oat and barley brans to lower serum cholesterol and to reduce the insulin requirement of diabetics. The polymer is made transiently. Not found in the meristem it begins to be synthesized when cells enlarge and is degraded extensively during further growth and differentiation. The β-glucan appears again in the cereal grain, but accumulates to a significant extent only in certain species. Our identification of the synthases and the enzymes that degrade β-glucan will be instrumental for us to use molecular engineering technologies to increase β-glucan content of cereal grains that are notably glucan-poor, such as rice and maize. Flours made from these glucan-enriched maize and rice grains will not only contain a nutritious dietary fiber, but they may also alter flour textures and baking textures that will create new uses of the flours for the food industry.

1. The composition and architecture of the primary cell walls of grasses, and cell wall dynamics during cell growth

Using the maize coleoptile as a biochemical model of cell elongation, we had reported the fine details of the changes in cell wall composition and architecture of the wall during the transition from meristematic activity to elongation and to cessation of growth. To summarize these findings, we found that β-glucan was essentially absent at the onset of coleoptile elongation, but quickly accumulated to as much as 20% of the total cell wall mass during maximal rates of elongation. The β-glucan was largely hydrolyzed and disappeared from the wall during cessation of growth and senescence. Concomitantly, we discovered a highly substituted glucuronoarabinoxylan (HS-GAX) was also made during maximal rates of cell elongation, and the loss of arabinose resulted in more tenacious binding the relatively unbranched xylans to cellulose *in vivo* and *in vitro*. Cessation of growth resulted in both extensive loss of arabinose and an enrichment in aromatic substances, primarily esterified and etherified residues of the hydroxycinnamic acid, ferulate.

In collaboration with Peter Kaufman (Univ. Michigan), we specifically found the graviresponse of the leaf-sheath pulvinus of oat (*Avena sativa*) involves an asymmetric growth response accompanied by several asymmetric processes, including degradation of starch and synthesis of cell wall (Gibeaut *et al.* 1990). To further understand the cellular and biochemical events associated with the graviresponse, changes in cell walls and their constituents and the activities of related enzymes were investigated in excised pulvini. Asymmetric increases in dry weight with relatively symmetric increases in wall weight accompanied the graviresponse. Starch degradation could not account for increases in wall weight. Most of the cell wall components increased proportionately during the graviresponse. However, β -glucan did not increase symmetrically, but rather increased in proportion in the faster growing lower halves of gravistimulated pulvini. These data were consistent with our observation of β -glucan increases during maximal rates of cell elongation in coleoptiles. Our data showing the accumulation of β -glucan as relevant to growth contrasted data from other laboratories indicating it was the hydrolysis of β -glucan load-bearing linkages that resulted in wall extension.

This debate continues today, but two of our more recent studies add considerable evidence that it is the *presence* of β -glucan that is growth permissive, not its hydrolysis and loss from the wall. First, in Kim *et al.* (2000) we describe the molecular cloning of a cell-wall exo- β -D-glucanase [described in more detail below in section 5.]. This enzyme specifically removes terminal glucose from fragments of β -glucan cleaved from the wall by an endo-glucanase. The enzyme activity and its specific transcript appear after maximal elongation rates and is associated with the disappearance of the glucan but *not* the rate of growth. Second, our studies of wall architecture (Carpita *et al.* 2001), which were just published in Plant Physiology, show the β -glucan to be thickly coating the microfibrils in growth-relevant regions of the wall and disappearing from the wall after growth. Together with colleagues at the John Innes Centre and the Institute of Food Research, Norwich, U. K., we developed the use of field-emission scanning electron microscopy (FESEM), immunocytochemistry, and Fourier transform infrared (FTIR) microspectroscopy to determine how these polymers are assembled in the primary walls of elongating maize coleoptiles (Carpita *et al.* 2001). Oxidation of the phenolic network followed by dilute alkali extraction resulted in removal of most of the GAXs. Such a treatment widened the pores substantially and permitted observation by FESEM of up to six distinct microfibrillar lamellae. β -Glucans remaining in the wall after this treatment were removed with a specific endo- β -D-glucanohydrolase. Sugar and linkage analyses of isolated cell types were used to define the polymers removed by sequential chemical extraction and enzyme digestion. Using this information, we were able to assign IR signals corresponding to carboxylic and phenolic esters, GAXs, β -glucans, glucomannans, and other cellulosic and non-cellulosic polysaccharides. Chemical imaging by microspectroscopy was then used to localize these molecules in tissue sections. We found an enrichment of phenolic substances in the vascular tissue, while glucomannans and esterified uronic acids were enriched in the epidermis to a greater extent than in the mesophyll cells. In contrast, β -glucan signals were more abundant in the mesophyll cells. The tissue-specific differences in abundance of β -glucan and pectic esters were confirmed by immunocytochemistry in the electron microscope and quantitative biochemical assays. These data allow us to define tissue-specific architectural frameworks for maize primary cell walls in the complex tissues of organs. Our work markedly alters our understanding of the specific spatial orientation of β -glucan around microfibrils.

We developed novel ways to trace the biosynthesis of the cell wall in intact cells and plants and used these techniques to demonstrate the selective turnover and alteration of cytoplasmic and cell wall polysaccharides of proso millet cells in liquid culture and maize seedlings (Gibeaut and Carpita 1991). Cells of proso millet in liquid culture and intact leaves of developing maize seedlings readily absorbed [U-¹⁴C]-D-glucose or [U-¹⁴C]-L-arabinose and incorporated a substantial amount of the radioactivity into cell wall polymers. Because of a salvage pathway that yields only nucleotide-pentoses, radioactivity from [U-¹⁴C]-L-arabinose accumulated in polymers containing arabinose and xylose. The fates of these radioactive polymers were followed for several hours or days. Using radiogas-proportional counting, we were able not only to follow the flux of carbon through individual cell-wall sugars, but through individual linkages! During pulse-chase of proso millet in liquid culture with arabinose, much of the radioactivity in soluble polymers disappeared, and there was a concomitant incorporation of radioactivity into the cell wall. Radioactivity incorporated into maize seedlings which were pulsed with [U-¹⁴C]-L-arabinose or [U-¹⁴C]-D-glucose when only the first primary leaf had expanded, permitted observation of cell wall metabolism in developing tissues for four days. Although disappearance of total radioactivity from the cell wall was not substantial, there were marked changes in the distribution of radioactivity in several sugars. Our data also demonstrated a constant turnover and recycling of several sugars from cell wall polymers during development. Although several polymers were synthesized throughout the four-day incubation period, over 60% of the label accumulated in the first hours of incubation was lost from this pool. These data provided the first evidence for turnover of specific polymers and sugars from growing tissue *in vivo*. The data also indicated that arabinose is selectively hydrolyzed from GAX, and recycling of the arabinose from the cell wall to cytosol results in concomitant accumulation of label in the xylosyl units. The change in ratio was most marked in fraction in which accumulation of label in xylose was coincident with marked reduction in substitution of the GAX. Water-soluble arabinogalactans are the most abundant polymers of the cytosolic fraction and Golgi vesicles, and their selective turnover was demonstrated. This finding provided further evidence for these molecules to be "glycochaperones" for cell-wall polymers during secretion and assembly onto cellulose at the cell surface (Carpita *et al.* 1996).

In collaborative work with Dr. Maureen McCann (John Innes Centre, U.K.) we developed Fourier transform infrared microspectroscopy as a means to determine cell-wall composition and architecture (McCann *et al.* 1997). These techniques were used to provide discrimination of polymer abundance in the walls of the maize coleoptile by chemical imaging (Carpita *et al.* 2001).

2. Novel techniques of polysaccharide analysis

Replacement of carbazole with *meta*-hydroxydiphenyl greatly improves determination of uronic acids in the presence of neutral sugars by preventing substantially, but not completely, the browning that occurs during heating of sugars in concentrated sulfuric acid and avoiding the formation of additional interference by the carbazole reagent. However, interference is still substantial when uronic acids are determined in the presence of excess neutral sugar, particularly because of the browning that occurs during the first heating before addition of the diphenyl reagent. The browning can be essentially eliminated by addition of sulfamate to the reaction mixture. Although others have reported that sulfamate and the diphenyl reagent were incompatible, we find that a small amount of sulfamate suppresses color production by 20-fold

excess of some neutral sugars without substantial sacrifice of the sensitive detection of uronic acids by the diphenyl reagent (Filisetti-Cozzi and Carpita 1991). Sodium tetraborate is required for detection of D-mannuronic acid and enhances color production by D-glucuronic acid. We proposed this modified sulfamate/*m*-hydroxydiphenyl assay as a rapid and reliable means for the assay of uronic acids, particularly when present in much smaller amounts than neutral sugars.

After derivatization of polysaccharides for glycosyl linkage analysis, considerable contamination is often present. We have devised a simple phase partition system which extracts virtually all phthalate esters and baseline contaminants into a carbon tetrachloride phase, but yields greater than 95% recovery of partially methylated alditol acetate derivatives in a 40% methanol-water phase (Gibeaut and Carpita 1992).

3. The biosynthesis of β -glucans and other polymers of the walls of cereals

Because the β -glucan was unique to the cereals and because its presence was associated with cell elongation, we launched a program to investigate the mechanism of synthesis *in vitro* of this polysaccharide. We began by making a substantial improvement in the separation of Golgi from contaminating membranes by flotation centrifugation (Gibeaut and Carpita 1990, 1994b). Membranes from etiolated maize seedlings were isolated using sucrose gradients for *in vitro* studies of polysaccharide synthesis. Following downward centrifugation, flotation centrifugation improved the purity of membrane fractions, in particular the Golgi apparatus. Based on naphthylphthalamic acid binding to plasma membrane and inosine-5'-diphosphatase activity in Golgi apparatus, flotation centrifugation removed about 70% of the plasma membrane which cosedimented with the Golgi apparatus in downward centrifugation. The addition of chelators during flotation centrifugation allowed separation of the Golgi apparatus from endoplasmic reticulum, as indicated by NADH cytochrome c reductase activity. Glucan and xylan synthase activities were measured as the radioactivity incorporated from either UDP- ^{14}C -glucose or UDP- ^{14}C -xylose into 80% ethanol insoluble materials. Glucan synthase activity at a substrate concentration of 1 mM UDP-glucose without CaCl_2 was greatest in fractions enriched in Golgi apparatus, but in the presence of 3 mM CaCl_2 the activity was greatest in fractions enriched in plasma membrane. Glucan synthase activity at a substrate concentration of 10 mM UDP-glucose in the presence of 3 mM MnCl_2 was greatest in fractions enriched in plasma membrane, but was also high in fractions enriched in Golgi apparatus. Xylan synthase activity, at a substrate concentration of 1 mM UDP-xylose in the presence of 3 mM MnCl_2 , was greatest in fractions enriched in Golgi apparatus. To further characterize these synthase reactions, the glycosyl linkages of the products formed were analyzed with a gas chromatograph coupled to a radiogas proportional counter. With the substrate, UDP- ^{14}C -glucose, and fractions enriched in Golgi apparatus, both (1 $\rightarrow$ 3)- and (1 $\rightarrow$ 4)-radioactive glucosyl linkages were formed, whereas the main linkage formed by fractions enriched in plasma membrane was (1 $\rightarrow$ 3)-glucosyl. With the substrate, UDP- ^{14}C -xylose, mostly (1 $\rightarrow$ 4)-xylosyl and some terminal-xylosyl linkages were formed by fractions enriched in Golgi apparatus. Only xylan synthase activity copurified with Golgi apparatus and, because plasma membrane lacked this activity, xylan synthase may be used as an indicator of Golgi apparatus.

Membranes of the Golgi apparatus from maize (*Zea mays* L.) were used to synthesize *in vitro* the mixed-linkage (1 $\rightarrow$ 3),(1 $\rightarrow$ 4) β -D-glucan (β -glucan) (Gibeaut and Carpita 1993a,b). The β -glucan was about 250 kDa, and was separated from a much larger (1 $\rightarrow$ 3) β -D-glucan (callose) by gel-permeation chromatography. Diagnostic oligosaccharides, released by a sequence-

dependent endoglucanase from *Bacillus subtilis*, were separated by HPLC, and by GLC. The trisaccharide, β -D-Glcp-(1 $\rightarrow$ 4) β -D-Glcp-(1 $\rightarrow$ 3)-D-Glc, the tetrasaccharide, [β -D-Glcp-(1 $\rightarrow$ 4)]₂- β -D-Glcp-(1 $\rightarrow$ 3)-D-Glc, and longer cellodextrin-(1 $\rightarrow$ 3)-D-Glc oligosaccharides were synthesized in proportions similar to those found in purified β -glucan. Activated charcoal, added during homogenization, enhanced synthesis of β -glucan presumably by removing inhibitory compounds. Synthesis was localized to the Golgi apparatus by a combination of downward and flotation centrifugations on sucrose step gradients. The rate of synthesis did not saturate at up to 10 mM UDP-Glc. Chelators completely abolished synthesis, but synthase activity was restored by addition of either MgCl₂ or, to a lesser extent, MnCl₂. Synthesis continued well over one hour, but addition of KOH to raise the pH from 7.2 to 8.0 during reactions increased the rate, indicating that a transmembrane pH gradient may facilitate synthesis of β -glucan. Short-lived activities of several polysaccharide synthases have been demonstrated previously, but this was the first stable synthesis *in vitro* of an authentic plant cell-wall polysaccharide made in the Golgi apparatus (Gibeaut and Carpita 1993).

We used two strategies to try to identify the β -glucan synthase. First, we developed a new affinity label, a dialdehyde of UDP generated by periodate oxidation of the ribosyl group. Affinity labels others have used relied on derivatization of the uridine, and these labels have not yielded labeled synthase proteins. We prepared the dialdehyde probe from [³⁵S]-thio-UDP, which we synthesize from [³⁵S]-thio-UTP in a phosphotransferase reaction *in vitro*, and find several polypeptides, particularly a 55 kDa, 65 kDa, and 98 kDa trio, that label in a Mg²⁺-dependent manner and whose labeling is competed by UDP-Glc. However, non-specific labeling was still quite high, and in light of the difficulty in differentiating minor bands that might represent the true β -glucan synthases, we have abandoned this approach until we have candidates for the β -glucan synthase catalytic subunits from our molecular cloning strategy.

In parallel studies, we have developed procedures for convenient large-scale isolation of the maize Golgi apparatus and have identified, by 2-phase detergent-aqueous partitioning, 3 major intrinsic Golgi-membrane polypeptides of molecular mass 47 kDa, 38 kDa, and 31 kDa. Five additional bands are observed at 35 kDa, 53 kDa, 61 kDa, 125 kDa, and 205 kDa, and we are scaling up the preparations to obtain enough protein for microsequencing. Two of these were abundant enough to obtain N-terminal amino acid sequence from a single run, and we are continuing to obtain protein for internal sequence. Our data indicate that the 31 kDa intrinsic protein shares homology with the tonoplast intrinsic protein, γ -TIP, and other membrane intrinsic proteins (MIPs), or "aquaporins", a large family of proteins thought to function in water transport across membranes. The 47 kDa polypeptide is another form of the MIP. Several plasma membrane and tonoplast MIPs have been identified and cloned, but by virtue of the size difference they are different from the Golgi proteins we identified. The second polypeptide of 38 kDa shares homology with internal sequence of a human Ca²⁺-channel protein. This could be an interesting protein related to the movement of secretory vesicles (unpublished work).

Delmer and her colleagues (Delmer and Amor, *Plant Cell* 7,987-1000 (1995); Amor *et al.* *Proc. Natl. Acad. Sci USA* 92, 9353-9357) reported that an 84 kDa protein, which was associated with the plasma membrane and affinity-labeled with UDP-Glc, was sucrose synthase and not a callose synthase. They showed that a large proportion of the sucrose synthase was bound to the plasma membrane and not the cytosolic fraction. They proposed that sucrose synthase was tightly associated with callose (cellulose) synthase where it provide a source of glucose as UDP-Glc directly from imported sucrose. From our observations of callose synthase associated with the Golgi apparatus of maize and the reaction conditions of β -glucan synthase and the callose

synthase, we proposed that the β -glucan synthase unique to the grasses may have arisen from an ancestral cellulose synthase gene [Gibeaut and Carpita 1994a,b). Similarly, sucrose synthase, as detected by antibodies (kindly provided by Dr. Prem Choury, Univ. Florida), is associated with both the Golgi and plasma membranes of maize shoots. Experiments in the last year of this project will focus on a possible linkage between sucrose synthase and the intrinsic Golgi membrane proteins and/or β -D-glucan synthase.

We then examined in greater detail the biochemical mechanism of synthesis in vitro of β -glucan). As mentioned earlier, about 90% of the β -glucan is composed of cellotriosyl and cellotetraosyl units linked by single (1 $\rightarrow$ 3) β -D-linkages, with the remainder composed of cellodextrins of DP 5 to 9 spaced between the (1 $\rightarrow$ 3)-linkages. Whereas the ratio of cellotriosyl units to cellotetraosyl units in the native polymer is strictly controlled between about 2 and 3, the ratios of these units in β -glucan formed in vitro vary from 1.5 at micromolar concentrations of UDP-Glc to over 11 when the substrate concentration is elevated to 30 mM (Buckeridge *et al.*, 1999, 2001). These results support a model in which three sites of glycosyl transfer occur within the synthase complex to produce the cellobiosyl-(1 $\rightarrow$ 3)-D-glucosyl units. We propose that the third site is of lower affinity so that failure to fill that site results in the iterative addition of one or more cellobiosyl units to produce the longer cellodextrin units in the polymer. Experiments designed to decrease UDP-Glc concentration in excised sections of maize coleoptiles did not result in wide variations in the ratios of cellotriosyl and cellotetraosyl units in β -glucan synthesized in vivo, indicating that other factors control delivery of UDP-Glc to the synthase. Our immunolocalization of sucrose synthase at Golgi membranes indicates that this enzyme may be involved in this control. Our ultimate efforts were directed towards use of limited proteolysis and detergents to determine the topologic location of the synthase. These experiments demonstrated that, unlike all other synthases of non-cellulosic polysaccharides at the Golgi apparatus, the catalytic domain of the β -glucan synthase is located on the exterior surface where it apparently extrudes its product into the lumen of the Golgi membranes (Urbanowicz *et al.* 2001)

4. New model systems of cell wall biogenesis

A comprehensive study was conducted to examine the cell-wall polysaccharides of developing flax (*Linum usitatissimum* L.) fibers and other tissues. These fibers are used to make linen and other fiber products. Flax is a very important source of fibers in regions where cotton cannot be grown. The fiber bundles can be stripped from the plant during development for biochemical analysis. Flax is similar to *Arabidopsis* as a genetic system. Like *Arabidopsis*, the flax genome is relatively small (1.5pg DNA /2C nucleus), generation time is short (60-90 days), and plant size is manageable. Cultivated flax self-pollinates but can be cross-pollinated. Flax is transformable by *Agrobacterium*, and transgenic plantlets can be propagated. We want to establish flax as a genetic system to study cell-wall development.

Flax (*Linum usitatissimum* L.) fibers originate from procambial cells of the protophloem and develop in cortical bundles that encircle the vascular cylinder. We analyzed the chemical composition of the cell walls from various organs of the developing flax plant, from fiber-rich strips peeled from the stem, and from the xylem (Gorshkova *et al.* 1995, 1996a,b). Sugar and linkage analyses showed that chelator-soluble polysaccharides from all tissues contained 5-arabinans with very low degrees of branching, rhamno-galacturonans, and polygalacturonic acid. The fiber-rich peels contained, in addition, substantial amounts of a buffer-soluble 4-galactan

with infrequent branching at the O-2 and O-3 positions with *t*-galactosyl units. The cross-linking glycans from all tissues were (fucogalacto)xyloglucan, typical of type I cell walls, xylans containing (1→4)β-D-xylosyl units with a low degree of branching exclusively at the xylosyl O-2 with *t*-(4-O-methyl)-glucosyluronic acid units, and (galacto)glucomannans. Tissues containing predominantly primary cell wall contained a larger proportion of xyloglucan. The xylem cells were composed of about 60% 4-xylans, 32% cellulose, and small amounts of pectin and the other cross-linking polysaccharides. Our studies indicate that flax contains only one type of synthase for each of these polysaccharides and that each synthase functions in all cells but at differing relative activities.

We discovered that a pectic galactan enriched in the fiber during early development turns over as the fiber cells mature and cement together (Gorshkova *et al.* 1997, 1998). Our hypothesis is that the galactan functions as a lubricant during intrusive growth but then is hydrolyzed extensively when growth ceases and secondary wall formation predominates. The selection of mutant plants with an altered cell wall composition and architecture is particularly useful because of the wide range of potential modifications and the possibility of uncovering novel genes that encode enzymes that participate in biosynthetic pathways, wall assembly, or in modifications to polymers *in muro*.

We optimized the hydrolysis and separation of xyloglucan oligomers by high performance (high pH) anion-exchange-HPLC, with detection by pulsed amperometry (Dionex). We found that digestion of Tamarind seed xyloglucans with *Trichoderma* endoglucanase left a group of limited digestion products that were apparent dimers of the expected group of monooligomers. We discovered that they uniquely contained arabinose residues in position that prevented hydrolysis by the *Trichoderma* enzyme. These results revived hypotheses that they may have physiological implications for the nature of their binding to cellulose (Niemann *et al.* 1997).

5. Cell-wall relevant genes

Our kinetic studies showed that β-D-glucan not only accumulates specifically during cell elongation but also is hydrolyzed so rapidly that only about one-third ever accumulates, and much of this is hydrolyzed once elongation has terminated (Gibeaut and Carpita 1991). A spin-off of our work on the synthesis of the β-glucan, has been an investigation of the enzymes that hydrolyze the β-glucan in the wall. The hydrolysis is catalyzed by two enzymes, an endo-(1→4)β-D-glucanase and an exoglucanase. We have purified these two enzymes from the cell walls of maize seedlings, prepared monospecific polyclonal antibodies against them, and obtained internal peptide sequence. From screening of maize expression libraries, we selected clones that encoded the expected peptide sequences. The antibodies detect the protein in great abundance wall preparations and "tissue-prints" of developing tissues but not from fully elongated or mature tissues (Kim *et al.* 2000). We have purified two cell-wall enzymes that degrade the β-glucans during growth, and we have characterized the activity, and cloned and sequenced a genomic clone of one of them, an exo-β-D-glucanase. The ExG is able to cleave the non-reducing terminal β-D-linked glucose from 2-, 3-, 4-, and 6-linkage position, but the enzyme cleaves (1→3)-β-D-linked terminal unit about twice as fast as a (1→4)-β-D-linked unit. Analysis of the deduced amino acid sequence of the ExG reveals a membrane "patch" near the C-terminus of the enzyme. Subsequently, we discovered an exoglucanase associates with the plasma membrane, and this enzyme is detected by the same antisera as is the wall-associated isoform. Using immunofluorescence detection, we determined that the enzyme is located on the

exterior surface of living protoplasts, and two-phase detergent partitioning established that the enzyme is an extrinsic membrane protein. The enzyme is active in the intact membranes and in detergent soluble fractions. The membrane-associated exoglucanase displays nearly the same relative activity as the wall-associated form against 2-, 3-, and 6-linked β -glucosyl substrates, but the activity against 4-linked substrates was severely attenuated. Membrane anchors or spans are rare among hydrolases. We are exploring the possibility that an isoform of the enzyme may serve an 'editing' function in concert with cellulose synthase, whereby the exoglucanase excises β -linkages other than (1 $\rightarrow$ 4)-linkages.

We also characterized cellulose synthase and β -glucan synthase genes in rice. Two cotton fiber cDNAs encoding a cellulose synthase have been identified based on the amino-acid identity of their substrate binding domains with those of *Acetobacter* cellulose synthase (J. R. Pear *et al.* 1996. *Proc. Natl. Acad. Sci USA* 93, 12637-12642). Our studies of the in vitro synthesis of β -glucans indicated that the synthase gene is derived from an ancestral cellulose synthase (Gibeaut and Carpita 1993, 1994a,b), and we developed a model mechanism to explain how a cellulose synthase is converted to a β -glucan synthase by acquisition of an additional glycosyl transferase (Carpita *et al.* 1996, Buckeridge *et al.* 1999, 2001; Carpita and Vergara 1998; Vergara and Carpita 2001). We have since identified five rice cDNAs that are cellulase synthase homologs, called *CesAs*, and we sequenced each of them. Analysis of these sequences show that the five *CesA* homologs fall into three separate sub-families. Using a *CesA* cDNA probe, we also selected over two dozen genomic clones, and in the coming year we will be using a PCR-based approach to characterize the unique ones as belonging to one of the three sub-families or to any others that may be part of the complete rice family. We are using gene-specific probes to determine cell specific expression, and we are using antibodies directed against unique peptide sequences to determine the sub-cellular location of the gene products. A gene product located at the Golgi apparatus rather than plasma membrane will constitute a strong candidate for a β -glucan synthase.

6. Comprehensive reviews of cell wall structure, architecture and biogenesis

Our major reviews during this period integrated information on the chemical structure, function and turnover of individual polymers with data obtained from new techniques which probe the arrangement of the polymers within the walls of individual cells. We provide structural models of two distinct types of walls in flowering plants consistent with the physical properties of the wall and its components (Carpita and Gibeaut 1993). Advances in determination of polymer structure and in preservation of structure for electron microscopy provide the best view to date of how polysaccharides and structural proteins are organized into plant cell walls (McCann *et al.* 1995; McCann and Carpita 1996). The walls that form and partition dividing cells are modified chemically and structurally from the walls expanding to provide a cell with its functional form. In grasses, the chemical structure of the wall differs from that of all other flowering plant species that have been examined (Carpita and Gibeaut 1993; Carpita 1996, 1997). Nevertheless, both types of walls conform to the same physical laws. Cell expansion occurs via strictly regulated reorientation of each of the wall's components that first permit the wall to stretch in specific directions and then lock into final shape.

We also have comprehensively and critically reviewed the classical and current literature related to the biosynthesis of cell wall polysaccharides in vitro (Gibeaut and Carpita 1994a), as

well as presented perspectives on how the cell interacts biologically with the cell wall at the plasma membrane-cell wall interface (Miller et al. 1997) and how cell-walls are potential targets for genetic engineering (Chapple and Carpita 1998).

Publications arising from this DOE support

Journal articles and major reviews (refereed)

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