THE EXTRACELLULAR MATRIX OF PLANTS: MOLECULAR, CELLULAR AND DEVELOPMENTAL BIOLOGY

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Poster Sessions

Structure and Chemistry of the Primary Cell Wall

A 001 **PRIMARY CELL WALL PECTIC POLYSACCHARIDES**, Alan G. Darvill[1], Malcolm O'Neill[1], Patrice Lerouge[2], Jinhua An[1], Rafael Guillen[1], William S. York[1], Andrew J. Whitcombe[1], Rudolf Toman[1], Velupillai Puvanesarajah[3], and Peter Albersheim[1], [1]Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, GA 30602-4712, [2]Currently at CNRS Laboratoire de Pharmacologie et de Toxicologie Fondamentales, Toulouse, France, [3]Currently at Analytical Biochemistry Laboratories, Columbia, MO.

The primary cell walls of plants contain several structurally complex matrix polysaccharides that, together with cellulose, constitute approximately 90% of the wall. Our research team has emphasized the study of the structures of the cell wall polysaccharides of cultured sycamore (*Acer pseudoplatanus*) cells with the goal of understanding the structures and functions of the cell wall. Specifically, we have investigated in detail the structure of the pectic polysaccharides rhamnogalacturonan I (RG-I), rhamnogalacturonan II (RG-II) and homogalacturonan, and the hemicellulosic polysaccharide xyloglucan. These studies utilize both chemical and enzymatic procedures to generate oligosaccharide fragments of sizes suitable for structural analysis. Results obtained with RG-I and RG-II released from the cell wall by treatment with *endo*polygalacturonase establish that these pectic polysaccharides are structurally complex. RG-I has a diglycosyl repeating unit backbone -4)-α-D-Gal*p*A-(1,2)-α-L-Rha*p* with many structurally different side chains with a wide variety of structures containing arabinosyl, fucosyl, galactosyl and hexuronosyl residues attached to C-4 of approximately 50% of the backbone rhamnosyl residues. The structures of these side chains are being studied using *endo*arabinase, arabinosidase, *endo*-galactanase, *exo*galactanase, *exo*galacturonidase, and various chemical methods. Structural differences in RG-I isolated from different pea tissues are also being investigated. Chemical methods including mild acid hydrolysis, lithium degradation, and periodate oxidation are being used to study the structure of RG-II. RG-II contains approximately 30 glycosyl residues with several side chains attached to a backbone of α-1,4-galacturonosyl residues. Much of the glycosyl sequence of RG-II is known, although the distribution of the side chains on the backbone remains to be elucidated. Our recent studies of RG-I and RG-II will be described.

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A 002 TISSUE-SPECIFIC, WALL-RELATED BIOCHEMISTRY, J.E. Varner, Department of Biology, Washington University, St. Louis, MO 63130.

In herbaceous plants the hydroxyproline-rich glycoproteins (HRGPs), glycine-rich proteins (GRPs) and proline-rich proteins (PRPS) are frequently associated with the walls of xylem, phloem fibers and sclerenchyma. In loblolly pine the secondary walls of mature wood are relatively rich in HRGPs. Because of this association of structural proteins with walls that become lignified an interest arose in some aspects of the process of lignification. In particular an in vivo assay has been used to study the developmental production of hydrogen peroxide. The assay procedure, a modification of the procedures used by T.A. Smith, (1970), R. Kaur-Sawhney, et al. (1981) and R. Angelini and R. Federico (1989) is simple; apply a solution containing 4% boiled potato starch and 0.2 M potassium iodide to the freshly cut surface of a free-hand section (500-1,000 microns) of the tissues to be examined. The blue-black color of the starch iodine complex appears in one to ten minutes on those cells producing hydrogen-peroxide. These cells are xylem, phloem fibers, sclerenchyma, epidermal and cells that have been severely damaged or crushed. Sectioning with a sharp double-edge razor blade elicits little hydrogen peroxide production from collenchyma, cortex and pith parenchyma cells. These same cells when wounded with an unsharpened end of the razor blade produce hydrogen peroxide abundantly. The site of the hydrogen peroxide production--the cut/damaged cells or the first layer of intact cells is not known. The source of electrons is not known nor is it known whether the hydrogen peroxide is produced directly or from superoxide anion. Hydrogen peroxide production has been examined in several different species. In some species lowering the pH of the test solution from 7.0 to 5.0 or 4.0 greatly increases the accumulation of the starch iodine complex on the cut surface. Also in some species the application of 10 mM malate at pHs from 4.0 to 7.0 greatly increases the accumulation of the indicator color. Whether this increased accumulation is due to increased hydrogen peroxide production or to decreased utilization is not known. By placing the section under a nitrogen atmosphere immediately after cutting and applying the starch-iodide reagent after various times it can be shown that utilization of the hydrogen peroxide in the tissue at the time of sectioning requires three to five minutes. Therefore one could estimate that in planta the moving transpiration stream contains hydrogen-peroxide in the concentration range of 10 to 100 μM and that the stomata might indirectly have some control over the hydrogen peroxide concentratins along the length of the xylem. It seems possible that the cutinization of the walls in the sub-stomatal cavity utilizes hydrogen peroxide from the xylem transpiration stream.

A 003 **STRUCTURAL DIVERSITY IN XYLOGLUCANS FROM HIGHER PLANTS: SPECTROSCOPIC TECHNIQUES FOR THE EXAMINATION OF THE STRUCTURE AND METABOLISM OF XYLOGLUCANS.** William S. York, Rafael Guillen, Giuseppe Impallomeni, Makoto Hisamatsu, Laura Kiefer, Peter Albersheim, and Alan G. Darvill. Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, Georgia, 30602-4712.

Oligosaccharide subunits of the xyloglucan produced by various species of higher plants were isolated and characterized by n.m.r. spectroscopy and fast atom bombardment mass spectrometry. *Endo*-(1,4)-β-glucanase-treatment of cell walls (containing xyloglucans) and of soluble xyloglucans prepared from the spent medium of suspension-cultured plant cells released complex mixtures of xyloglucan-derived oligosaccharides. The oligosaccharides and/or their corresponding oligoglycosyl alditols (produced by borohydride reduction) were purified by a combination of gel-permeation (Bio-Gel P-2) chromatography, normal phase h.p.l.c., reverse phase h.p.l.c. and high pH anion exchange (h.p.a.e.) chromatography. The primary structures of the oligosaccharides were established by a combination of chemical methods, fast atom bombardment mass spectrometry and n.m.r. spectroscopy. The oligosaccharides (four of which are shown below) have β-(1,4)-linked glucopyranosyl backbone (□) substituted with α-xylopyranosyl residues (△) at C-6. The identities and attachment sites of other structural components, such as β-galactopyranosyl- (■), α-fucopyranosyl- (●), α-arabinofuranosyl- (○), and β-xylopyranosyl- (▲), residues, were established for over thirty oligosaccharides or oligoglycosyl alditols. Comparison of the ^{1}H-n.m.r. spectra of these oligomers allowed correlations between specific xyloglucan structures and the positions of specific ^{1}H- and ^{13}C-resonances to be deduced. Familiarity with these correlations greatly facilitates the structural determination of a wide range of xyloglucans and their subunit oligosaccharides. Furthermore, assignment of the n.m.r. spectra of xyloglucans and xyloglucan oligosaccharides is a prerequisite to the use of n.m.r. as a tool to probe their conformational properties. The application of these techniques to the examination of the metabolism and secondary structure of xyloglucans will be discussed.

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Biosynthesis of the Extracellular Matrix

A 004 CELLULOSE SYNTHESIS IN *ACETOBACTER XYLINUM*, R. Malcolm Brown, Jr.[1], Krystyna Kudlicka[1], Inder Saxena[1], HePing Chen[1], Susan Cousins[1], Shigenori Kuga[2], and Richard Drake[3], [1] Department of Botany, The University of Texas at Austin, Austin, Texas 78713-7640, [2] Department of Forest Products, The University of Tokyo, Japan,[3] Department of Biochemistry and Molecular Biology, The University of Arkansas for Medical Science,4301 W. Markham Street, Little Rock, Arkansas 72205.

Cellulose biosynthesis has been very difficult to resolve. Only with appropriate model systems has the biosynthetic pathway been elucidated. *Acetobacter xylinum*, a gram negative bacterium, is an excellent resource for investigations of cellulose biosynthesis and cellulose structure. Through a row of spinneret pores, the bacterium assembles a ribbon of cellulose microfibrils into the medium. Time lapse video demonstrates the pattern of ribbon synthesis. Some strains (NQ-5) exhibit a periodic reversal of ribbon synthesis.If Tinopal LPW, a fluorescent brightening agent is added during synthesis, tubular non crystalline glucan chain sheets will form. Delayed crystallization can be induced by acid washes or photoisomerization. If glucan chain order is preserved, native cellulose I can be crystallized. In microgravity environments, cellulose ribbon synthesis is disturbed, leading to extensive splaying of microfibrils and their bundles. *In vitro* cellulose synthesis leads to the assembly of cellulose II crystallites. If sufficient substrate is used, ordered glucan chain aggregates can be produced, thus satisfying essential conditions for parallel glucan chain orientation and subsequent crystallization into the native cellulose I allomorph. A new mutant of *Acetobacter* has been discovered which synthesizes abundant "native band" cellulose *in vivo*. This cellulose recently has been described as folded chain cellulose II. The enzymes for cellulose assembly have been isolated, purified, identified, and the genes for these have been cloned, sequenced, and expressed. Cellulose biosynthesis in *Acetobacter* is under the control of an operon with 4 genes: AcsA, AcsB, AcsC, and AcsD. The functions of these genes in polymerization and crystallization will be described. Microbial cellulose promises to be a new industrial source. New fermentation technologies are being developed in anticipation for the application of microbial cellulose for novel product applications. Microbial cellulose assembly can be a useful system to predict more complex assemblies of this polymer in plant cell walls.

A 005 SYNTHESIS OF CELLULOSE AND CALLOSE IN HIGHER PLANTS, Deborah P. Delmer, Yehudit Amor, Andrawis, Andrawis, Mazal Solomon, Tamara Potikha, Patricia Ohana, Rafael Mayer, and Moshe Benziman, Departments of Botany and Biological Chemistry, The Hebrew University, Jerusalem 91904, Israel.

In recent years, much progress has been made in the characterization of the genes and gene products involved in the biosynthesis of cellulose in the bacterium Acetobacter xylinum. In brief, an operon of 4 genes has been cloned, two of which code for a catalytic and regulatory subunit of the cellulose synthase. The bacterial synthase is specifically activated by cyclic diguanylic acid (c-di-GMP) via its interaction with the regulatory subunit. In higher plants, a similar synthase activity has not been detected, but, using cotton fibers, we have identified an 84 kD polypeptide which is a likely candidate for the catalytic subunit, and two polypeptides which specifically bind c-di-GMP. The c-di-GMP-binding polypeptides share antigenic, and some sequence homology, with the bacterial regulatory subunit, and also show striking developmental regulation. Based upon these findings, several groups, including our own group, have been involved in attempts to clone genes coding for subunits of the plant synthase using strategies based upon the assumption of some sequence homology between the plant and bacterial genes. The current status of these efforts in plants will be discussed. In addition, a mutant of Arabidopsis which appears to be impaired in the ability to synthesize and/or organize the deposition of cellulose and callose will be described.

Callose is another β-glucan which is synthesized by plants in response to wounding, stress, or pathogen invasion, and also at certain specific stages of natural development in plants. Callose synthase activity is readily detected in vitro, and the enzyme shows an absolute requirement for both micromolar calcium and a β-glucoside for activity. Activation of callose synthase in vivo presumably involves a redistribution and elevation of the cytoplasmic levels these two activators. We have recently identified β-furfuryl-β-glucoside as a potent endogenous activator of the enzyme, and have evidence that it is largely localized in the vacuole. Conditions which stimulate callose synthesis in vivo (lowering cytoplasmic pH, elevating cytoplasmic calcium, and elicitor treatment) lead to a redistribution of FG such that the cytoplasmic concentration is substantially elevated. Callose synthesis is also often highly localized, suggesting that the enzyme might interact with the cytoskeleton. We have recently identified an annexin-like protein which can interact with the cotton fiber callose synthase and influence its activity. Since at least one member of the annexin family in animals has been shown to interact with actin, we suggest that the cotton fiber annexin may play some role in localizing the callose synthase within specific regions of the plasma membrane (e.g., plasmodesmata or the cell plate where callose deposition is particularly intense). Based upon all these, and others' findings, an overall model for regulation of callose synthesis during development or after cell perturbation will be presented.

A 006 XYLOGLUCAN METABOLISM AND ITS BIOLOGICAL SIGNIFICANCE. Gordon Maclachlan, Biology Dept., McGill University, Montreal, Que., CANADA, H3A 1B1.

Xyloglucan (XG) is deposited in primary cell walls of growing dicots and ripening fruits, where it is tightly bound to cellulose, and in the periplasmic spaces of cotyledons in certain seeds, from where it is mobilized during germination. XG-depolymerizing enzymes develop in these tissues in response to hormones (auxin or ethylene). Internal cleavage of XG chains may result in a "loosening" of constraints in the extracellular matrix and thereby contribute to expansion growth. It may reduce the "firmness" of a tissue as in the softening component of fruit ripening. Hydrolysis of XG to its constituent subunits by 1,4-β-glucanase may result in the production of oligosaccharins which regulate various aspects of morphogenesis. Further hydrolysis by glycosidases to free sugars may have nutritional value. Thus, the enzymes which form and degrade XG could control important physiological processes.

All XGs contain a backbone of up to several thousand 1,4-β-linked glucose units with a repeating subunit of cellotetraose that is substituted with two or three 1,6-α-linked xylose branches. This structure has been synthesized *in vitro* using microsomal preparations supplied with UDP-glc plus UDP-xyl. XG : xylosyltransferase (XT) will not act with preformed 1,4-β-glucan as acceptor, and XG: glucosyltransferase (GT) will not add more than a few glucose units to endogenous acceptors unless the growing chain is xylosylated. Accordingly, any XG synthase complex that accomodates these properties would appear to require at least three glycosyltransfer sites. One GT site is needed for chain elongation when a binding site in the complex transmits the information that recently formed parts of the chain are xylosylated. Two XT sites appear to be necessary: one to xylosylate every free C_6 group on one side of the cellulosic ribbon generated by GT, and the other to xylosylate every second free C_6 group on the other side, thereby leaving one unsubstituted glucose unit per cellotetraose. Endo-cellulases, XG-ases and XG : transglycosylases all cleave XG at the unsubstituted glucose, which is therefore essential for XG catabolism. Where XG fragments are incorporated into longer chains of XG by transglycosylation at points where XG is cleaved, the net effect is depolymerization with transitory chain rearrangements. These may have localized biological value in re-establishing XG cross-linkages after a period of wall loosening and XG-chain displacement, but cannot lead to an increase in average degree of polymerization or take over the functions of XG synthase.

Most XGs are galactosylated via 1,2-β-linkage to xylose side chains with a gal:xyl ratio that ranges from approx. 1:6 in pea stem to 1:2.2 in tamarind seed. XG subunits with 0 to 2 galactose units have been isolated from many sources. It is not known whether one or two galactosyltransferases are required to catalyse these additions or whether such enzymes are obligatory components of the XG synthase complex. However, their substrate specificity will determine the location of fucose units along the XG backbone. XG fucosyltransferase is readily solubilized in detergents and assayed by providing non-fucosylated full-length XG as fucosyl acceptor, or fragments as small as three subunits (25 sugars). In pea stems, the enzyme is active in trans elements of the Golgi apparatus and seems to be the last step in processing XG before secretion. It is possible that XG fucosylation is a signal that is required for secretion, even in those tissues that contain no fucose in the final extracellular XG. Nasturtium and tamarind seeds, for example, contain XG α-fucosidase activity which could be responsible for defucosylating XG after it is secreted.

A 007 ORGANIZATION AND DYNAMICS OF THE GLYCOPROTEIN AND POLYSACCHARIDE SYNTHETIC PATHWAYS IN THE PLANT GOLGI APPARATUS, L.A. Staehelin, A. Driouich, T.H. Giddings, and G.F. Zhang, Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, CO 80309-0347, U.S.A.

The plant Golgi apparatus is the site of synthesis of complex polysaccharides and glycoproteins. Research to date on this organelle has been hampered by the lack of precise information of how the synthetic pathways of N- and O-linked glycoproteins, and of complex polysaccharides are organized within Golgi stacks as well as the lack of knowledge of the traffic patterns through and around these stacks. We have used highly characterized antibodies in conjunction with immunogold staining of high pressure frozen/freeze-substituted suspension cultured sycamore maple cells to localize the sites of appearance of specific sugar groups on defined polysaccharides and N- and O-linked glycans. Our findings indicate that 1) individual Golgi stacks can process simultaneously glycoproteins and polysaccharides; 2) *cis* cisternae are involved in O-linked arabinosylation of hydroxyproline-rich glycoproteins, and the synthesis of the backbone of pectic polysaccharides; 3) medial cisternae add xylose to N-linked glycoproteins, extend the backbone of pectic polysaccharides, and methyl-esterify the polygalacturonic acid domains of pectic polysaccharides; 4) *trans* cisternae initiate and complete the backbone of the hemicellulosic polysaccharide, xyloglucan (XG), add terminal fucose residues to some XG sidechains and to N-linked glycans, and add sidechains to pectic polysaccharides; 5) the *trans* Golgi network completes the fucosylation of XG.

During differentiation of root tip cells the Golgi stacks undergo distinct structural changes caused by alterations in the enzymatic makeup of the cisternae. We have also investigated the effects of the fungal metabolite Brefeldin A (BFA) on the structure and function of the Golgi apparatus of plant cells. In animal cells, BFA inhibits protein secretion by causing a complete disassembly of Golgi stacks and the resorption of the cisternae into the ER. Although BFA also inhibits protein secretion in plant cells, the Golgi stacks do not disappear. Instead, the ER cisternae swell, additional *trans* cisternae are formed, dense secretory vesicles accumulate, and the Golgi stacks aggregate into large clusters. Based on fractionation experiments we postulate that the main block in secretion occurs between the Golgi apparatus and the plasma membrane.

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Mechanisms of Cell Wall Growth

A 008 ROLE OF ACIDIFICATION IN CELL WALL EXPANSION, Robert E. Cleland, Dept. of Botany KB-15, University of Washington, Seattle, WA 98195.

The acid-growth theory states that cell elongation is initiated by an effector-mediated acidification of the apoplast, which activates enzyme-mediated cell wall loosening. The theory makes three predictions: 1) wall acidification will occur in response to the effector, 2) exogenous acidity will cause elongation if infiltrated into the walls, and 3) neutral buffers, if infiltrated into the walls will block effector-mediated elongation. Evidence to support this theory has been obtained for auxin- and fusicoccin (FC)-induced growth of stems and coleoptiles, light-induced growth of bean leaves and gravity-mediated growth of stems and roots. Acid-induced wall loosening is not the only mechanism of auxin-induced elongation, however. A study of prolonged growth of *Avena* coleoptile sections showed that acid-extension was responsible for elongation during the first 90 minutes, but thereafter a second process with a less acidic pH optimum (5.5-6.0) became limiting. Use was taken of the ability of FC to cause in vivo wall acidification to show that about 50% of auxin-induced growth of *Avena* coleoptiles can be accounted for by acid-extension.

Recent studies have provided some insight into the mechanisms of both acid-mediated wall loosening and auxin-induced proton excretion. Frozen-thawed walls, when under tension, can extend for prolonged periods if given acidic conditions. This extension is not due to breakage of calcium crosslinks in the walls. It has been possible to solubilize proteins responsible for this wall loosening, and progress has been made in characterizing them. It has been assumed that the role of acid is to activate the wall loosening enzymes, but there is evidence that suggests that the acid is increasing the availability of substrate for the enzyme instead. The proton excretion appears to be mediated by a plasma membrane ATPase. The effect of auxin may be to increase the amount of ATPase in the PM. Alternatively, it may be to activate preexisting ATPase. An activation via a decrease in cytoplasmic pH now seems unlikely, but an activation via protein kinase-mediated enzyme phosphorylation or by change in surrounding lipids is still possible.

A 009 STIMULUS-DEPENDENT OXIDATIVE CROSS-LINKING OF A PROLINE-RICH PLANT CELL WALL PROTEIN: A NOVEL, RAPID DEFENSE RESPONSE AND CONTROL POINT IN CELL MATURATION

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Treatment of bean or soybean cells with fungal elicitor or glutathione causes a rapid insolubilization of pre-existing (hydroxy)proline-rich structural proteins in the cell wall. This insolubilization, which involves H_2O_2-mediated oxidative cross-linking, is initiated within 2 minutes and complete within 10 minutes under optimal conditions, and hence precedes the expression of transcription-dependent defense responses such as the synthesis of phytoalexin antibiotics and lytic enzymes. We present data that demonstrate that elicitor causes a rapid hardening of the cell wall that can be ascribed to the oxidative cross-linking of these structural proteins. Moreover, this cross-linking was observed in the early stages of an incompatible interaction, but not a compatible interaction, between soybean and Pseudomonas syringae pv. glycinea. Cross-linking is also under developmental control associated with cellular maturation during hypocotyl growth and in tissues subject to mechanical stress such as the stem : petiole junction. Stimulus-dependent oxidative cross-linking of plant cell wall structural proteins is a novel site of cellular regulation with potentially important functions in the flexible realization of the final functional architecture of cell walls during development and rapid toughening of cell walls in the initial stages of plant defense.

A 010 CELL WALL GROWTH IN TIP GROWING CELLS, Eberhard Schnepf, Zellenlehre, Universität Heidelberg, Im Neuenheimer Feld 230, W-6900 Heidelberg, F. R. Germany

In tip growing cells, wall expansion and apposition of wall material depends on the local exocytosis of Golgi vesicles which deliver matrix material and incorporate cellulose (or chitin) synthase complexes into the plasma membrane. Various current hypotheses on the mechanisms of tip growth (1) are confronted with observations in tip growing moss protonemata. The apical apposition of wall material and the lateral expansion of the wall in the tip region results in an extension of a given wall portion which is also shifted from the inside to the outside of the wall. The number of cellulose synthase complexes, represented by rosettes in the PF of the plasma membrane, corresponds with the rate of cellulose synthesis. The synthase complexes are apically inserted into the plasma membrane and are then believed to move laterally within the plasma membrane, ahead of the emerging elementary fibril, necessarily more or less in basal direction. In consequence the elementary fibrils would have the reducing ends of the glucan chains apically, generally within the wall or at its surface, whereas the non-reducing ends would be more basally situated and closer to the plasma membrane. In consequence the elementary fibrils would not be arranged parallel to the wall surface, as in thickening walls or in walls expanding by intercalary growth. It is suggested that a single rosette produces a single elementary fibril and disintegrates thereafter. Rough calculations based on growth rate and wall production of protonema cells as well as on experiments with the ionophore monensin indicate both that the cellulose synthase complexes are short-lived (15 min) and that they migrate within the plasma membrane at a rate of 900nm per minute. The cell diameter depends not only on the size and position of the area, where wall material as well as wall loosening factors are secreted, but also on the equilibrium between turgor pressure and wall rigidity (and its increase from apical to basal). When in germinating spores the synthesis of polysaccharides is disturbed by feeding with galactose in high, but non-plasmolytical concentrations, the apical dome becomes instabile and the cell diameter is increased. Mild plasmolysis likewise results in wider cells with looser walls. The firmness of the wall can obviously be modulated by the cell. Plasmolysis initially causes the rosettes to disappear transiently; the formation, transport and exocytosis of Golgi vesicles are likewise transiently blocked. Increase of cytosolic calcium by the ionophore A23187 also results in wider caulonema tips. Similar growth irregularities are caused in pollen tubes when the calcium sequestration by the ER is inhibited. It is concluded that the growth of the cell wall is controlled in rate and site by ion channels and ion pumps in the plasma membrane, which also seem to be short-lived.

(1) Heath, I.B. (edit): Tip Growth in Plant and Fungal Cells. Academic Press, San Diego, 1990.

A 011 CYTOKINESIS IN TOBACCO BY-2 CELLS, Hiroh Shibaoka, Tetsuhiro Asada, Seiji Sonobe, and Tatsuo Kakimoto, Department of Biology, Faculty of Science, Osaka University, Toyonaka, Osaka, Japan

The cell cycle of tobacco BY-2 cells was synchronized by the method in which the cell cycle was arrested at the transition between the G_1 and the S phases by aphidicolin and then at metaphase by propyzamide. By using this method, a large number of cells with phragmoplasts were obtained. Cells with phragmoplasts were treated with glycerol to permeabilize the plasma membrane and fluorescent-dye-labeled tubulin (DTAF-tubulin) was introduced into the cells. A thin fluorescent band appeared at the equatorial plane and the width of the fluorescent band increased with time, indicating that DTAF-tubulin polymerized onto "plus" end of microtubules (MTs) at the equatorial plane of the phragmoplast. In addition to the fluorescent band at the equatorial plane, weak bands of fluorescence appeared at the distal portion of the phragmoplast. DTAF-tubulin also polymerized onto free "minus" end of microtubules. The weak bands and the bright band were separated by non-fluorescent bands. As the width of the bright band at the equatorial plane increased, the weak bands moved away from the equatorial plane, suggesting that translocation of MTs takes place concomitantly with the polymerization of tubulin at the equatorial plane. AMP-PNP and GMP-PNP that did not inhibit the polymerization of tubulin inhibited the translocation of microtubules. GTP effectively caused the translocation of MTs. ATP also caused the translocation of MTs, but ATP was far less effective than GTP. Phragmoplasts were isolated from the protoplasts prepared from tobacco BY-2 cells with phragmoplasts. Isolated phragmoplasts incorporated radioactivity from UDP-[U-^{14}C]glucose into insoluble material. The radioactivity associated with the insoluble material was solubilized by 1,3-β-glucanase and the solubilized radioactivity is associated with glucose, indicating that most of the product is 1,3-β-glucan. Autoradiograms of sections of isolated phragmoplasts after incubation with UDP-[6-^{3}H]glucose showed that silver grains were present at the equatorial plane. Electron microscope autoradiograms demonstrated that silver grains were present only at the equatorial plane and no grains were observed on the ER or on the Golgi apparatus. It seems unlikely that 1,3-β-glucan was synthesized at the Golgi apparatus or at the ER and was transported to the equatorial plane. Isolated phragmoplasts were incubated with UDP-[6-^{3}H]xylose in the absence and in the presence of unlabeled UDP-glucose. Isolated phragmoplasts incorporated radioactivity from UDP-[6-^{3}H]xylose, and the incorporation was accelerated by the presence of the simultaneously applied UDP-glucose, an indication that xyloglucan can be synthesized in isolated phragmoplasts. Autoradiograms of sections of isolated phragmoplasts which had been incubated with UDP-[6-^{3}H]xylose and unlabeled UDP-glucose revealed that the majority of silver grains was present on the Golgi apparatus and only small number of grains was found at the equatorial plane. It is likely that xyloglucan is synthesized at the Golgi apparatus, packed in Golgi vesicles and transported to the equatorial plane in the living cells.

Role of the Extracellular Matrix in Plant Development

A 012 STRUCTURAL DYNAMICS OF THE CELL WALL DURING DEVELOPMENT, Nicholas C. Carpita, David M. Gibeaut, and Jong-bum Kim, Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907

The major polysaccharides of the primary cell walls of flowering plants have been catalogued, the fine structure of the polysaccharides is increasingly better understood, and images of the cell wall polysaccharides in the electron microscope have never been crisper. Despite this progress, we still have a rudimentary understanding of the dynamic changes that occur in the cell wall during cell expansion and differentiation, and the specific changes in structure responsible for expansion are still controversial. We know that the primary walls of grasses and related monocots are composed of different structural polysaccharides and proteins than those of all other flowering plants. Even though the growth physics of grasses and dicots are similar, the changes responsible for wall expansion must be mediated by different structural elements. Structural changes in pectins (de-esterification, depolymerization) and xyloglucans (depolymerization, transglycosylation) are associated with expansion in dicots, whereas the appearance of a mixed-linkage β-D-glucan coincides with the expansion in grasses. Pulse labeling studies with intact maize seedlings shows remarkable turnover of arabinose from glucuronoarabinoxylans and glucose from the β-D-glucan despite a seemingly steady accumulation of radioactivity in the wall. In this lecture, we will review the present state of knowledge of the dynamics of the expanding and differentiating cell wall with emphasis on the metabolism of the structural framework, possible roles of pectic substances in control of that metabolism, and enzymic and structural proteins related to growth processes.

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A 013 ENZYMIC CROSS-LINKING OF POLYMERS IN THE PRIMARY CELL WALL, Stephen C. Fry, John A. Brown and Graham Wallace, Centre for Plant Science, University of Edinburgh, Daniel Rutherford Building, The King's Buildings, Mayfield Road, Edinburgh EH9 3JH, U.K.

Non-cellulosic polysaccharides and glycoproteins are *difficult to extract* from growing cell walls but are water-soluble after extraction. This indicates that in the wall matrix the polymers were cross-linked. We are studying the structure and biosynthesis of four potential cross-links and the wall enzymes that may catalyse their formation:

(a) Intermicrofibrillar xyloglucan chains. Xyloglucans can hydrogen-bond to cellulose and are long enough to tether two or more microfibrils. The formation of H-bonds is non-enzymic, but the action of xyloglucan endotransglycosylase (XET) suggests that it may enable the integration of newly-secreted xyloglucan chains into a pre-existing xyloglucan-cellulose network [1,2]. This idea is supported by the fact that DCB, a cellulose-synthesis inhibitor, fails (for several hours) to prevent the firm integration of newly secreted xyloglucan into the cell wall in vivo.

(b) Tyrosine dimers. Dimerisation of tyrosine residues in extensin to form the potential cross-link, isodityrosine, is probably peroxidase-catalysed. In vivo the reaction is very precisely steered, resulting in the formation of isodityrosine but no dityrosine, whereas in vitro horseradish peroxidase + H_2O_2 generates mainly dityrosine. Factors responsible for this specificity will be discussed [3].

(c) Benzyl ether linkages between feruloyl-polysaccharides and other wall polymers. Evidence for this type of reaction was obtained through studies of the fate of [^{14}C]cinnamate in vivo [4]. Ether formation is proposed to be due to the reaction of quinone methides (formed by the action of peroxidase on feruloyl-polysaccharides) with nucleophiles such as polymer-bound -OH groups. Attempts to reproduce this type of reaction in vitro will be discussed.

(d) Galacturonoyl esters. We have characterised several O-galacturonoyl derivatives of neutral sugars as models of potential novel cross-links [5]. Some of the galacturonic acid residues of pectins in vivo are esterified via their -COOH groups to alcohols other than methanol [6 & unpubl]; data on the nature of these alcohols will be discussed.

In conclusion, several diverse extraprotoplasmic enzymes must contribute to the assembly of the plant cell wall.

[Supported AFRC research grants.]

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A 014 CELL SURFACE GLYCOPROTEINS AND PLANT MORPHOGENESIS, J. Paul Knox, Centre for Plant Biochemistry & Biotechnology, University of Leeds, Leeds, West Yorkshire, LS2 9JT, United Kingdom.

The retention of an extracellular matrix by plant cells as a cohesive cell wall closely associated with the plasma membrane has important consequences for plant morphogenesis (1). The molecular mechanisms controlling plant cell development, adhesion and morphogenesis are not understood. However, the derivation and use of monoclonal antibodies recognizing surface components - particularly glycan components - are providing insight into, and markers for, these morphogenetic events (2). Monoclonal antibodies recognising carbohydrate epitopes of plasma membrane glycoproteins (also carried by extracellular arabinogalactan proteins) indicate that complex glycosylational changes occur at the outer face of the plasma membrane as cells diverge in fate at the root meristem. The dynamic patterns of expression of combinations of these arabinogalactan-protein epitopes reflect both the temporal differentiation of cell types and also the establishment of spatial distinctions between cells and tissues. Such molecular events may represent a pre-pattern of the emerging tissue pattern i.e. they may indicate cell to cell signalling mechanisms responsible for the control of cell fate (3). Several monoclonal antibodies recognizing cell wall glycoproteins have also been developed. These antibodies cross react with carrot extensin and potato and tomato lectins. The patterns of expression of these wall glycoproteins also reflect the development of cellular complexity at the root apex. In certain cases the epitopes have been localized to the primary cell wall in the region of specific intercellular spaces. The development of intercellular space is an important aspect of plant morphogenesis (1). The biogenesis of intercellular spaces and their anatomical significance will be discussed. Evidence will also be presented demonstrating that the precise developmental locations of both the plasma membrane and the cell wall glycoprotein epitopes differ between species.

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Biochemistry of Extracellular Matrix Components

A 015 LIGNIN AND RELATED PHENYLPROPANOIDS: ASSEMBLY, STRUCTURE AND DEGRADATION, Norman G. Lewis, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

The regulatory mechanisms controlling monolignol partitioning into lignin, lignan and neolignan, and perhaps suberin/sporopollenin pathways are poorly understood. Such limitations are further confounded by a lack of definition of the enzymology involved in the latter stages of formation of such metabolites. This paper critically evaluates our existing knowledge of four processes: (1) the underlying factors involved in initiating and regulating monolignol formation; (2) monolignol partitioning into lignin and lignan/neolignan metabolites, with particular attention being placed upon the stereoselectivity, enantiospecificity and substrate specificity of novel phenylpropanoid coupling (and related) enzymes, i.e., pinoresinol synthase, laccase, coniferyl alcohol oxidase, polyphenol oxidase, peroxidase, etc.; (3) the process of lignification (enzymes, intermediates and structures) in response to secondary cell wall thickening and wounding; and (4) the suberization process. Lastly, following plant death, biodegradation then ensues; a summary of our current knowledge of this yet poorly understood process is also given, with particular reference to the claims that "lignin" peroxidases are essential.

A 016 SEED STORAGE POLYSACCHARIDES - MODELS FOR THE STUDY OF CELL WALL MATRIX BIOSYNTHESIS AND TURNOVER

J S Grant Reid, University of Stirling, Scotland, United Kingdom.

In the seeds of many plants, the extracellular matrix is used for the storage of reserve polysaccharides. The stored carbohydrates (Cell Wall Storage Polysaccharides, CWSP)[1] are structurally similar to, or identical with individual non-cellulosic matrix components of the cell walls of vegetative tissues, from which they have almost certainly been derived in the course of evolution. During seed development there is intensive biosynthesis of CWSP, and germination is followed by their programmed breakdown. We are using CWSP-containing seeds as model systems to study the biochemical mechanisms of biosynthesis and turnover of individual cell wall matrix components. Xyloglucan breakdown in nasturtium cotyledons, and galactomannan biosynthesis in developing legume-seed endosperms will be considered.

Numerous seeds, including that of nasturtium (Tropaeolum majus L) store xyloglucan in cotyledonary cell walls[1]. The CWSP xyloglucan possesses most of the structural features of plant primary cell wall xyloglucan. Studies on xyloglucan mobilisation in nasturtium[2] have enabled the purification to homogeneity of three enzymes of novel specificity -a xyloglucan-specific endo-(1→4)-β-D-glucanase[3] which acts as a powerful xyloglucan endo-transglycosylase, a xyloglucan oligosaccharide-specific α-xylosidase[4] and a xyloglucan-active β-galactosidase[5]. Closely similar enzymes are involved in the processes of xyloglucan turnover which occur in primary walls[6,7], and which are believed to regulate both plant cell wall mechanical properties and elongation-growth. We are engaged in a collaborative programme aimed at the isolation, from nasturtium tissues, of the genes encoding the xyloglucan-modifying enzymes with a view to modulating their expression in transgenic plants.

Galactomannans are CWSP in the endosperms of leguminous seeds. They have a (1→4)-linked β-D-mannan backbone which carries single-unit (1→6) α-D-galactosyl substituents. This structural pattern (a linear β-linked backbone with a distribution of short side-chains) is typical of the main hemicellulosic polysaccharides of cell walls. The galactomannans from different species have different mannose/galactose (Man/Gal) ratios which are genetically controlled (natural range of Man/Gal ratios 1.1 to 3.5). We are studying galactomannan biosynthesis in three species (fenugreek (Trigonella foenum-graecum, Man/Gal = 1.1), guar (Cyamopsis tetragonoloba, Man/Gal = 1.6 and senna (Senna occidentalis, Man/Gal = 3.3), with emphasis on the molecular mechanisms underlying the control of Man/Gal ratio. Evidence will be presented a) that in fenugreek and guar the regulation of Man/Gal ratio is determined only by the specificities of interacting membrane-bound mannosyl- and galactosyltransferases, and b) that in senna the Man/Gal ratio of the primary biosynthetic product is regulated as in the other species, but the final Man/Gal ratio is determined to a significant extent by a process of post-depositional modification catalysed by a late-development regulated α-D-galactosidase[8-10]. This is a model project to test the feasibility of altering the structures of complex, non-cellulosic cell wall polysaccharides in vivo by manipulating their pathways of biosynthesis.

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A 017 THE USE OF MUTANTS TO DISSECT THE STRUCTURE AND FUNCTION OF THE CELL WALL

Clint C.S. Chapple, Wolf-Dieter Reiter, and Chris R. Somerville, DOE Plant Research Laboratory, Michigan State University, East Lansing, MI, USA, 48824-1312.

Mutants of *Arabidopsis thaliana* have been very useful in elucidating the biochemical and molecular regulation of physiological processes. We have used this approach to examine the plant cell wall by isolating mutants with biochemically altered cell wall polymers. Two approaches have been taken. Mutants with altered polysaccharide composition were identified by gas chromatographic screening of leaves from a mutagenized population of *Arabidopsis*. Mutants were identified on the basis of altered monosaccharide composition of total leaf hydrolysates. A collection of 38 mutant lines, representing at least 10 distinct loci was obtained. Several of the mutant lines are now being studied in detail. One such line, defective at a newly identified locus called *FUS1* (*fu*cose *s*ynthesis locus 1), is incapable of synthesizing fucose in its shoots, although the fucose level in root tissue is near that of wild type. This mutation results in the production of xyloglucan and rhamnogalacturonan II that lacks the fucose and 2-*O*-methylfucose found in the wild type polysaccharides. The *fus1* mutants also show a significant decrease in tensile strength of the flowering stem when compared to wild type, possibly due to the production of altered cell wall polysaccharides.

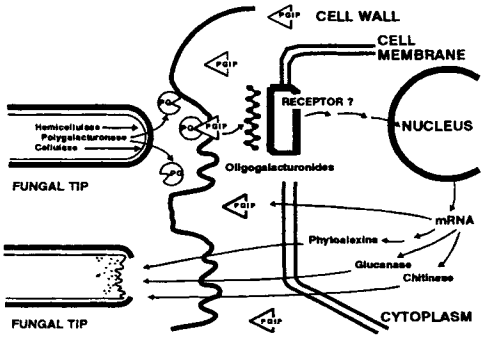
A second approach identified a mutant of *Arabidopsis* blocked in the general phenylpropanoid pathway. This mutant fails to hydroxylate ferulate to 5-hydroxyferulate, and is thus unable to produce sinapic acid and the guaiacyl:syringyl lignin typical of wild type plants. This mutant offers the opportunity to clone the defective gene, possibly ferulate-5-hydroxylase, and to examine the impact of syringyl units on the physico-chemical characteristics of lignin in an otherwise isogenic background.

A 018 *Abstract Withdrawn*

Workshop: Emerging Techniques for Studying Cell Walls and Cell Wall Macromolecules

A 019 THE INTERACTION BETWEEN ENDOPOLYGALACTURONASE AND THE PLANT CELL WALL PGIP: A MOLECULAR APPROACH, Giulia De Lorenzo, Department of Plant Biology, University "La Sapienza", Piazzale Aldo Moro, 00185 Rome, Italy, and Botany Institute, University of Bari, Italy.

*Endo*polygalacturonase is an important pathogenicity factor of fungi. The enzyme catalyzes the fragmentation and solubilization of plant cell wall homogalacturonans, thereby assisting in the colonization of the plant tissue and providing nourishment for the fungus. On the other hand, *endo*polygalacturonase is a potential avirulence factor, as it can activate plant defense responses by generating, from the plant cell wall homogalacturonans, oligogalacturonides that elicit the synthesis and accumulation of phytoalexins and other defense responses. All dicots thus far examined contain a cell wall-associated protein (PGIP) that inhibits fungal *endo*polygalacturonases. The interaction between polygalacturonases and PGIP favours the formation of oligogalacturonides of the proper size to elicit plant defense responses. A fungal gene encoding *endo*polygalacturonase (*pgA*) and a plant gene encoding PGIP have been cloned from *Fusarium moniliforme* and bean, respectively, and characterized. We are currently investigating the regulation of the expression of both genes. Synthesis of PGIP in bean is induced at the transcriptional level by wounding, elicitors and fungal infection, suggesting a novel mechanism of active defense response of plants. The hypothesis that the interaction between polygalacturonase and PGIP may function as an autocatalytic defense mechanism that plays an important role in the resistance of plants to fungi will be discussed (see figure). Transgenic expression experiments have been undertaken i) to enhance or decrease PGIP levels in tomato plants, and ii) to analyse the regulation of the *pgip* gene (using fusion of the *pgip* promoter with the reporter gene *uidA*). Transgenic expression of the *pgA* gene in yeast has been performed to study the structure-function relationship of the polygalacturonase by site-directed mutagenesis.



Possible involvement of fungal polygalacturonase and PGIP in resistance of plants to fungi

A 020 COMPUTER SIMULATIONS OF CELL WALL POLYSACCHARIDE CONFORMATION. Samuel Levy[1], William S.York[2], Bernd Meyer[2] and L. Andrew Staehelin[1]. [1]Department of Molecular, Cellular and Developmental Biology, University of Colorado, Boulder, Colorado 80309-0347. [2] Complex Carbohydrate Research Center, University of Georgia, 220 Riverbend Road, Athens, GA 30602.

The application of theoretical potential energy calculations, employing the concepts of static and dynamic equilibrium, allow us to elucidate global and local energy minima and to obtain a quantitative description of preferred conformations of oligosaccharide and represetative polysaccharide fragments. To this end, we have employed the GEGOP software as one approach to studying the molecular details of the interaction between two plant cell wall polysaccharides, xyloglucan (XG) and cellulose. XG binds and crossslinks cellulose microfibrils with a high specificity and affinity. The exact nature of this interaction is fundamental in regulating the physical properties of the cell wall and consequently plant cell growth. XG possesses a 1-4-ß-glucan containing up to 75 % substitution with 1-6 linked mono-, di- and tri-saccharide sidechains. The nona- (mono- and tri-saccharide sidechains) and hepta-saccharide (monosaccharide sidechains) fragments constitute the major components of XG in dicotyledonous plants. The static equilibrium conformations of a representative oligosaccharide containing these fragments were determined for a flat, cellulose-like backbone, and twisted, cellbiose-like backbone. A nona- and hepta-saccharide with a flat backbone possesses sidechains folded to one surface of the backbone, defined as the $H1^S$ face, primarily in the nonasaccharide region of the oligosaccharide. This leaves the other surface, the $H4^S$ face, sterically accessible which we postulate could bind to cellulose. In contrast, the trisaccharide sidechain folds onto the $H4^S$ face of the twisted glucan backbone conformation. The structural features displayed by the twisted glucan backbone conformation correlated well with preliminary n.m.r. data on nonasaccharide fragments isolated from sycamore suspension-cultured cells. Thus, the calculated twisted glucan backbone form could represent one conformational family of XG in solution.

Metropolis Monte Carlo (MMC) simulations performed on a nona- and hepta-saccharide fragment allows a statistical sampling of the range of permitted conformations. MMC simulations on a fully relaxed molecule indicated that a specific sidechain orientation positively correlates with a flattening of the twisted glucan backbone. These results suggests that by inducing a flattening of the glucan backbone, which permits binding of this region of XG to cellulose, the trisaccharide sidechain indirectly plays a role of initiating cellulose binding. Following the binding initiation by the nonasaccharide region of XG a stepwise flattening of adjacent heptasaccharide region could occur, due to the low energetic cost of folding monosaccharide sidechains and the increase in favorable interaction between two flat ß-glucan backbones. On the basis of these conformational preferences we hypothesise that the nonasaccharide region is a binding initiator and the heptasaccharide region is a binding stabilizer. In addition to the nona- and hepta-saccharide fragments several other oligosaccharide fragments have been defined for XG from sycamore suspension-cultured cells. Some of these fragments may be responsible for inducing the crosslinking of cellulose by XG. One potential oligosaccharide domain that could induce the "lifting-off" of XG from cellulose is the relatively rare pentasaccharide. This structure is characterized by containing an odd number of backbone glucosyl residues (3) compared to the even number (4) found in all other XG oligosaccharides thus far defined.

Preliminary calculations indicate that any oligosaccharide fragment attached to the non-reducing end of the pentasaccharide, which contains di- or tri-saccharide sidechains has a low probability of binding with the cellulose surface in proximity to the pentasaccharide due to unfavorable steric close contacts. Static equilibrium structures indicate that any post-pentasaccharide domain could adopt, at a single glycosidic linkage, a conformation which projects this non-binding domain away from the cellulose surface. MMC simulations will indicate to what extent these "non-binding" conformations are populated and therefore specify their likelihood of occurence.

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A 021 METHODS FOR LOOKING AT THE ORIENTATIONS OF WALL MACROMOLECULES, Maureen C. McCann[1], Nicola J. Stacey[1], Reg Wilson[2], and Keith Roberts[1], [1]John Innes Institute, Norwich, U.K. and [2]Institute of Food Research, Norwich, U.K.

Direct visualisation of extracted cell wall polymers has shown many of them to be flexuous rods of up to 700nm in length (1). Stringent packing constraints must operate if such polymers are to be accommodated in a cell wall of, say, 200nm thickness. It seems likely that the re-orientation of cellulose microfibrils that occurs during elongation must also result in the re-orientation of many other cell wall components.

We have applied two novel methodologies to elucidate the changes that occur in composition and orientation of cell wall polymers during the processes of growth, development and decay. Many techniques currently applied to cell walls involve analysis of bulk samples or of material that has undergone chemical treatment. Fourier-Transform Infra-Red microspectroscopy is a non-invasive physical technique that provides a rapid and powerful assay for the presence of specific chemical bonds from a defined area of a single cell wall (2). Insertion of polarisers into the path of the Infra-Red beam before and after passing through a cell wall sample mounted on the stage of the microscope accessory permits determination of the orientation of particular functional groups with respect to the direction of elongation of the cell.

We can also directly visualise polymers in the cell wall at high resolution using the fast-freeze, deep-etch, rotary-shadowed (FDR) replica technique (3). By combining the technique of immunogold labelling of specific epitopes with monoclonal antibodies with the FDR replica technique, it should be possible to identify unambiguously the fibres imaged in replica and to define particular wall domains. When we consider the compositional heterogeneity between different tissues within the plant, and between different domains within the wall around a single cell, it is important to note that both of these techniques enable us to define changes in the architecture of elongating cells at the level of a single cell wall.

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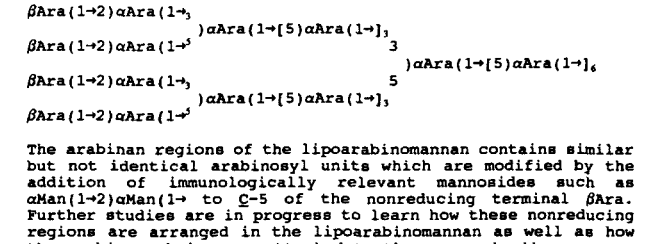
A 022 CHEMICAL AND ENZYMATIC METHODS DECIPHER INTRICATE NON-REPEATING STRUCTURES IN MYCOBACTERIAL CELL WALL POLYSACCHARIDES, Michael McNeil*, Gurdyal S. Besra*, Delphi Chatterjee*, Mamadou Daffe‡, Kay-Hooi Khoo,§ Anne Dell,§ Howard R. Morris,§ and Patrick J. Brennan*, *Department of Microbiology, Colorado State University, Fort Collins, Colorado 80523 U.S.A. §Department of Biochemistry, Imperial College of Science, Technology, and Medicine, London SW7 2AZ, U.K., ‡Centre de Recherche de Biochimie et de Genetique Cellulaires du C.N.R.S. Toulouse, France.

The cell wall polymers arabinogalactan and arabinomannan of *Mycobacterium tuberculosis* lack both a repeating unit structure and functional groups for specific degradations. They are thus similar to a number of plant polysaccharides. Nevertheless partial acid hydrolysis coupled with appropriate alkylations, chromatographic separations, mass spectrometry and NMR allow the recognition of several oligosaccharide units or structural motifs; again similar to plant cell wall rhamnogalacturonans and to some extent xyloglucans. Recently we have probed the arrangements of these structural motifs in the entire polysaccharide molecules using FAB/MS of mild acid generated large per-O-alkylated oligosaccharides. The power in this technique arises from the fact that appropriate alkylations allow fragments derived from the non-reducing end of the molecule by a single glycosyl cleavage to be distinguished from internally derived fragments produced by two or more glycosyl cleavages. The galactan region of the arabinogalactan is known to contain approximately 30 linear D-Galf units and 1 or 2 branched D-Galf units where the arabinan is attached. The FAB/MS studies were able to reveal that these galactosyl units are arranged such that the branched residues are located very near the reducing end of the galactan chain. This is true because linear galactan chains of at least 25 residues formed by a single acid cleavage and thus containing the original non-reducing end were detected during FAB/MS. The same approach yielded information on the arabinan and has allowed the recognition of the following tricosaarabinoside ("23'mer") from the arabinogalactan:



The arabinan regions of the lipoarabinomannan contains similar but not identical arabinosyl units which are modified by the addition of immunologically relevant mannosides such as αMan(1→2)αMan(1→ to C-5 of the nonreducing terminal βAra. Further studies are in progress to learn how these nonreducing regions are arranged in the lipoarabinomannan as well as how the arabinan chains are attached to the mannan backbone.

A 023 EXTRACTION OF DEFINED FRAGMENTS OF PECTIN BY SELECTIVE CLEAVAGE OF ITS BACKBONE ALLOWS NEW STRUCTURAL FEATURES TO BE DISCOVERED, Andrew J. Mort[1], Niels O. Maness[2], Feng Qiu[1], Gabriel Otiko[1], Jinhua An[3], Paul West[4], and Padmini Komalavilas[5], [1]Dept. of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078-0454, [2]Dept. of Horticulture, Oklahoma State University, Stillwater, OK 74078-0454, [3]CCRC University of Georgia, Athens, GA, [4]Dept. of Chemistry, Oklahoma State University, Stillwater, OK 74078-0454, [5]Dept. of Pharmacology, University of Southern Alabama, Mobile, AL.

Only two sugars are known to be in the backbone of pectins: galacturonic acid (galA) and rhamnose (rha). The galA is α(1-4) linked to other galA residues and infrequently α(1–2) linked to rha residues. The rha residues are always linked α(1-4) linked to galA residues. Thus, initially pectin appears to have a very simple backbone. However, different regions of pectins contain very different proportions of galA and rha and the galA residues are modified by acetylation and methyl esterification to varying degrees. It is our expectation that determining the structure of the backbones of the various sections of pectins will help us to understand their functions. Also, knowing their structures is a prerequisite to the determination of the ways in which they are biosynthesized. Studying the structure of pectins has encouraged us to develop several new methods of looking at polysaccharide structures and to adapt recently introduced methods for use with pectins. Two of these will described: 1) Application of HF solvolysis for specific cleavage of the backbone of pectins to allow the distribution of rha residues to be determined. 2) Reduction of methyl esterified galA residues to allow quantitation and localization of methyl esterified galA residues. We have shown that anhydrous liquid HF can be used at sub-zero temperatures to cleave particular glycosidic linkages with high specificity. In the case of pectins, rha linkages are far more labile than those of galA. Thus the backbone of pectins can be broken wherever rha residues occur. After treating cotton suspension culture cell walls with HF at -23° we obtain three major fractions containing galA: 1) the disaccharide galA→rha which comes from rhamnogalacturonan I, 2) a homogalacturonan fraction ~40% methyl esterified, and 3) a homogalacturonan ~10-15% methyl esterified. This shows that methyl esters are not evenly distributed in pectins, but are concentrated in some regions. By reducing methyl esterified galA residues quantitatively to galactose we can permanently label those positions which were esterified. We can then use the degree of conversion to tell us the percentage esterification. Since only microgram quantities of samples are needed for sugar analysis by GLC, we can determine the degree of esterification of very small pectin samples. Another result of converting methyl esterified galA residues into galactose is their lability to liquid HF is drastically increased. Thus treatment of the reduced pectins with liquid HF under appropriate conditions cleaves the polymer at all galactosyl linkages. The resulting oligosaccharides ($galA_n$-gal) which can be fractioned by anion exchange HPLC and quantitated indicate the runs of contiguous non-esterified galA residues in the original pectin.

(Supported by DOE grant DE-FG05-86 ER - 13496)

Roles of Proteins in Controlling Cell-Cell Interactions and Cell Wall Differentiation

A 024 THE EXTRACELLULAR MATRIX AS A FUNCTIONAL MEETING POINT FOR SYMBIONTS, N.J.Brewin, A.L. Rae, S. Perotto, John Innes Institute, Norwich NR4 7UH, Great Britain.

In common with other legumes, peas establish a nitrogen-fixing symbiosis with *Rhizobium*, specifically with *R.leguminosarum* bv *viciae*. In common with many other terrestrial plants, they also establish a mineral-scavenging symbiosis with the vesicular-arbuscular (VA) mycorrhizal fungi. Both symbioses are characterised by an intimate and co-ordinated growth of host and microbial cells and by progressive differentiation of the extracellular matrix. There is an initial invasive phase in which plant cell growth is reorganised to create an inwardly growing tunnel of plant cell wall and plant cell membrane which ensheathes the invading endosymbiont as it traverses root epidermal and cortical cells. Subsequently, there is a phase of metabolic integration in which the endosymbiont is more-or-less integrated into the host plant cytoplasm, although the microorganism is always enclosed by a differentiated form of host cell plasma membrane and hence is retained in a specialised form of extracellular matrix. An important characteristic shared by both *Rhizobium* and mycorrhizal symbioses is that cell and tissue invasion involves co-ordinated cell growth rather than cell damage or cell death: hence the hypersensitive response is not induced and antimicrobial phytoalexin compounds are not synthesised. Evidence for common components of symbiotic interaction comes from the discovery that nearly half of the non-nodulating pea mutants that have been isolated are also unable to establish the mycorrhizal symbiosis. Moreover, lupins, which do not form transcellular infection threads with *Rhizobium*, do not establish a mycorrhizal symbiosis.

What distinguishes the *Rhizobium* and mycorrhizal symbioses is that the former, but not the latter, stimulates the formation of a mass of nodule tissue by inducing cortical cell divisions which subsequently differentiate into an organised apical meristem. The active "elicitor" is a chemically defined lipo-oligosaccharide secreted by *Rhizobium* following stimulation by specific flavonoids present in host legume root exudates. This *Rhizobium*-derived signal molecule is an oligochitin (tri- to penta- N-acetylglucosamine). Host specificity is determined by strain-specific sugar modifications, either at the reducing end (e.g. sulphation, fucosylation or acetylation) or at the non-reducing end (e.g., acetylation and, in particular, the nature and degree of unsaturation of an O-linked long chain fatty acid residue).

The mode of action of the lipo-oligosaccharide Nod-factor has not been elucidated. The only clues lie in the nature of the biological response. The earliest observed effect of the application of Nod-factor is the deformation and curling of growing root hairs (3-4 hours): this perhaps implies an effect of Nod-factor on the co-ordination of cell wall growth through modification of the plasma membrane or of the underlying cytoskeleton. (In alfalfa root hair cells, membrane depolarisation has been observed within 20 minutes of the application of Nod-factor.) Another early response to the application of Nod-factor is the development of cortical cells with centralised nuclei and transcellular cytoplasmic strands. In the inner cortex, these pre-mitotic structures predetermine the orientation of cell plate formation following cell division in the nodule primordium: in the outer cortex, similar cytoplasmic strands predetermine the pathway for transcellular infection thread growth (without prior cell division).

Finally, it should be pointed out that nodule initiation can be experimentally uncoupled from nodule invasion either by the application of purified Nod-factor or by inoculation with *Rhizobium* mutants defective in cell surface polysaccharides (e.g., acidic extracellular polysaccharide, beta 1,2-glucans or lipopolysaccharides). Moreover, nodule initiation can be experimentally induced in the absence of Nod-factor, either by application of TIBA (tri-iodobenzoic acid, an auxin transport inhibitor) or by genetic breeding for alfalfa lines in which uninvaded (*Rhizobium*-independent) nodule-like structures arise spontaneously and may serve as root storage organs for starch. These observations imply that *Rhizobium*-derived Nod-factor may serve as a key which unlocks the door to a pre-existing host-encoded pathway for root nodule development.

Reference

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A 025 THE ROLE OF EXTRACELLULAR MATRIX MOLECULES IN POLLEN-STIGMA INTERACTIONS, Marilyn A. Anderson, Antony Bacic, Jan L. Lind, Angela H. Atkinson, Alison M. Gane and Adrienne E. Clarke, Plant Cell Biology Research Centre, School of Botany, University of Melbourne, Parkville, Victoria 3052, Australia.

Many flowering plants have a mechanism which prevents inbreeding and promotes outcrossing, known as self-incompatibility (SI). In many plants, this is controlled by a single locus (*S*-locus) with multiple alleles[1]. We are working with *Nicotiana alata*, an ornamental tobacco, as the experimental system to understand the molecular basis of SI. During pollination, the pollen tubes grow extracellularly through the transmitting tissue cells of the female pistil to the ovary. We have identified a number of components of the extracellular matrix of the pistil which are relevant to self-incompatibility as well as the probable defence of these tissues against microbial pathogens. These include an allelic series of self-compatibility glycoproteins (*S*-RNases), a 120 kD glycoprotein and a family of arabinogalactan-proteins which are major style components, and a proteinase inhibitor which is expressed in the stigma. Structural information on these matrix components will be presented and their possible functions discussed.

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A 026 WALL PROTEINS AND THE GROWTH AND DIFFERENTIATION OF PLANT CELLS, Abraham Marcus, The Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, PA.

The proteins of the cell wall are comprised of enzymatic factors that synthesize and modify the various components of the extracellular matrix, structural proteins that interact with the nonprotein components of the extracellular matrix to generate structures that are unique to specific cell types, and a network that either modulates the deposition of extracellular matrix components or prevents the further extension of the cell wall. Early growth of the soybean seedling provides a system allowing analysis of aspects of these functions. When maintained in the dark through 5d, the seedling hypocotyl grows rapidly in an almost unrestrained manner. Transfer of the seedlings to light after 2d, results in limited growth between d3 and 4 with a sharply reduced rate of growth thereafter. Analysis of the salt-extractable proteins shows that growth or lack of growth of the hypocotyl has little effect on the major "resident" proteins. In contrast, the levels of both the mRNA coding for the 33 Kd protein, PRP2, and the protein itself are affected. With continued rapid growth, both the mRNA and the protein remain at a high level, whereas when growth is curtailed both the mRNA and the protein rapidly disappear. These results suggest that PRP2 may play an important role in determining the growth of the hypocotyl. We have also purified three of the major salt-extractable proteins of the hypocotyl cell wall. The proteins, 50 Kd, 30 Kd, and 28 Kd, are all strongly hydrophilic, completely lack hydroxyproline, and contain 6-8% tyrosine. We have also found that the non-extractable residue contains 20% hydroxyproline. Assuming that the salt-extracted proteins mirror the precursor pool, this suggests a strong selectivity in the cross-linking process and a major requirement for hydroxyproline.

Molecular and Functional Studies of Cell Wall Hydrolases

A 027 EVOLUTION OF ß-GLUCAN ENDOHYDROLASE FUNCTION, Peter B. Hϕj, Lin Chen, Peilin Xu, Patricia Mirabile, Maria Hrmova and Geoffrey B. Fincher, Department of Biochemistry, La Trobe University, Bundoora, Victoria 3083, Australia.

The (1→3,1→4)-ß-glucan 4-glucanohydrolases (EC 3.2.1.73) and the (1→3)-ß-glucan endohydrolases (EC 3.2.1.39) constitute two classes of endohydrolases that catalyse the hydrolysis of cell wall polysaccharides of plant and fungal origin. The (1→3,1→4)-ß-glucanases hydrolyse (1→4)-ß-glucosyl linkages, only where the glucosyl residue is linked at the C(O)3 position. These enzymes are therefore specific for (1→3,1→4)-ß-glucans with adjacent (1→3)- and (1→4)-ß-glucosyl linkages. The (1→3)-ß-glucanases hydrolyse (1→3)-ß-glucosyl linkages, but usually require several contiguous, unsubstituted (1→3)-ß-linked glucosyl residues for activity. Thus, the (1→3)-ß-glucanases cannot hydrolyse the isolated (1→3)-ß-linkages found in most (1→3,1→4)-ß-glucans. The (1→3)-ß-glucanases are widely distributed in plants, where they participate in a number of developmental processes, including the removal of wound and dormancy callose. They are also classified amongst the "pathogenesis-related" proteins that are expressed in response to microbial attack. The (1→3)-ß-glucanases are believed to provide the plant with a degree of protection against fungal invasion through their ability to hydrolyse the (1→3)- and (1→3,1→6)-ß-glucans that are common components of fungal walls. In contrast, the distribution of the (1→3,1→4)-ß-glucanases is limited to the cereals and to some grasses, where they function in the metabolism of (1→3,1→4)-ß-glucans found in the cell walls of these plants.

Comparative studies on the (1→3)-ß-glucanases and (1→3,1→4)-ß-glucanases from barley have revealed a high degree of structural similarity, both between the enzymes and between their corresponding genes. Positional identities of amino acid sequences range from 45-50% and several highly conserved regions have been identified. The availability of two closely related polysaccharide hydrolases, isolated from a single plant species but probably serving widely different functions, has presented an opportunity to examine the molecular basis for the evolution of subtle differences in their substrate specificities. Specificity is determined not only by whether the polysaccharide is bound to the enzyme, but also by whether the glycosidic linkage of the bound polysaccharide is correctly positioned in relation to the catalytic amino acids. We have used active site-directed inhibitors to define the catalytic amino acids of the two enzymes. Common structural features of the genes, including conserved sites of intron insertion and characteristic patterns of codon usage, provide additional evidence that the genes encoding barley (1→3)- and (1→3,1→4)-ß-glucanases share a common evolutionary history, which might be related to the co-evolution of cell wall composition in plants and fungi.

A 028 GLUCURONOXYLANASE DEGRADATION OF COMPLEX XYLANS, Donald J. Nevins[1], Kazuhiko Nishitani[2], Naoki Sakurai[3] and Masihiro Inouhe[4], [1]Department of Vegetable Crops, University of California, Davis, CA 95616, [2]Kagoshima University, Kagoshima, Japan, [3]Hiroshima University, Hiroshima, Japan, [4]Ehime University, Matsuyama, Japan.

The major xylan polysaccharides of higher plant cell walls are linear mainchains with arabinose, galactose and glucuronic acid attached as branches at specific positions. Secondary substitution of arabinose with ferulic acid and glucuronic acid with methyl ethers are also common. Conventional extraction of xylan components of the cell wall with alkali modifies some of these constituents and dissociates ester bonds. To circumvent these degradation events, enzymes offer an alternative. The administration of microbial xylanases to cell wall preparations mediates limited hydrolysis of the xylan main chain because the high level of substitution interferes with enzymic binding. Under these circumstances xylanase treatment of walls will liberate those xylans that may exist as homopolymers and a certain proportion of complex xylans will be released because of the presence of occasional open xylosyl sequences. In any case xylan degradation is restricted to a relatively small fraction. An alternative treatment to enhance the xylanase yield is to subject the wall to mild acid hydrolysis to remove sufficient arabinose to allow better enzyme access.

Glucuronoxylanase, β-(1->4)-glucuronoxylan xylanohydrolase, mediates extensive hydrolysis of diverse xylans because of recognition of glucuronic acid attached at the 2-0-xylosyl position. Hence a relatively abundant yet specific population of xylans may be targeted for hydrolysis within a milieu that includes xylans lacking glucuronic acid.

Xylans with different compositions are liberated from diverse taxa because of the conservation of glucuronic acid associations with xylans.

Relatively uniform fragments have been isolated from the glucuronoxylanase digests. The fragments retain features of the intact polymer including labile constituents otherwise lost during extraction. The most abundant fragments have been isolated and conjugated to BSA for the preparation of monoclonal antibodies. Antibodies screened against the glucuronoarabinoxylan fragments were administered to cross sections of stems and detected by a fluorescent second antibody. Binding of the antibody was observed throughout the tissue but a higher fluorescence intensity was observed in walls of epidermal cells. When the tissue was pretreated with glucuronoxylanase, antibody binding was abolished.

Glucuronoxylanase functions to excise selected components from the wall and selected fragments may be isolated to serve as antigens. Enzyme treatment of cell walls excises the binding epitope and assists in confirming antibody specificity *in situ*.

Role of Plant Cell Walls in Pathogenesis

A 029 THIONINS, K. Apel, S. Bohl, H. Bohlmann, S. Bunge, B. Mollenhauer, G. Schrader
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Leaf thionins have been identified in barley (**Hordeum vulgare**) as a group of highly abundant polypeptides with antifungal activity. The existence of leaf thionins has been predicted from cDNA sequences encoding Mr 15000 thionin precursor polypeptides. These transcripts are highly abundant in leaves of dark-grown barley seedlings. During illumination, however, the concentration rapidly declines. Mature thionins could be detected in the cell wall and the central vacuole of barley leaf cells. The toxicity of these thionins for phytopathogenic fungi and their predominance in the outer cell wall of the epidermal cell layer of etiolated barley leaves indicated that thionins may be part of a resistance mechanism of barley plants against pathogens. In mature light-adapted barley plants thionins mRNAs are hardly detectable. However, when exposed to the fungal pathogen **Erysiphe graminis** (powdery mildew) plants began to reaccumulate thionin mRNAs transiently. At the same time marked changes in the distribution of thionins at the site of infection occurred. One of the first host reactions to powdery mildew is the formation of the papilla, a locally restricted cell wall apposition, that begins to form in the epidermal cell below the fungal appressorium. In susceptible barley leaves the papilla was almost completely devoid of thionins while in the cell walls close to the infection site the concentration of thionins was reduced. In resistant leaves, however, thionins accumulated in the papilla and in the cell wall close to the infection site the thionin concentration remained high. These results suggest that leaf thionins take part in the defense mechanism of barley plants.

A 030 TWO PATHWAYS FOR SIGNAL TRANSDUCTION IN PLANT PATHOGENESIS RESPONSE, Vered Raz, Yoram Eyal, Yael Meller, Guido Sessa, Rachel Green, Tamar Lotan and Robert Fluhr, Department of Plant Genetics, The Weizmann Institute of Science P.O. Box 26, Rehovot 76100, Israel.

We have used the plant pathogenesis response, exemplified by the induction of pathogenesis-related (PR) genes, as a paradigm to investigate elicitor-dependent signal transduction in the plant cell. Two general pathways from elicitor to gene induction have been defined. One pathway dictates coordinate regulation of PR gene expression and involves ethylene. This pathway is exemplified by elicitors whose actions are abrogated in the presence of ethylene-action inhibitors and includes elicitors such as salicylic acid. The second pathway activates PR proteins accumulation in an ethylene-independent manner. This pathway is exemplified by the elicitor endoxylanase, whose mode of action presumably involves release of plant cell wall fragments as secondary elicitors. Signal transduction in these pathways differ fundamentally in their requirement for calcium and cellular phosphorylation. Blocking calcium fluxes with chelators arrested ethylene-dependent induction of PR proteins but not ethylene-independent induction. Artificially increasing cytosolic calcium levels by treatments with the calcium ionophore, ionomycin, or the calcium pump blocker, thapsigargin, stimulated PR proteins accumulation. Preventing cellular phosphorylation events with kinase inhibitors, blocked the ethylene-motivated response but not the response induced by endoxylanase. Perturbation of the cellular phosphorylation by application of okadaic acid, a phosphatase inhibitor, led to induction of PR gene expression, which was abrogated by simultaneous addition of kinase inhibitors. Our results define two pathways to plant pathogenesis response and delineate essential steps in ethylene-mediated signal transduction.

A 031 CUTICLE AND SUBERIN: EXTRACELLULAR MATRIX UNIQUE TO PLANTS, P. E. Kolattukudy, Ohio State Biotechnology Center, 1060 Carmack Road, Columbus, OH 43210

Cuticle that is attached to aerial parts of plants is composed of a biopolyester cutin, as the structural component and associated waxes. The soluble components of this extracellular matrix trigger differentiation of germinating fungal spores into infection structure called appressorium. Fungal genes triggered by these plant signals have been cloned and characterized. These genes will be discussed. Enzymatic assistance in breaching the structural barrier of the plant is essential for infection of plants by some fungi. Expression of fungal cutinase genes involved in pathogenesis is triggered by hydroxy fatty acids from the plant. The mechanism of regulation of this gene expression by the plant signal will be discussed. Fungal degradation of extracellular carbohydrate barriers is necessary for successful invasion of the host. Fungal pectate lyase has been shown to play an essential role in this process. Pectate lyase genes involved in this process have been cloned and characterized. This family of genes found in pathogenic fungi will be discussed. The extracellular matrix components of plants and fungi trigger expression of plant peroxidase genes that are involved in the reinforcement of plant cell walls. These gene products probably catalyze crosslinking of cell wall components and deposition of a phenolic matrix on the wall. The biological consequences of overexpression of this gene and suppression of expression by an antisense approach will be discussed. The highly anionic peroxidase previously thought to be expressed only upon wounding or fungal attack was recently found to be constitutively expressed in a tissue specific manner and was found to be developmentally regulated. How a normally developmentally regulated peroxidase gene is recruited to respond to the fungal and plant cell wall signals for defense purposes will be discussed.

The Oligosaccharin Signalling Pathway of Plants

A 032 **OLIGOSACCHARINS - OLIGOSACCHARIDES WITH REGULATORY FUNCTIONS**, Peter Albersheim, Christopher Augur, Carl Bergmann, Russell W. Carlson, Jong-Joo Cheong, Stefan Eberhard, Michael G. Hahn, Kyung-Sik Ham, Veng-Meng Ló, Victòria Marfà, Bernd Meyer, Debra Mohnen, Malcolm A. O'Neill, Mark D. Spiro, Herman van Halbeek, William S. York, and Alan Darvill, Complex Carbohydrate Research Center, The University of Georgia, 220 Riverbend Road, Athens, Georgia, 30602-4712.

Plants and animals have evolved signaling mechanisms to regulate the expression of genes essential for their growth, development, and defense against pests. Some of these signals or regulatory molecules are oligosaccharides. Oligosaccharides with regulatory activities are called *oligosaccharins*. This report will describe the structures and functions of several of the six well-characterized oligosaccharins, of which two originate as components of polysaccharides of the primary cell walls of plants, two as components of polysaccharides of the walls of fungal pathogens of plants, one as the side chain of glycoproteins secreted by fungi, and one family of oligosaccharins is synthesized by a family of bacterial symbionts of plants.

The oligosaccharins to be described are able to regulate plant growth, organogenesis, or defense against pathogens. The results of oligosaccharin research provide evidence that plants utilize the structural complexities of oligosaccharides to regulate important physiological processes. Cell wall polysaccharides of plants and microbes are a rich source of oligosaccharins, and the walls also contain glycanases and glycosidases capable of generating oligosaccharins.

Considerable evidence supports the hypothesis that oligosaccharins are important regulatory molecules in plants, although much work still remains to evaluate the *in vivo* role and importance of these molecules. With one exception, most of the data on the biological activities of oligosaccharins has been obtained in bioassays. Studies with intact plants are needed, perhaps using plants transformed with genes encoding enzymes, receptors or other proteins that alter the *in situ* activity of oligosaccharins. Indeed, studies on the enzymes that release and process oligosaccharins, on receptors of oligosaccharins, and on the effects oligosaccharins have on membranes and membrane-associated proteins should elucidate the events that initiate oligosaccharin activities and lead to a better understanding of the signal pathways that transduce the effect of these regulatory molecules.

Progress in this new area of biology is partly due to the development of sophisticated analytical techniques for purifying and determining the structures of complex carbohydrates and the collaborative research of physiologists, biochemists, molecular biologists, and organic chemists. The results of this interdisciplinary research are prompting plant scientists to re-evaluate their concepts of development, defense mechanisms, and functions of cell walls. These studies may also lead to biotechnology-based environmentally friendly approaches to improve resistance to microbial and insect pests and to control the growth and development of plants.

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A 033 LOCAL AND SYSTEMIC SIGNALLING IN A PLANT DEFENCE RESPONSE

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The defence response that will be discussed involves the events induced in tomato plants following perception of a wound stimulus. Injury to the lamina of one leaf on a tomato plant triggers changes in gene expression at the local injury-site and systemically in distant unwounded aerial regions of the plant. The response has mainly been studied using expression of proteinase inhibitor (pin) genes as wound-inducible markers (reviewed 1).

Changes in level of pin mRNA, pin protein and pin activity can also be induced by the application of defined compounds to tomato plants using a bioassay. These include glycan elicitors, abscisic acid (ABA) jasmonic acid and a peptide, called 'systemin'. The bioassay, as well as other approaches, has also been used to determine the ability of compounds to inhibit pin induction of wounding or by chemical application. Inhibitors include salicylic acid and a structurally-specific group of hydroxybenzoic acids, ion transport inhibitors, fusicoccin and auxin (IAA). In total, these studies have led to a numbert of models to describe the events that are induced in planta when a leaf is wounded (reviewed 2). However as yet, the molecular mechanism underlying the systemic wound response is not known.

At Leeds, we have been interested in the cell-specificity of the wound-response and the role of cell wall fragments in signalling events that give rise to the local and systemic changes in gene expression (3,4). Two gene markers have been used : pin 2, and pTOM13 recently shown to encode ethylene-forming enzyme (5,6). Both genes are known to be wound-inducible, but in situ hybridization shows the cell specificity of response to be very different. Interestingly, when the effects of two different glycan elicitors were assayed : these were also found to differ. Pectic fragments (dp10-20) induced pin 2 but with little or no effect on pTOM 13; oligogalacturonides (dp 2-9) induced pTOM13, but with little or no effect on pin 2. The differential response of the two genes to the wound stimulus, and to the different glycan elicitors leads to the possibility that in planta, a range of cell wall fragments are released at the injury-site and act as specific signals to induce different genes (7).

It is known from earlier data that cell wall fragments applied to the injury-site on a leaf do not exit the lamina and cannot therefore represent the long-distance systemic signal (8). In principle there are two potential mechanisms for propagation of a systemic response : chemical or physical (2). The former involves long-distance movement of a chemical signal, such as through transport in the phloem. Two candidates exist for a physical system : hydraulic signals and electrical signals. These possibilities will be discussed in the light of recent data from a number of laboratories (9-11).

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A 034 **SPECIFIC RECOGNITION OF OLIGOGLUCOSIDE ELICITORS BY BINDING PROTEIN(S) IN SOYBEAN PLASMA MEMBRANES**, Michael G. Hahn[1], Jong-Joo Cheong[2], Rob Alba[1], and François Côté, The University of Georgia, Complex Carbohydrate Research Center and Departments of Botany[1] & Biochemistry[2], 220 Riverbend Road, Athens, Georgia, 30602-4712, USA.

A detailed understanding of the role of oligosaccharins (oligosaccharides with regulatory activities) will require elucidation of the cellular signaling pathways induced by these signal molecules. We are studying the cellular signaling pathway induced by a branched hepta-β-glucoside originally isolated from the mycelial walls of the phytopathogenic fungus, *Phytophthora megasperma* f. sp. *glycinea*. This oligosaccharin induces an important defense response in soybean, namely the biosynthesis and accumulation of anti-microbial pterocarpan phytoalexins. The hepta-β-glucoside elicitor induces half-maximal accumulation of phytoalexins in soybean cotyledons at concentrations of ~10 nM. Structurally related oligoglucosides are only active at 10- to 10^5-fold higher concentrations. Our research has focused on an early step in this signal pathway, the specific recognition of the hepta-β-glucoside elicitor by binding protein(s) (presumptive receptors) in soybean cells. Proteinaceous binding sites for a radio-iodinated tyramine derivative of the hepta-β-glucoside elicitor are present in membranes prepared from all major parts of soybean seedlings. These elicitor binding proteins (EBPs) co-migrate with a plasma membrane marker (vanadate-sensitive ATPase) in linear sucrose density gradients. Binding of the radiolabeled hepta-β-glucoside elicitor was specific, reversible, saturable, and of high affinity (apparent K_d = 0.75 nM). Competitive displacement of the radio-labeled hepta-β-glucoside elicitor with a number of chemically synthesized elicitor-active and structurally related, but less active oligoglucosides demonstrated a direct correlation between the ability of an oligoglucoside to displace the labeled elicitor and its elicitor activity. Thus, the EBPs recognize the same structural elements of the hepta-β-glucoside elicitor that are essential for its phytoalexin-inducing activity, suggesting that the EBPs are physiological receptors for the elicitor.

The EBPs have been solubilized using several non-ionic detergents, including n-dodecylsucrose, n-dodecylmaltoside, and Triton X-114. The n-dodecylsucrose-solubilized EBPs retain the binding affinity (apparent K_d = 1.8 nM) for the radiolabeled elicitor and show the same specificity for elicitor-active oligoglucosides determined previously for the membrane-localized EBPs. Current research is directed toward the purification and cloning of the hepta-β-glucoside EBPs. Techniques being utilized include ligand affinity chromatography and cloning by functional expression in a heterologous cell type. Purification and characterization of the EBPs or their corresponding cDNAs is a first step toward elucidating how the hepta-β-glucoside elicitor triggers the signal transduction pathway that ultimately leads to the synthesis of phytoalexins in soybean.

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A 035 OLIGOURONIDES, SYSTEMIN AND JASMONATES AS SIGNALS FOR PLANT DEFENSIVE GENES, C. A. Ryan, Edward E. Farmer, Barry McGurl, Greg Pearce and Scott Johnson, Institute of Biological Chemistry, Washington State University, Pullman, WA 99164-6340.

Several signailing molecules have been identified that regulate the expression of genes coding for proteinase inhibitor proteins that are synthesized in plant leaves in response to predator and/or pathogen attacks. Regulatory molecules include oligosaccharide fragments of plant and fungal cell walls, small molecules, such as abscisic acid, salicylic acid, auxin, jasmonates, and a recently discovered polypeptide of 18 amino acids, called systemin. Oligouronides are considered among the early signals for plant defensive genes, released by enzymes secreted by attacking pathogens. Oligouronides are not mobile and therefore are unlikely systemic signals for defensive genes in response to pest attacks. Systemin, a potent inducer of two proteinase inhibitor genes in tomato and potato is readily transported in the phloem of young tomato plants when placed directly on leaf wounds, consistent with the properties of a systemic signal. Recently, jasmonic acid and its methyl ester and their biosynthetic intermediates have beeen shown to induce proteinase inhibitor genes in plants. A model has been proposed for the activation of the inhibitor genes by oligouronides and systemin in plants via receptors that result in the activation or synthesis of lipase activity and the subsequent release of linolenic acid from membrnaes, resulting in the synthesis of jasmonic acid and the activation of the genes. The properties of oligouronides, systemin and jasmonates will be presented in the context of the proposed model. (Supported in part by Washington State College of Agriculture and Home Economics Project #1791 and grants from the National Science Foundation.)

Late Abstract

IDENTIFICATION OF A MUTANT OF *ARABIDOPSIS THALINA* BLOCKED IN THE CONVERSION OF HIGH MANNOSE TO COMPLEX ASPARAGINE-LINKED GLYCANS, Arnd Sturm[1], Antje von Schaewen[2] and Maarten J. Chrispeels[3], [1]Friedrich Miescher-Institut, Basel, Switzerland, [2]Universität Osnabrück, Osnabrück, Germany, [3]University of California, San Diego, CA.

The biosynthesis of many secretory plant proteins and their movement along the secretory pathway is accompanied by the attachment of high mannose glycans to specific asparagine residues, and the subsequent modification of these glycans in the endoplasmic reticulum and the Golgi apparatus. Glycosidases and glycosyltransferases in the Golgi convert typical $Man_9GlcNAc_2$ high mannose glycans into complex glycans that have less mannose residues and additional sugars such as α(1-3)fucose, β(1-4)galactose, and β(1-2)xylose. Glycoproteins can contain a heterogeneous array of different glycan structures, and the extend of Golgi modifications differ for different proteins, for various glycosylation sites on a single protein, and are not necessarily uniform for a single glycosylation site. This diversity is illustrated by three vacuolar proteins made by the storage parenchyma cells of developing bean. The two abundant polypeptides of the storage protein phaseolin have mostly high mannose chains at their two glycosylation sites, α-amylase inhibitor has primarily complex glycans at 4 glycosylation sites, and phytohemagglutinin has one high mannose and one complex chain.

In the course of our work on the function of N-glycosylation in plants we generated an antiserum that is specific for β(1-2)xylose residues found on plant glycoproteins. With this antiserum we isolated a mutant in *Arabidopsis thaliana* that is unable to complete the conversion of high mannose to complex glycans and which accumulates $Man_5GlcNAc_2$ glycans. In the presence of deoxymannojirimycin, an inhibitor of mannosidase I, the mutant cells synthesize $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$ glycans, suggesting that the biochemical lesion in the mutant is not in the biosynthesis of high mannose glycans in the ER, but in their modification in the Golgi. These data are consistent with the absence of GlcNAc transferase I activity from the mutant. The mutant plants are able to complete their development normally, suggesting that the complex glycans are not essential for normal developmental processes under optimal growth conditions. We must, therefore, consider the possibility that complex glycans are needed only on a small subset of glycoproteins which are synthesized when plants are grown under non-laboratory conditions, and subjected to particular biotic or abiotic stresses.

Structure and Chemistry of the Primary Cell Wall;

Biosynthesis of the Extracellular Matrix

A 100 THE HEMICELLULOSES AS STRUCTURE REGULATORS IN THE PLANT CELL WALL, R.H. Atalla and J.M. Hackney, USDA Forest Forest Service, Forest Products Laboratory, Madison, WI 53705, and D.L. VanderHart, National Institute of Science and Technology, Gaithersburg, MD, 20899

Models of cellulose in higher plants implicitly regard the aggregation of cellulose during biogenesis as independent of the environment within which it occurs. Our recent studies have shown, in contrast, that the hemicelluloses can modify the patterns of aggregation of cellulose when they are present during biogenesis. In particular, we have shown that xyloglucan can change the aggregation of cellulose produced by Acetobacter xylinum from the I_α type characteristic of bacterial and algal celluloses into the I_β type typical of higher plant celluloses. The effects are evident in both the Raman and the solid state ^{13}C NMR spectra.

In the present work, we have extended the study to include other hemicelluloses. We find that a threshold level for activity, between 0.5% and 1.0%, occurs for all of the hemicelluloses studied, which include, in addition to the xyloglucan, a glucomannan and a xylan and a mannan of low molecular weight. Each of the hemicelluloses produced a distinct modification of structure as indicated by the x-ray diffraction patterns recorded in transmission. These observations are consistent with the hypothesis that the hemicelluloses are not passive components in the development of the cell wall, but active participants in determining patterns of aggregation and tertiary structures.

A 101 A XYLEM CELL WALL LACCASE IS ASSOCIATED WITH LIGNIFICATION IN LOBLOLLY PINE, Wuli Bao, Ross Whetten, David O'Malley and Ronald Sederoff , Department of Forestry, P. O. Box 8008, North Carolina State University, Raleigh, NC 27695

Lignin is believed to arise from an enzymatically catalyzed free radical polymerization of substituted cinnamic acid precursors, the monolignols. Both peroxidase and laccase enzymes may be involved in the polymerization step. We have purified a laccase from cell walls of differentiating xylem of loblolly pine (*Pinus taeda*). This enzyme is capable of making dehydrogenation polymers (DHP), a lignin like material, from coniferyl alcohol and sinapyl alcohol *in vitro*. The laccase contains type I copper based on ESR data and absorbance at 610 nm. The enzyme uses a wide variety of substrates, including the monolignols coniferyl alcohol and sinapyl alcohol. It also uses catechol, 4-methylcatechol and syringaldazine, but not p-cresol, tyrosine or ferulic acid. This enzyme is inhibited by EDTA and NaCN, but not by 1 mM phenylhydrazine or diethyldithiocarbamic acid. PH optimum for the laccase is 5.9. The Km for O_2 is 37 uM and Km values for coniferyl alcohol and sinapyl alcohol are approximately 10 mM. DHP made from coniferyl alcohol or sinapyl alcohol consists of trimers, tetramers, pentamers, hexamers and higher order polymers. The enzyme is not detectable in soluble extracts of differentiating xylem or crude homogenates of non-lignifying tissues, such as embryos or megagametophytes. Histochemical staining shows laccase activity next to the cambium in a layer of about 16 xylem cells that are lignifying or going to be lignified. The actively lignifying cells show more intense staining. We propose that this laccase is involved in the polymerization of lignin.

A 102 CELLULOSE SYNTHESIS IN THE CELLULAR SLIME MOLD *DICTYOSTELIUM DISCOIDEUM*, Richard L. Blanton, Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131

The cellular slime mold *Dictyostelium discoideum* holds great promise as a model organism for the study of eukaryotic cellulose synthesis: (1) cellulose biosynthesis is a significant activity of differentiating cells; (2) an *in vitro* assay system for the cellulose synthase exists, and (3) it is a well-known experimental organism. The utility of *D. discoideum* for studying cellulose synthesis will be demonstrated by presenting results from three projects:

(1) The biochemistry of the cellulose synthase: The *in vitro* cellulose synthase activity approaches *in vivo* rates. The product of the *in vitro* reaction has been characterized biochemically and biophysically.

(2) The developmental regulation of cellulose synthesis: *Dictyostelium discoideum* presents the opportunity to study the spatial and temporal control of cellulose synthesis during development. All of the differentiating cells will synthesize cellulose found in at least one of the following structures: (1) in the slime sheath surrounding the slug; (2) in the stalk tube that surrounds the stalk cells; (3) in the stalk cell walls; and (4) in the spore coats. In monolayer culture amoebae will differentiate to form stalk cells in the absence of morphogenesis. This culture system has permitted a study of the conditions that influence stalk tube versus stalk cell wall synthesis.

(3) The effort to use *D. discoideum* to generate probes useful for exploring the molecular regulation of cellulose synthesis: The greatest potential for *Dictyostelium* lies in the ability to apply genetic methods to the cellulose synthase problem. Ongoing efforts will be described, including strategies for generating, screening, and characterizing mutants in genes involved in cellulose synthesis and in generating and characterizing antibody probes for proteins involved in cellulose synthesis.

This work was supported by grants from the U.S. Department of Energy (DE-FG05-90ER20006) and the National Science Foundation (DCB 91-05737).

A 103 GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST PLANT CELL WALL POLYSACCHARIDES, Michael G. Hahn, Jörg Puhlmann, Michael J. Swain, Wolfram Steffan, Peter Albersheim and Alan G. Darvill, University of Georgia, Complex Carbohydrate Research Center, 220 Riverbend Road, Athens, GA 30602-4712, USA.

Monoclonal antibodies (McAbs) are useful tools to probe the structure of plant cell wall polysaccharides and to localize these polysaccharides in plant cells and tissues. We have initiated a systematic effort to generate a large and diverse library of McAbs against each plant cell wall polysaccharide that has been defined. Dot blot and enzyme-linked immunosorbent assays are being used to screen for the McAbs. One approach has been to generate a library of McAbs from mice immunized with a non-covalent complex of methylated bovine serum albumin and rhamnogalacturonan I, a pectic polysaccharide isolated from suspension-cultured sycamore maple (*Acer pseudoplatanus*) cells. The epitopes recognized by two of these McAbs have been characterized using competitive ligand binding assays with poly- and oligosaccharides. McAb CCRC-M1 recognizes an epitope containing terminal fucosyl residues that is present principally in the hemicellulosic polysaccharide xyloglucan, and to a lesser extent in rhamnogalacturonan I. McAb CCRC-M7 recognizes an epitope of branched arabinosyl residues that is present in rhamnogalacturonan I, and in a variety of plant-derived gums and plant glycoproteins. CCRC-M7 is representative of the largest group of McAbs obtained from the initial round of immunizations/fusions, and appears to recognize an immunodominant epitope. A second round of immunizations/fusions has been initiated using rhamnogalacturonan I that was enzymatically or chemically de-arabinosylated (to remove 95% or more of the immunodominant arabinose-containing epitopes) and then covalently coupled to bovine serum albumin (to increase the immunogenicity of the polysaccharide). Several additional McAbs reactive with native rhamnogalacturonan I have been obtained, and the epitopes recognized by these new McAbs are being characterized. We are also investigating alternative strategies to greatly increase the number of McAbs specific for individual plant cell wall polysaccharides or epitopes within those polysaccharides.

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A 104 CELL WALL CHANGES IN APPLE FRUIT HELD AT 20°C OR 38°C

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Prestorage heating (38°C for 4 days) of apples leads to enhanced retention of fruit firmness during ripening compared to unheated apples. Cell walls extracted with phenol:acetic acid:water from apples held at 20 or 38°C for four days were sequentially extracted to give 3 pectic (water-, CDTA-, Na_2CO_3-soluble) and 2 hemicellulosic (GTC- and KOH-soluble) fractions. Pectic fractions from fruit held at 38°C lost neutral sugars with no accompanying decrease in galacturonic acid, while at 20°C both neutral sugars and galacturonic acid decreased. The molecular size distribution of the components of CDTA- and Na_2CO_3-soluble fractions were similar at both temperatures. The GTC fraction, when separated on a molecular size column (CL Sepharose 4B) consisted of two major polymers, one with high levels of xylose and glucose, and the other with high levels of mannose and glucose. The xyloglucan peak decreased in cell walls from fruit held at 20 but not at 38°C. We conclude that holding fruit at 38°C inhibits the loss of uronic acid from pectic moieties, and that of xyloglucan from hemicellulosic fractions, while not affecting loss of neutral sugar side chains.

A 105 CELL WALL ULTRASTRUCTURAL STUDIES WITH A MONOCLONAL ANTIBODY TO (1→3,1→4)-β-D-GLUCANS, P. J. Meikle[1], B. A. Stone[1], N.J. Hoogenraad[1], I. Bönig[2], and A.E. Clarke[2] - [1]Department of Biochemistry, La Trobe University, Melbourne, 3083, and [2]Plant Cell Biology Research Centre, The University of Melbourne, Melbourne, 3052, Australia

A monoclonal antibody has been generated against barley (1→3,1→4)-β-D-glucan. The monoclonal antibody showed no cross-reactivity against a (1→3)-β-glucan-BSA conjugate and only slight cross-reactivity was observed against a cellopentaose-BSA conjugate in an indirect ELISA. The antibody binding site has been mapped by competitive inhibition studies using a range of oligosaccharides generated from (1→3,1→4)-β-D-glucan by (1→4)- and (1→3,1→4)-β-D-glucan endohydrolases. The optimal epitope has been identified as the heptasaccharide G(1→3)G(1→4)G(1→4)G(1→3)G(1→4)G(1→4)G. The K_a of the antibody for this unit structure has been determined, by a non-competitive ELISA, to be $3.8 \times 10^4\ M^{-1}$. The monoclonal antibody has been used to develop a sensitive sandwich ELISA for the specific quantification of (1→3,1→4)-β-glucans. The assay operates in the range 1-10 ng/ml and shows no significant cross-reaction with xyloglucan, arabinoxylan or carboxymethyl-pachyman ((1→3)-β-glucan). When used with a second-stage gold-labelled, rabbit anti-mouse antibody and viewed under the electron microscope, the monoclonal antibody probe was found to bind strongly to the walls of aleurone, pericarp and seed coat cells in thin sections of immature wheat (*Triticum aestivum* cv. Millewa) grains. A specific (1→3)-β-glucan antibody (Meikle *et al.*, Planta, 181, 1-8, 1991) bound to discrete patches within the endosperm and the pericarp layer.

A 106 BIOSYNTHESIS OF α-1,4-LINKED HOMOGALACTURONIDES USING TOBACCO CELL-FREE MEMBRANE EXTRACTS, Mohnen, D., Liljebjelke, K., Adolphson, R., Baker, K., and Kumar, A., Complex Carbohydrate Research Center & Dept. of Biochemistry, University of Georgia, Athens, GA, 30602.

Polygalacturonides (PGAs) are α-1,4-linked D-galactosyluronic acid homopolymers that form the backbone of three pectic polysaccharides: rhamnogalacturonan II, homogalacturonan and methylesterified homogalacturonan. Pectic polysaccharides are produced in rapidly dividing cells, are thought to be critical for cell enlargement, and are a source of biologically active oligosaccharides that regulate plant development and defense responses. The study of the enzyme(s) that synthesize PGA (galacturonosyltransferase(s)) is of fundamental importance for understanding pectin structure and function.

Particulate enzyme preparations from radish roots were used to epimerize commercially available UDP-[^{14}C]glucuronic acid (UDP-[^{14}C]GlcA) to UDP-[^{14}C]galacturonic acid (UDP-[^{14}C]GalA), the substrate for PGA synthesis. Maximum epimerization ranged from 30%-50%. The reaction yielded a UV-absorbing peak with a retention time (HPLC) and an R_f (TLC) equivalent to that of authentic UDP-GalA. The identity of the UDP-GalA produced was confirmed by composition analysis. HPLC-purified UDP-[^{14}C]GalA was used to assay for synthesis of PGA using total membrane extracts from tobacco suspension cultured cells. Incorporation of UDP-[^{14}C]GalA into precipitable product was time dependent with maximal incorporation occurring within 60 minutes of incubation at 30°C. Analysis of the product by TLC confirmed the time dependent appearance of a major radiolabeled spot with an R_f similar to commercially available PGA. Digestion of the product with a purified endopolygalacturonase yielded ~90% decrease in precipitable product compared to undigested product. Further analysis of the product and measurement of the enzyme kinetics will be presented. The results suggest that the product recovered is largely homogalacturonide and that the enzyme activity measured is that of α-1,4-galacturonosyltranserase.

A 107 ENZYMIC DEGRADATION OF HAIRY REGIONS OF PECTIN

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Pectic hairy regions contain a rhamnogalacturonan backbone with neutral sugar side-chains attached to it (1). Schols et al. (2) isolated and characterised pectic hairy regions out of apple juice, produced with the enzymic liquefaction process, and proposed a structure. The common pectolytic enzymes are not able to degrade the pectic hairy regions. Schols et al. (3) described a novel enzyme: rhamnogalacturonase, produced by Aspergillus aculeatus, that was able to degrade the pectic hairy regions. In the commercial preparation from Aspergillus aculeatus we are searching for other enzymes that are active on pectic hairy regions, and on rhamnogalacturonan fragments as produced by the enzyme rhamnogalacturonase. Pectic hairy regions were prepared from Golden Delicious as described (2). Rhamnogalacturonan fragments were prepared from the hairy regions using rhamnogalacturonase. Activity was determined with HPSEC and HPAEC. Several enzymes, present at our department, were tested on the substrates. No activity towards the pectic hairy regions could be detected with polygalacturonase, pectin lyase and pectate lyase from various microbial sources. Endo-arabinanase, arabinofuranosidase B and endo-galactanase from Aspergillus niger released small amounts of sugars from these structures. A ß-xylosidase from Aspergillus awamori was not active. A ß-galactosidase from Aspergillus niger was able to release at least 50% of the galactose from the hairy regions, and at least 75% of the galactose from the rhamnogalacturonan oligomers. We started the purification of several rhamnosidases and galacturonosidases from Aspergillus aculeatus, that were able to degrade the rhamnogalacturonan oligomers almost completely. Aspergillus aculeatus therefore appears to produce various enzymes that can degrade pectic hairy regions and rhamnogalacturonan oligomers.

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A 108 SECONDARY WALL SYNTHESIS IN XYLEM TRACHEARY ELEMENTS TRANSDIFFERENTIATING FROM *ZINNIA ELEGANS* MESOPHYLL CELLS, Gary F. Peter and Ian M. Sussex, Department of Plant Biology, University of California, Berkeley, CA 94720

The goal of this study is to determine whether xylem tracheary elements (TEs) differentiating from meristematic cells and TEs transdifferentiating from mesophyll cells follow the same pathway. TE differentiation has been divided into four ontogenetic stages: 1) cell origin and commitment, 2) cell enlargement and patterning of the secondary wall, 3) secondary cell wall formation, and 4) cell death and wall lysis (1). We have standardized the single cell culture system of *Zinnia* and are obtaining up to 60% differentiated TEs in 72h (2, 3). As a prerequisite for correlating cellular changes with altered gene expression, we are characterizing the temporal sequence of cytoplasmic changes in transdifferentiating *Zinnia* mesophyll cells. We are determining when the cytoskeleton reorients, when the secretory pathway is stimulated, when secondary wall synthesis commences, when peroxidase-mediated lignification occurs and when the cells lyse. To characterize the temporal sequence of gene expression, cDNA libraries were constructed in lambda-YES from mRNA isolated from 52h and 72h transdifferentiating tissue. These libraries will be used to isolate cDNAs for genes both known to be expressed in differentiating TEs and new genes expressed at earlier stages of differentiation. These libraries have been screened by hybridization with probes synthesized by PCR with degenerate primers to phenylalanine ammonia lyase, peroxidases and a single stranded nuclease. We are in the process of determining the structure of these cDNAs and designing gene specific probes for use in northern blots with RNA isolated at various times during differentiation and by *in situ* hybridizations to individual cells. Using a variety of approaches, we are also attempting to isolate cDNAs expressed in during cell wall patterning and synthesis. Once we obtain probes for mRNAs expressed in a temporal sequence, we will hybridize medial sections in the differentiating zone of roots to determine if these same genes are expressed in a similar sequence during differentiation of TEs from undifferentiated cells of meristematic origin.

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A 109 A SEARCH FOR ARABIDOPSIS MUTANTS IMPAIRED IN CELL WALL BIOSYNTHESIS, Tamara Potikha and Deborah P. Delmer, Department of Botany, The Hebrew University, Jerusalem 91904, Israel

We have focused our interests on a search for mutants impaired in the biosynthesis of cellulose, callose, or lignin. For development of a suitable screening technique, we first grew wild-type Arabidopsis seedlings in the presence of an inhibitor of cellulose (DCB) or lignin (AIP, inhibitor of PAL) synthesis and studied the resulting phenotype which we reasoned should resemble mutations in these pathways. Growth on DCB resulted in highly swollen root tips and leaf trichomes, a glossy appearance of leaves, and reduced birefringence in the walls of trichomes and xylem elements. During growth on AIP, leaf size was reduced, structured xylem elements were not visible, and no wound-induced lignification could be obtained. Screening of mutagenized M2 seedlings involves a procedure in which leaves are excised and wounded, kept overnight in a salt solution, and examined for: 1) altered shape or birefringence of trichomes and/or xylem; 2) ability to synthesize lignin in xylem and/or at sites of wounding; 3) wound-induced callose synthesis. Screening includes a search for both constitutive and temperature-sensitive mutations. One of the most interesting mutants obtained to date has a phenotype which suggests it is impaired in the ability to synthesize beta-glucans. This constitutive, dominant mutation results in plants which totally lack birefringence in the walls of leaf trichomes, and have reduced birefringence in most other cell walls, including xylem elements, but the shape of the trichomes is normal. Preliminary analyses indicate that the level of total leaf cellulose is reduced by about 40% compared to wild-type leaves. In addition, this mutant does not synthesize the organized rings of callose at the base of the trichomes in respnse to wounding which are synthesized by wild-type plants. Total plant size is reduced, but the mutant is fertile and sets seeds normally. (Supported by a grant from the Israel Academy of Sciences, a fellowship from the Israeli Ministry of Interior, and a contract from the U.S. Department of Energy.)

A 110 A GLYCINE-RICH PROTEIN IN WHEAT ALEURONE CELL WALLS AND A SOLUBLE PROTEIN FROM DEVELOPING WHEAT GRAINS THAT CROSS-REACTS WITH AN ANTISERUM AGAINST A GLYCINE-RICH PROTEIN, David I. Rhodes and Bruce A. Stone, Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia

Walls isolated from wheat aleurone cell preparations and treated with SDS and phenol-acetic acid-H_2O to remove cellular protein fluoresce strongly with the protein fluor, Ponceau Red 2R, and contain ~1% w/w protein. Extraction of walls with water at 90°C removes some polysaccharide and leaves a residue containing 2% w/w protein. Further treatment with xylan and (1→3,1→4)-β-glucan hydrolases yields a soluble fraction containing protein and oligosaccharides and a residue representing 30% of the water-extracted wall, containing 4% w/w protein. Propan-2-ol-water (3:2, v/v) extracts part of the protein from this residue. This protein and the protein solubilized after polysaccharide hydrolase treatment are similar in composition (glycine 23%, tyrosine 6%) and behave similarly in HPLC.

During wheat grain development a water-soluble protein that cross-reacts with a rabbit polyclonal antiserum raised to a glycine-rich protein from bean (*Phaseolus vulgaris* L.) that had been expressed as a lacZ fusion protein (Keller *et al.*, 1988, EMBO J., 7, 3625-3633) was observed in a Western analysis. This 43 kD protein increases in abundance from 10 days post-anthesis (d.p.a.) to a maximum at 20 d.p.a., when aleurone cells are differentiated from starchy endosperm cells, thereafter there is a steady decline until 45 d.p.a. when the protein is barely detectable.

A 111 SECRETION OF CELL WALL POLYSACCHARIDES IN *VICIA* ROOT HAIRS, D.Janine Sherrier and Kathryn A. VandenBosch, Department of Biology, Texas A&M University, College Station, TX 77843-3258

Newly emerging root hair cells are the susceptible site for the establishment of symbiosis between legumes and rhizobia. These tip-growing cells secrete new cell wall components at the apex, and the biogenesis of this region may provide material needed to establish a successful infection. To characterize the distribution of some of the components in *Vicia* root hair cell walls, we have combined freeze-substitution and immunogold electron microscopy. Cryofixation and freeze substitution provide an excellent means for preserving transient events in the exocytic pathway as well as the epitopes of components destined for the cell wall. Monoclonal antibodies which recognize methyl-esterified pectin (JIM 7) and de-esterified polygalacturonic acid (JIM 5) epitopes of pectin were used to locate these epitopes within the exocytic pathway and in the cell wall. A monoclonal antibody which recognizes xyloglucan was used to locate this cell wall component. We found that pectin is secreted in the methyl-esterified form, and de-esterified *in muro*. Xyloglucan co-localizes with methyl-esterified pectin in Golgi bodies, secretory vesicles, and in the cell wall.

A 112 SINGLE, DOMINANT GENE, Gmr(t), REGULATES THE BIOSYNTHESIS OF GLUCOMANNAN IN RICE ENDOSPERM CELL WALL BY A TISSUE AND VARIETY SPECIFIC MANNER

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Plant cell wall has complicated architecture consisting mainly of polysaccharides of diverse structure. Although the properties of the cell wall have been indicated to correlate with the growth, differentiation and physical properties of plant cells and tissues, few information is available about the genetic regulation of its chemical structure.

Rice endosperm cell wall consists of hemicellulosic polysaccharides such as neutral and acidic arabinoxylans, β-1,3-, 1,4-glucan and xyloglucan[1], rhamnogalacturonan-I-type pectic polysaccharide and cellulose microfibril. We recently found that the chemical structure of this cell wall changes distinctly depending on the genetic background of each varieties. This phenomenon was characterized chemically as the expression of a polysaccharide, glucomannan, in the endosperm cell wall in the positive varieties. The amount of glucomannan in the cell wall was almost constant at around 10% in the positive varieties and practically undetectable in the negative varieties, suggesting the presence of on-off like genetic regulation. The glucomannan is a β-1,4-linked linear polysaccharide consisting mainly of D-mannose and a small amount of D-glucose. Most of the glucomannan was recovered in the cellulose fraction, suggesting its association with cellulose microfibrils. Immunohistochemical analyses with the antiserum specific for β-1,4-linked mannooligosaccharide as well as chemical analyses indicated the tissue and variety specific expression of this polysaccharide. Segregation analysis showed that this phenomenon is governed by a single, dominant gene, which we propose to call Gmr(t). Combining the chemical analyses of the cell walls with RFLP mapping technology, we could localize the gene on the linkage map of rice chromosome 9. Trials for the establishment of isogenic lines concerning to this gene and also the biochemical studies on the critical step regulated by this gene is now underway.

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A 113 STRUCTURAL FEATURES OF A GLUCURONO-ARABINOXYLAN FROM SORGHUM.

M.A. Verbruggen, G. Beldman and A.G.J. Voragen, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands.

Recently more attention has been paid to sorghum as an industrial brewing raw material. Different from barley, cell wall material in sorghum hardly degrades during the malting (ref.1) and brewing processes. The presented study focusses on the extraction and enzymic degradation of a glucuronoarabinoxylan from a crude water-unextractable cell wall preparation (WUS, ref.2) from sorghum.

Treatment of the WUS with saturated $Ba(OH)_2$-solutions extracted 40% of the amount of arabinose and xylose present. The extract contained 44% arabinose, 43% xylose, 2% galactose, 3% glucose and 7% uronic acids, being mainly glucuronic acid with minor amounts of 4-O-methylglucuronic acid. This subfraction seemed to be quite homogeneous. Only one population of polysaccharides with a broad distribution in molecular weight could be found, consistent of arabinoxylans practically all substituted with uronic acid groups. Methylation analysis showed that the ß-(1→4)-linked xylose backbone was highly substituted with single-unit arabinose side chains at O-3 or at both O-2 and O-3 of xylose. The glucuronic acid units are probably linked at O-2.

In order to investigate the detailed structure, the $Ba(OH)_2$-extract has been digested with pure arabinofuranosidases, xylanases, a glucuronidase and combinations thereof. Degradation is followed by HPSEC and HPAEC. Oligomeric endproducts will be purified by preparative HPAEC and identified by NMR.

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This research is supported by the Technology Foundation (STW) and Heineken Technical Services.

A 114 TOPOGRAPHICAL PROPERTIES AND SUBUNIT COMPOSITION OF HIGHER PLANT CALLOSE SYNTHASES AS DETERMINED BY LIMITED PROTEOLYSIS,

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Limited proteolysis and polypeptide depletion were used to probe the structural properties of callose synthase (CS) from red beet. Using plasma membrane (PM) vesicles of defined sidedness, coupled with latency measurements, it was shown that CS in vesicles of cytoplasmic face-out orientation were preferentially hydrolyzed. In contrast, CS activity in an apoplastic side-out enriched PM vesicle fraction was relatively resistant to proteolysis. Thus, the existence of a proteolytically-sensitive site (or sites) on the CS enzyme complex oriented towards the cytoplasmic surface of the PM was directly demonstrated.

Pronase E was found to be a powerful tool for probing the polypeptide composition of CS. Over 70% of CS activity was retained following treatment with Pronase E, however several polypeptides (92, 83, 57, and 43-kDa) previously implicated as components of the beet CS complex were extensively hydrolyzed and are therefore excluded as proteins required for catalysis. Three closely-migrating proteins of 31-, 29- and 27-kDa resisted hydrolysis by Pronase E, implicating one or more of these as the minimum components of the functional complex required for expression of CS activity. Purification of CS from Pronase E-treated vesicles by solubilization, glycerol gradient centrifugation and product-entrapment confirmed these results. We are currently investigating whether the 31-, 29- or 27- kDa proteins that are enriched in purified Pronase E-treated beet CS fractions bear any relationship to the recently described 31-kDa polypeptide of *Lolium*, and the possible existence of CS isoforms.

Polypeptide-depletion experiments, where activity loss is correlated with the release of specific polypeptides following exposure to detergents and chaotropes, were conducted to establish the relative hydrophobicity of polypeptides in a high purity CS fraction. These experiments established that proteins peripheral to the membrane do not play a significant role in the CS reaction, and that each of the major polypeptides co-fractionating with CS are highly hydrophobic integral membrane proteins.

A 115 SOLUBILIZATION AND PARTIAL PURIFICATION OF A PEA XYLOGLUCAN SYNTHASE, Yi Xin, Alan R. White, and Vida Pezeshk, Department of Botany, North Dakota State University, Fargo, ND 58105-5517, USA

Xyloglucan is a major hemicellulose plant cell wall polysaccharide that is synthesized in the Golgi apparatus. Cellular membranes from etiolated pea seedlings, *Pisum sativum* L. cv Alaska, were prepared by ultracentrifugation of homogenates on linear sucrose gradients. Samples were collected by gradient fractionation and subcellular fractions were assayed for membrane markers and glycosyl transferase activities. Products collected by ethanol precipitation were methylated and partially methylated alditol acetates with ^{14}C-glucose incorporated were injected on a GC with effluent split between a flame ionization detector and a radiogas proportional counter (RPC) to determine which peaks contained radioactive label. Glycosyl linkages of radiolabeled reaction products were determined by injecting the same samples on a similar column in a GC-MS with a similar temperature program (combined GC-RPC/GC-MS assay).

Glucan synthase (GS) showed peak activity coinciding with Golgi membrane markers and specific activity of GS increased about 14 fold in these fractions. The combined GC-RPC/GC-MS assay showed that GS reaction products from these Golgi fractions had ^{14}C-glucose incorporated into 1,4-linked ethanol-insoluble polymer. Size of reaction products was characterized by gel filtration chromatography. GS activity was solubilized from membranes with various detergents with digitonin, CHAPS, and octanoyl-N-methylglucamide giving the highest solubilized activities. Structural analysis of GS reaction products by the combined GC-RPC/GC-MS assay confirmed that ^{14}C-glucose was incorporated into 1,4-linked glucan by these fractions. The solubilized GS proteins were further purified by affinity chromatography. Active fractions were pooled, applied to Affi-Gel Blue and UDP-hexanolamine affinity columns, and eluted with 1.0 M NaCl. Proteins eluted were analyzed by SDS-PAGE and incorporation of ^{14}C-glucose into 1,4-linked polymer was determined by the combined GC-RPC/GC-MS assay.

Supported by National Science Foundation grant DCB-9005590.

Mechanisms of Cell Wall Growth;

Role of the Extracellular Matrix in Plant Development

A 200 AN ARABINOGALACTAN-PROTEIN FROM CARROT CELL CULTURES, Timothy C. Baldwin, Maureen Mc Cann and Keith Roberts. Department of Cell Biology, John Innes Institute, Colney Lane, Norwich NR4 7UH, U.K.

An extracellular secreted arabinogalactan-protein (AGP) from cultured cells of *Daucus carota* (carrot) has been purified by immuno-affinity chromatography of the culture medium using an anti-AGP monoclonal antibody (MAC 207) bound to cyanogen bromide-activated Sepharose 4B. The apparent molecular weight of this protein is 70-100 kD as judged by SDS-PAGE gels. Direct visualisation in the electron microscope showed putative monomers of the AGP to be approximately ellipsoidal (25nm by 15nm), and immunogold (MAC 207) negative staining confirmed the identity of the imaged molecules. These proteoglycans show a strong tendency to aggregate, sometimes forming higher order structures. Upon dessication, the AGP forms para-crystalline arrays, visible in the light microscope: polarised Fourier Transform Infra-Red (FTIR) microspectroscopy of these arrays suggests that the sugar moieties under these conditions demonstrate a high degree of polarisation. The data presented is consistent with the "Wattle-Blossom" model for AGP structure as proposed by Fincher *et al* (1983). However, the polarised FTIR data suggest there may be more ordered structure in the sugar side-chains and in their relationship to the protein backbone than that proposed by Fincher.

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A 201 GENES ENCODING FLOWER-PREDOMINANT PROLINE-RICH PROTEINS. Alice Y. Cheung. Department of Biology, Yale University, P.O.Box 6666, New Haven, Ct. 06511.

We have characterized cDNAs and some of their genes for two classes of Pro-rich proteins from N. tabacum . The first class, FPP (flower predominant Pro-rich proteins) cDNAs and genes, is comprised of a large gene family and encodes proteins that have the motif Ser Pro_{3-7}, similar to that found in the hydroxyl-proline rich glycoproteins which typically have $SerPro_4$ repeats. Contrary to tobacco extensin mRNA which accumulates to very high levels in roots, stem and tissue culture cells, these FPP mRNAs accumulate almost exclusively in flowers. The primary structure of the encoded proteins as derived from nucleotide sequence information from around 10 cDNAs and genes has the following overall characteristics: a N-terminal signal peptide, N-terminal and C-terminal regions of the putative mature protein with average amino acid composition, and a Pro-rich central region composed of Ser Pro_{3-7} motifs. Pro residues constitute about 25% of the entire protein; but over 65% of the central domain of these proteins are comprised of Pro. The most significant divergence among these deduced proteins is in the number of Ser_{3-7} motifs in their central region. These differences account for the range in size of these proteins (between 150 to 210 amino acids). The extreme C-terminal regions of these proteins are occupied by multiple and contiguous Glu and Lys residues. We are currently examining the deposition pattern of these proteins during floral development and its response to environmental stimuli. We are interested in understanding the significance of the length variation in the Pro-rich regions of these proteins and in also examining whether the highly charged C-terminal regions of these proteins are functionally or structurally significant.

The second class, TTS (transmitting tissue-specific)-1 and -2, encodes proteins that have multiple X Lys Pro Pro and thus are similar to what are collectively known as Pro-rich proteins (PRPs) which usually have repeats of Val Tyr Lys Pro Pro. TTS-1 and -2 are probably the only two members of this gene family. The Pro-rich domain is at the N-terminal half of the protein and is composed of many X Lys Pro Pro motifs. Transmitting tissues are known to be rich in many glycoproteins, some of which are Pro-rich, that may participate in pollen-pistil interactions. We are currently examining whether these PRPs are deposited in the cell walls or in the intercellular matrix of transmitting tissue cells. This information should help to assign whether these PRPs are involved in the architecture or in the functions of the style.

A 202 GENETIC MANIPULATION OF THE CELL WALL EXTENSIN NETWORK. J.B. Cooper, D. Halverson, C. Coldren, and P. Sullivan. Department of Biological Sciences, University of California, Santa Barbara, CA 93106

Extensins are rod-shaped hydroxyproline-rich glycoproteins thought to form a crosslinked network in the primary cell wall. This network may be important in cell wall function. We utilized a cloned extensin gene to construct defined mutations in the expression and the structure of extensin. Overexpression mutants were constructed by fusing heterologous promoters (CaMV 35S and soybean SSU) to the coding and 3' sequences of extensin. Structural mutations, designed to alter the length of extensin glycoprotein rods from the wild type length (~90 nm) to between 20 and 140 nm, were constructed by deleting or inserting sequences encoding the proline-rich peptide motifs of extensin. Transgenic *Nicotiana tabacum* plants were regenerated containing each of these constructs linked to a Kan^r marker. A "dwarf seedling" phenotype segregated out in the R2 progeny of two independent trangenic plants containing several copies of the 35S-extensin gene construct. This dwarf phenotype cosegregated with high levels of transgene expression, and with high levels of hydroxyproline in transgenic shoots. All of the transgenic tobacco plants constitutively expressing the extensin length mutations exhibited extremely abnormal patterns of shoot development. Transgenic plants expressing short-extensin constructs synthesized and secreted short extensins. The incorporation of these mutant glycoproteins into the cell wall network is now being investigated. Transcripts from the long-extensin construct were spliced abnormally. We are also expressing the extensin length mutations in carrot cell protoplasts and examining both the structure of the mutant extensin networks, and the effects of these mutations on cell wall regeneration and cellular development. Our results should lead to a better understanding of the role of extensin glycoprotein networks in plant cell growth and development.

A 203 THE INFLUENCE OF PLANT GROWTH REGULATORS ON CELL WALL ORGANIZATION IN DEVELOPING COTTON FIBERS. David C. Dixon, United States Department of Agriculture, Agricultural Research Services, Southern Regional Research Center, New Orleans, LA 70124.
The development of cotton fibers can be divided into three specific stages, initiation, elongation and maturation. Each of these stages is characterized by a reorganization of cellulose microfibrils in the developing cell wall. During initiation the microfibrils are arranged in a random manner. The microfibrils orient transverse to the long axis of the fiber during elongation and during maturation, which is characterized by the onset of secondary wall synthesis, the cellulose microfibrils are arranged in a steep helical pattern with respect to the long axis of the fiber. The reorientation of cellulose microfibrils is correlated with a nearly identical reorientation of cortical microtubules within the cytoplasm of the developing fiber, suggesting that the cytoskeleton may be controlling the organization of microfibrils in the cell wall.
The effects of plant growth regulators on fiber development and cellulose microfibril organization are being tested on *in vitro* grown cotton fibers. Treatment with abscissic acid (ABA) limits fiber growth and appears to cause a premature reorientation of cellulose microfibrils from a transverse orientation to a helical pattern. This reorientation of cellulose microfibrils is also correlated with a reorientation of microtubules. Because it appears that the microtubules are involved in the ABA induced reorganization of cell wall microfibrils, microtubule preparations from ABA induced and uninduced fibers are being analyzed for changes in expression of tubulin and microtubule associated proteins.

A 204 SECRETED PROTEINS IN EMBRYOGENIC SUSPENSION CULTURES OF NORWAY SPRUCE (*PICEA ABIES*)

Ulrika Egertsdotter, Håkan Mo and Sara von Arnold, Dept. of Forest Genetics, Swedish University of Agricultural Sciences, Box 7027, S - 750 07 Uppsala, Sweden.

Embryogenic cultures are maintained as different cell-lines. Each cell-line originates from one zygotic embryo, and generally shows characteristics typical for the cell-line. The types of cell-lines differ in many aspects, notably maturation of the somatic embryos. Only cell-lines consisting of large, well organized embryos (type A) can go through maturation and develop plantlets, whereas the cell-lines comprised of smaller and comparatively less organized embryos (type B) cannot. Secreted proteins concentrated from the suspension culture medium of a type A cell-line have earlier been found to alter the morphology of type B embryos towards more organized structures. The protein content of the culture medium was examined by SDS PAGE, and secreted proteins were detected by in vivo labelling experiments. In general, a total of 25 secreted proteins can be detected in the suspension culture medium. Four secreted proteins of 26 kD, 62 kD, 66 kD and 85 kD appear specifically in the medium from the type A cell-line, and these cannot be found in cell-lines of the B type. By N-terminal sequencing of the 20 first amino acids, the 26 kD protein secreted to the medium of the type A cell-line showed 60 % homology to zeamatin, an antifungal protein found in several monocot species.

A 205 SECONDARY CELL WALL PATTERNING IN TRACHEARY ELEMENTS IS PARTLY MEDIATED BY SELF-ASSEMBLY,Candace H. Haigler, Linda T. Koonce, and James G. Taylor, Department of Biological Sciences, Box 43131, Texas Tech University, Lubbock, TX 79409

It has long been known that banded arrays of cortical microtubules play a critical role in establishing the pattern of tracheary element secondary cell wall thickenings. By use of *tracheary elements* differentiating in suspension culture (from mesophyll cells of *Zinnia elegans*), we have obtained evidence that the critical role of microtubules occurs very early in secondary wall deposition. Once localized cellulose microfibril deposition has been initiated, the microtubules are no longer required for wall patterning to be sustained. Three kinds of supporting evidence will be presented: (a) analysis of tracheary elements formed after sequential addition of the microtubule antagonist, amiprophosmethyl, during the onset of semi-synchronous differentiation; (b) changes in appearance of cortical microtubules as differentiation progresses; and (c) maintenance of a plasma membrane associated antigen in a pattern even after addition of amiprophosmethyl. Such an early role for microtubules is consistent with alterations in secondary wall composition induced by addition of the cellulose synthesis inhibitors dichlorobenzonitrile (DCB) or isoxaben. Tracheary elements formed in the presence of these drugs have patterned secondary walls that have little or no cellulose and xylan in the thickenings. Xylan appears to be synthesized, but it diffuses away from the thickening sites in the absence of cellulose. In contrast to normal tracheary elements with patterned lignin, lignin is dispersed in tracheary elements that lack cellulose and xylan in the thickenings. These results suggest the following model, reflecting partial self-assembly of the secondary cell wall: (a) cortical microtubules set in place a molecular template that can maintain the localized synthesis of cellulose in their absence; (b) the localization of cellulose mediates the fixation of xylan and possibly other wall components into the thickenings; and (c) one or more normal components of the secondary cell wall is required for patterned localization of lignin. This research was supported by NSF grants DCB-87-10243 and 91-06704 to CHH.

A 206 EFFECT OF XYLOGLUCAN OLIGOSACCHARIDES ON THE WALL MORPHOLOGY OF CELLS CULTURED IN SUSPENSION, Jean-Paul Joseleau, Gérard Chambat, Ahmed Faïk, Katia Ruel, Centre de Recherches sur les Macromolécules Végétales, (CERMAV), CNRS, associé à l' Université Joseph Fourier, B.P. 53 X, 38041 Grenoble cedex, France.

The development of the walls of cells grown in suspension is under the intrinsic control of the cell and of the external medium, and this is regulated *via* endogenous and exogenous signals. Recent work on the biological activity of cell wall originating oligosaccharides has shown that oligosaccharides derived from the hemicellulosic xyloglucans had significant growth-regulating function and accumulated in the culture medium of suspension-cultured cells (1). In the present work the accumulation of xyloglucans and xyloglucan oligosaccharides in the culture medium of *Rubus fruticosus* cells in suspension was followed during a cell culture cycle (2). The presence in the medium of numerous hydrolases could account for oligomers formation from the extracellular xyloglucans. The origin of the latter as a result of ageing of the cells was studied by electron microscopy with immunocytochemical techniques. The activity of xyloglucan fragments as elicitors or activators of wall-bound glyco and glycanohydrolases was demonstrated and their effect on the morphology of the cell walls was examined at the ultrastructural level. 10 Minutes after addition of the nonasaccharide XG9 at about 10^{-8} M concentration the external surface of the cell walls showed evidence of delamination and defibrillation with the appearance of an abundant material which detached from the wall into the culture medium. This material could be identified by immunocytochemistry as rich in xyloglucan. This modification of the wall surface of 10 day old cells after XG9 treatment resemble that of 27 d old untreated cells, suggesting that XG9 had an ageing effect on the cell walls. The fate of the added oligosaccharide was examined by use of a chemically activated XG9 which could thus be specifically reacted for electron microscopy examination. It appeared that after 10 to 30 minutes of contact with the culture, XG9 remained associated to the surface of the cell with virtually no penetration in the wall.

(1) McDougall G.J. and S.C. Fry (1991). J. Plant Physiol. 137 : 332-336.
(2) Joseleau J.P., N. Cartier, G. Chambat, A. Faïk and K. Ruel (1992). Biochimie, 74 : 81-88.

A 207 ARABINOGALACTAN-PROTEINS ARE ESSENTIAL IN SOMATIC EMBRYOGENESIS OF *DAUCUS CAROTA* L.,

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Daucus carota L. cell lines secrete a characteristic set of arabinogalactan-proteins (AGPs) into the medium. The composition of this set of AGPs changes with the age of the culture. This is determined by crossed-electrophoresis with the specific AGP binding agent, the ß-glucosyl Yariv reagent.

Addition of AGPs isolated from the medium of a non-embryogenic cell line to an explant culture initiated the development of the culture to a non-embryogenic cell line. Without addition of AGPs or with addition of carrot seed AGPs an embryogenic cell line was established.

Three months old embryogenic cell lines usually contain less than 30% of dense, highly cytoplasmic cells, i.e. the embryogenic cells. When carrot seed AGPs were added this percentage increased to 80%.

Addition of carrot seed AGPs to a 2 years old, non-embryogenic cell line resulted in the reinduction of embryogenic potential.

These results show that specific AGPs are essential in somatic embryogenesis and are able to direct development of cells.

A 208 VITRONECTIN-LIKE PROTEINS AND mRNAS IN PLANTS I, Elizabeth M. Lord and Linda L. Walling, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124

Substrate adhesion molecules, SAMs, are extracellular matrix glycoproteins which confer dynamic capabilities to animal matrices. One such SAM, vitronectin (VN), is involved in a number of processes including cell movement in animals. We have reported the presence of a vitronectin-like molecule in all organs of the plant using human VN antibodies and cDNA probes (Sanders *et al.*, 1991). Hybridizations with a human vitronectin cDNA probe and genomic DNA from *Vicia faba*, *Glycine max*, and *Lycopersicon esculentum* revealed vitronectin-like sequences. Human vitronectin antibodies detected vitronectin-like proteins in leaf and root protein extracts from *Lilium longiflorum*, *V. faba*, *G. max*, and *L. esculentum*. In addition, immunocytochemical staining of frozen sections of *L. longiflorum* leaf and *V. faba* gynoecium demonstrated that vitronectin-like proteins were localized to the ECM on the cell surface. A model is provided, describing how a plant SAM could function to facilitate pollen tube extension in the style. Here, we show that in lily, two VN-like proteins are heterogeneous. A 55-kD and a 41-kD protein consist of three isoforms in roots and leaves with an additional two isoforms of the 41-kD protein occuring in the roots alone. Heterogeneity in these VN-like proteins is also demonstrated by the identification of two species of VN-like mRNA, both in lily leaves and soybean roots. We have used different preparations of human VN antisera to identify these two proteins. Goat anti-human VN serum strongly recognizes the 41-kD protein, while rabbit anti-human VN serum recognizes the 55-kD protein. Monospecific antibodies to either 41-kD or 55-kD root proteins cross react with human VN, but the reciprocal is the case only for the 55-kD protein. We are continuing our efforts to establish that the 41-kD protein is recognized by human VN antibodies, and we are in the process of sequencing both proteins. Preliminary data show that the 55-kD protein is highly hydrophobic. We are also producing antibodies from both plant proteins for immunogold labeling of tissues at the TEM level and for the screening of expression libraries.

A 209 AN ARABIDOPSIS CELL MUTANT AFFECTING CELL WALL PROPERTIES

Regina S. McClinton, Valerie Vreeland, Z. Renee Sung. Department of Plant Biology, University of California, Berkeley, Berkeley, California 94720.

teratoma is a single gene recessive mutant of Arabidopsis thaliana, generated by both EMS and gamma-ray mutagenesis. *teratoma* makes the same organs as wild type Arabidopsis, at the correct time and position, but the organs are not correctly shaped. *teratoma* is very reduced in height, has lobed leaves, and roots that are short, thick, and look callused, all causing it to resemble a brussel sprout. *teratoma* is also unable to grow in soil, and is sterile. *teratoma* has improperly shaped cells, cuboidal and non-elongating, which is distinguishable in heart stage embryos. Thus the failure to establish proper cell shape results in incorrect organ shape, but does not alter the plant's ability to follow the genetic pattern of organogenesis. Tissue prints done with antibodies against pectin show that *teratoma* pectin is both more soluble and more highly methyl esterified than wild type Arabidopsis. Also, *teratoma* produces short polygalcturonic acid fragments not seen in wild type Arabidopsis. The Teratoma gene has been mapped to chromosome 5, and is closely linked to the marker gene ttg. We are working to isolate the gene, in hopes of determining the nature of the mutation. It will most likely be one of these classes: cytoskeletal, carbohydrate enzyme, or regulatory.

A 210 A POSSIBLE ROLE FOR COATED PITS IN PLANT CELL WALL SYNTHESIS, Brent G. Mersey, College of Biological Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

Since the mid-1960's ultrastructural studies have demonstrated that coated pits are ubiquitous in plant cells, yet even today, their function remains unknown. Ultrastructural studies correlated the presence of coated pits with newly formed cell wall. Biochemical studies showed that coated vesicle fractions were specifically enriched in ß, 1-4, glucan synthetase, probably involved in the synthesis of either xyloglucan or cellulose. Coated pits at the plasma membrane are closely associated with cortical microtubules. Animal studies have reported the presence of tubulin in coated vesicle fractions. The orientation of newly synthesized cellulosic microfibrils parallels cortical microtubules. This has lead to the hypothesis that microtubules on the cytoplasmic side of the plasma membrane are somehow involved in guiding the deposition of the cellulosic component of the cell wall. Finally, although functional cellulose synthetase has never been purified, there is ample evidence from biochemical and freeze-etch ultrastructural studies that synthetically active cellulose synthetase is a multi-unit protein complex organized as a "rosette" which spans the plasma membrane. The diameter of the cellulose synthetase rosette would fit precisely within a typical plasma membrane coated pit. Therefore, clathrin-coated pits may be the means by which cellulose synthetase rosettes are organized as a functional complex in the plasma membrane and linked to cortical microtubules which orient the cellulose microfibrils of the newly forming cell wall.

A 211 THE EXPRESSION OF INOSITOL-CONTAINING GLYCOLIPIDS ASSOCIATED WITH THE PLANT CELL SURFACE IN PEA ROOT NODULES IS REGULATED DURING NODULE DEVELOPMENT, Silvia Perotto1, Bjorn Drobak2, Neil Donovan2 and Nick J.Brewin2, 1 Department of Plant Biology, University of Turin, Italy, 2 Department of Genetics, John Innes Institute, Colney Lane, Norwich, UK.

Pea root nodules are symbiotic plant organs which provide a useful model to investigate the expression of plant cell surface antigens involved in plant/microbe interactions and in the process of plant development. A novel class of plant cell surface components was identified in pea root nodules using monoclonal antibodies (McAb) as molecular probes. The biochemical characterisation of the JIM 18 antigen suggests a molecular structure related to glycosyl-phosphatidyl inositols (GPIs), a class of inositol-containing membrane glycolipids not previously described in plants. An antigen of this class of components, recognised by JIM 18 McAb, was found to be associated with the plasma membrane and with the plant-derived membrane surrounding endosymbiotic bacteria in the pea nodule, the peribacteroid membrane. The expression of the JIM 18 epitope on the peribacteroid membrane was regulated during the development of the symbiotic compartment. It was in fact present on the peribacteroid membrane surrounding early released symbiotic bacteria, but it could not be detected in a slightly more differentiated stage of symbiotic relationship. However, infected cells that had lost expression of the JIM 18 epitope on the peribacteroid membrane, still retained this epitope on their plasma membrane. The early and specific loss of the JIM 18 epitope from the peribacteroid membrane suggests that it may be involved in the structural and functional differentiation of the plant glycocalyx associated with this plant membrane during nodule development.

A 212 CHARACTERIZATION OF A cDNA CLONE FROM *Arabidopsis* HIGHLY HOMOLOGOUS TO A LAMININ-RECEPTOR, Rafael Pont Lezica[1], Claude Bardet[2], Bernard Lescure[2] and Michèle Axelos[2].
[2]Laboratoire de Biologie Moleculaire des Relations Plantes-Microorganismes, GDR 1003-CNRS, Castanet-Tolosan and [1]URA 1457 CNRS/UPS, Toulouse, France.

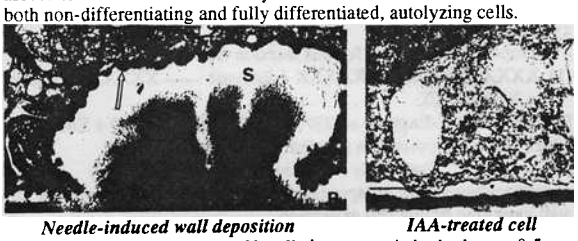
Laminin, a major constituent of animal basement membranes, interacts with cells through different cell-surface glycoproteins. Laminin-receptors cloned from diverse mammals code for highly conserved proteins of about 33 kD.
A cDNA clone (1091 bp) was isolated from an *Arabidopsis* cell culture library, covering an open reading frame for a protein of 298 amino acids. Two hundred residues at the amino terminus of the *Arabidopsis* protein show extensive homology (88%) to mammal laminin receptors (human, mouse, bovine) as well as to a protein from the freshwater celenterate hydra (76 %). No homology was found between the carboxy-terminal residues from plants, hydra and mammals. Putative Asn-glycosylation, phosphorylation and myristoylation sites are found on the sequence.
Animal laminin receptors are highly expressed on transformed cells as it is their plant homologue (3 %). On the other hand the hydra receptor is associated with the cytoskeleton, the function of the plant laminin-like receptor is unknown and experiments are been carried out to understand the role of this protein in plant cells.

A 213 PRIMARY SUSPENSION CULTURES FROM *ZINNIA* AS A MODEL FOR INVESTIGATING THE REGULATION OF MESOPHYLL CELL EXPANSION. Alison W. Roberts, Marie Goguen, Benjamin Knight, Botany Department, University of Rhode Island, Kingston, RI 02881

Despite the importance of the palisade mesophyll as the primary photosynthetic tissue of most dicots, little is known about the mechanisms that control the extent and direction of cell expansion during development, and thus give rise to an efficiently functioning tissue. Leaf area expansion is controlled by the mechanical properties of epidermal cell walls, but the expansion of mesophyll cells follows a different time course and must be regulated independently. The deficiency of research in this area is due in part to a lack of a convenient methodology. We are using primary suspension cultures of *Zinnia* mesophyll cells as a model for investigating mesophyll cell expansion. Cell expansion has been quantified by computer image analysis of individual cells. Mesophyll cell expansion *in vivo* has also been quantified for comparison with the expansion rates of cultured cells. Individual *Zinnia* mesophyll cells can be released from leaves by gentle maceration yielding a suspension with cell walls unaltered by hydrolytic enzymes. Unlike serially transferred suspension cultures, these cells expand in response to auxin. Cultured mesophyll cells are also induced to expand by light, cytokinin, and buffering at pH 5.5-6.0. Increasing the solute concentration of the culture medium with mannitol inhibits cell expansion. Preliminary experiments indicate the cells isolated from mature leaves expand in culture at the same rate as cells isolated from unexpanded leaves when the culture media contain auxin and cytokinin. Experiments are underway to determine the minimum auxin and cytokinin concentrations required to induce expansion.

A 214 ULTRASTRUCTURAL CHANGES ASSOCIATED WITH SECONDARY-WALL DEPOSITION DURING INDUCED TRACHEID DIFFERENTIATION IN CONIFERS, *Rodney A. Savidge*, Department of Forest Resources, University of New Brunswick, Fredericton, NB, E3B 6C2, Canada, and *John R. Barnett*, School of Plant Sciences, University of Reading, Whiteknights, Reading RG6 2AS, UK.

A pair of needles on a dormant stem cutting of *Pinus contorta* Dougl. induced tracheid differentiation in cambial cells at the junction of needle trace with the vascular cambium, differentiation occuring in the absence of preceding cell division or cell expansion and progressing both apically and basally. Secondary walls were deposited as ribs of annular and spiralled thickenings and also as structures resembling bordered pits but devoid of margo and torus. The walls lacked the tri-laminar structure of normal secondary-xylem tracheids, but deposition of oriented cellulose microfibrils nevertheless occurred at the plasmalemma. Cortical microtubules were not seen by transmission electron microscopy (glutaraldehyde, OsO_4 fixation; uranyl-acetate, lead-citrate staining) at any stage of secondary-wall growth. The cytoplasm of differentiating tracheids was enriched in mitochondria and polyribosomes, and the endomembrane system was more active than in either non-differentiating or auxin- (indol-3-ylacetic acid, IAA) treated cells. Plasmalemma at sites of wall growth was in dynamic flux with vesicles derived from both rough endoplasmic reticulum (ER) and dictyosomes. Electron-dense exocytotic bodies were invariably associated with active secondary-wall formation and were absent from both non-differentiating and fully differentiated, autolyzing cells.



Needle-induced wall deposition ***IAA-treated cell***

P, 1° wall; S, developing 2° wall; ⇑, exocytotic body; bars = 0.5 µm.

A 215 AUXIN-INDUCED CELL-WALL PROTEINS IN MAIZE COLEOPTILE SEGMENTS, Thomas Schindler & Peter Schopfer, University of Freiburg, Botanical Institute II, Schaenzlestr.1, D-7800 Freiburg, Germany.

Addressing the still unresolved question of how auxin stimulates growth we started from two observations. Clearly protein synthesis is required for the induction of auxin-induced growth and the rapid growth response (12 - 15 min) must be accompanied by rapid changes in the cell-wall extensibility i.e. structure. Therefore three protein fractions are investigated. One fraction of particular interest are the highly glycosylated Arabinogalactan Proteins (AGP), furthermore a fraction of salt-extractable cell-wall glycoproteins and a fraction of unextractable proteins in the cell wall are under investigation. Tracer experiments with labeled arabinose, fucose, galactose, proline and leucine were performed. Results: AGPs: Neither fucose nor leucine prooved to be good markers for buffer-soluble AGPs as identified by Yariv-reagent and binding of the anti-AGP antibody MAC 207. Galactose labeled AGPs less specifically than proline and arabinose. In no case a substantial effect of hormone treatment (after 0.5 h, 1h, 2h) could be detected. Salt-extractable cell-wall polymers: No hormone effect on the incorporation of arabinose, fucose and galactose could be detected. Using leucine and proline an increase in radioactivity of 40 % and 30 % respectively, above the control (H_2O) was found. For leucine this was the case for both 0.5 h and 1 h treatment with 10^{-5} mol l^{-1} IAA. SDS-PAGE analyses and subsequent autoradiography revealed that several proteins in the range of 30 - 80 kDa are induced. Cell-wall bound polymers: With fucose and galactose no effect of hormone treatment could be detected. After incubation with arabinose and proline a minor effect of increased residual radioactivity after hormone treatment was obtained. With leucine a substantial increase (30 % - 40 %) could be observed both after 0.5 h and 1 h IAA treatment. Conclusion: Auxin induces a rapid incorporation of leucine in the cell wall in comparison with the control (H_2O). The majority of the leucin radioactivity is not extractable with 4 M salt thus indicating a covalent linkage in the cell wall.

A 216 TIP SYNTHESIS VERSES INTERCALARY GROWTH IN YOUNG DEVELOPING COTTON FIBERS, Robert W. Seagull, Southern Regional Research Center, USDA/ARS, New Orleans, LA. 70124

Developing cotton fibers provide a good model system for the study of cell extension and wall development. Fibers are single cell extensions, derived from the epidermal cell layer of the growing seed. The literature documents circumstantial evidence to support that cotton fiber exhibits growth characteristics that are consistent with cell expansion via both intercalary growth and tip synthesis. The observations that support intercalary growth include deposition of new wall material along the entire length of the cell and the apparent reorientation of wall microfibrils in the primary wall during fiber elongation. Observations that support tip synthesis include intense incorporation of wall material in the tip region of the cell and continued fiber elongation after the initiation of secondary cell wall synthesis. The relative contributions of these mechanisms to final fiber length has yet to be determined.

A quantitative ultrastructural analysis was conducted to provide further documentation of the growth mechanism(s) contributing to the final length of the cotton fiber. Young cotton fibers, between 3 and 7 days post anthesis (DPA) were examined with electron microscopy, using both conventional glutaraldehyde fixation and rapid freezing-freeze substitution (RF-FS) fixation. Quantitative analysis of organelle distribution in the tip and sub-apical regions of developing cotton fibers shows that both Golgi bodies and mitochondria are evenly distributed throughout the cytoplasm, except for the extreme end of the cell (within 1 um of the tip). Secretory vesicles appear to bind preferentially at the apex of the cell. Microfibrils are transversely oriented (mimicking the microtubule organization) in the inner most layer of the wall and axially oriented in the outer most wall. The even distribution of organelles in the fiber and the organization of wall microfibrils are consistent with an intercalary mechanism of fiber elongation. The distribution of secretory vesicles is consistent with a tip synthesis mechanism of fiber elongation. Therefore, during the early stages of elongation, fibers grow via a combination of tip and intercalary synthesis.

A 217 REAL TIME ASSESSMENT OF CELL WALL ION BEHAVIOR AND SWELLING BY ELECTRICAL GAUGING, **Ilan Shomer, Department of Food Science, Agricultural Research Organization, The Volcani Center, P.O.Box 6, Bet Dagan 50250, Israel**

Direct electric measurements of potato tuber single ghost cells supported evidence for the existence of an electric potential in plant cell wall (CW), while eliminating the disturbance of viable protoplasmic compartments. The electric potential exhibited a typical cation exchange response, namely the Donnan potential. The potential was sensitive to electrolyte concentration, valence of the cations and the pH. CW potentials rendered by changes in electrolyte concentrations were 3, 3, 25 and 840 mV/mM for Na^+, K^+, Ca^{++} and Al^{+++} cations, respectively. NaCl resulted in a slightly higher potential than KCl, and $MgCl_2$ resulted in a higher potential than $CaCl_2$. According to the Donnan concept , a lower potential indicates a slightly stronger attraction of the cation with the anionic groups present in the CW. Potentials measured with sulfate anion salts with the same cation were significantly more positive than those measured with chloride anion, indicating a balance between negative and positive charges. Donnan potential increased with the pH, indicating the presence of carboxylic groups in the CW. The Donnan potential described above could serve as a tool to characterize behavior of CWs.

A 218 PROTEINS IN THE CELL WALLS OF ELONGATING AND MATURE COLEOPTILES OF RICE, Liz Smith[1], Ershen Lin[1], Brian Atwell[1] and Fiona Corke[2], [1] School of Biological Sciences, Macquarie University, NSW, 2109, Australia, [2] Department of Cell Biology, John Innes Institute, Norwich, NR4 7UH U.K.

The role of proteins in cell elongation is being investigated in rice coleoptiles which have a zone of elongating cells adjacent to the seed. Tissues more than 6mm from the seed do not grow. Cell walls prepared from these respective tissues (Atwell and ap Rees, 1986) have been extracted with 0.5% (w/v) SDS and proteins separated electrophoretically. The patterns obtained show that the cell walls which are growing have an abundant complement of proteins in the 10-20 kD range; these proteins become very scarce once the cells cease growing. Antisera to various cell wall protein fractions (< M_r 20kD) have been raised and immunofluorescence studies show recognition of cell walls. Western blots confirm that the antisera recognise primarily proteins in the walls of elongating cells.

One of the antibodies was used to screen an etiolated rice cDNA library, producing at least one positive clone of around 0.5 kb. cDNA from the positive clone was then used to probe Northern blots prepared from growing and mature zones of coleoptiles, primary leaves and roots. An mRNA species which was present at high levels in the growing zone of coleoptiles was almost undetectable in mature tissues of coleoptiles. The same mRNA species was also detected in growing leaf bases. Evidence for this tissue-specific pattern of gene expression and the sequence of the clone will be presented.

Atwell, B.J. and T. ap Rees (1986) J. Plant Physiol. 123: 401-408.

A 219 XYLOGLUCAN ENDOTRANSGLYCOSYLASE ACTIVITY IN THE APOPLAST OF PLANT CELL SUSPENSION CULTURES, R.C. Smith[1], S.C. Fry[2]. [1]Plant Science Centre, ANU, Canberra, Australia; [2] ICMB, University of Edinburgh, Scotland, UK.

Xyloglucan molecules are beleived to form load-bearing crosslinks between cellulose microfibrils in the plant primary cell wall (Hayashi and Maclachlan, 1984). Cleavage of these crosslinks is thought to loosen the wall, enabling cell expansion. The possibility that loosening may involve transglycosylation reactions has been proposed(Albershiem, 1976).

We report the presence of a xyloglucan endotransglycosylase activity in the apoplast of plant cell suspension cultures.

Spinach (*Spinacia oleracea* L.) cell suspension cultures were incubated with [*reducing terminus*-^{3}H]xyloglucan nonasaccharide (XG9: Glc_4, Xyl_3, Gal, Fuc) for up to 72h. During this incubation negligible [^{3}H]XG9 was brocken down, the majority of ^{3}H-labelled material remained soluble and extracellular. The major ^{3}H-labelled product formed was a high molecular weight, soluble, apoplastic polymer (molecular weight by g.p.c., 20-30kD). [^{3}H]XG9 underwent little re-arrangement on incorportion into the polymeric product, and could be released from the product by *Trichoderma* cellulase digestion. This suggests that the high molecular weight ^{3}H-labelled product was not synthesised within the protoplasm of the cells; and that [^{3}H]XG9 became associated with a soluble apoplastic polymer via (or at least near to) a cellulase labile bond. The high molecular weight incubation product was found to bind to cellulose, being removed by 1M-NaOH, in a similar manner to xyloglucan.

The proposed mechanism for this reaction is as follows:

(1). XXXXXXX-Glc-XXXXXXX + Enzyme —— XXXXXXX-Enzyme + XXXXXXX

(2). XXXXXXX-Enzyme + XG9 —— XXXXXXX-XG9 + Enzyme

where, XXXX represents a segment of xyloglucan.

We propose that this reaction is catalysed by xyloglucan endotransglycosylase; an apoplastic enzyme which may contribute to cell wall loosening during cell expansion.

Hayashi and Maclachlan (1984) Plant Physiol. 75:596-604: Albersheim (1976) In: Plant Biochemistry, eds J.Bonner and J.E. Varner, pp. 225-247

A 220 ISOLATION AND DIFFERENTIAL EXPRESSION OF PROLINE-RICH CELL WALL PROTEIN GENES IN *ARABIDOPSIS*, Mary L. Tierney, Thomas J. Fowler, June Traicoff, Agronomy Department and Biotechnology Center, Ohio State University, Columbus, OH 43210

Proline-rich proteins (PRPs) are plant cell wall proteins presumed to have a structural function by the nature of their predicted rigid conformations and their demonstrated ability to be insolubilized in the cell wall matrix. In a species, there can be several PRP genes that are regulated differentially by growth, development, and environmental signals. These selective patterns of expression are thought to reflect the participation of PRPs in the formation cell-type specific matrices within the cell wall. We have isolated four PRP clones from an *A. thaliana* ecotype *Landsberg* genomic library and are using these clones to dissect the differential expression of PRPs during development, using a molecular genetic approach. Each clone corresponds to one of four distinct *Eco*RI restriction fragments identified by genomic Southern blot analysis. The four clones can also be subdivided by their cross-hybridization strengths into two groups (AtPRP1 and AtPRP2), each having two members. Based on DNA sequence analysis, one AtPRP1 group clone has a derived amino acid sequence containing imperfect repeat units of PPVYTPPVHKPTLP. In contrast, an AtPRP2 group clone has a derived amino acid sequence with a motif of PIYKPPV in the carboxy-terminal 127 amino acids of the open reading frame linked to approximately 100 amino acids of derived sequence at the amino-terminus that lacks the repetitive unit. Probes constructed from each of the two sequenced clones were used in northern blot hybridization analysis. Strand-specific probes were used to show that the mRNAs encoded by these two clones correspond to the proline-rich open reading frames. The AtPRP1 probe hybridized to an approximately 1480 nt transcript in total RNA preparations from roots, while the AtPRP2 probe hybridized to a similarly sized transcript in total RNA preparations from 2-6 day old dark grown seedlings, and from flowers and stems. We will be determining the expression pattern of these genes in other tissues and at several times during development to characterize these genes more fully.

A 221 THE INTERCELLULAR ADHESION IN PLANTS IS NOT MEDIATED BY "EGG BOXES", P. Van Cutsem and F. Liners, Unité de Biotechnologie théorique, Facultés Universitaires de Namur, Rue de Bruxelles 61, B-5000 Namur, Belgium.

A monoclonal antibody has been raised to the supramolecular conformation of homopolygalacturonic acid chains dimerized through calcium cations, probably according to the "egg box" model (Grant GT., Morris ER., Rees DA., Smith PJC. and Thom D, 1973 FEBS Lett 32: 195-198). It recognizes oligomers of DP $\geq$ 9, and PGA with a degree of methylesterification of 30% (random) or 40% (blockwise) (Liners F, Thibault J-F and Van Cutsem P, 1992 Plant Physiol. 99: 1099-1104). Immunogold labelling of EM sections of suspension cultured cells of *Daucus carota* showed that PGA dimers were essentially located on the middle lamella material expanded at cell corners or lining intercellular spaces and on all parts of the cell walls in contact with the external medium, but not in primary walls. Middle lamellae far from junction zones and intercellular spaces were not recognized. However, all layers of the cell walls were labelled after on grid enzymatic deesterification. Walls of dead cells were heavily labelled, indicating a pectin methylesterase release. The pattern of labeling was the same in root apices, the root cap mucilage being also labelled. These observations suggest that either calcium-associated pectin dimers do not contribute to the wall stability (Liners F and Van Cutsem P, 1992 Protoplasma *in press.*), or the deesterified pectins are readily degraded to loosely held oligomers.

A 222 PROLINE-RICH PROTEINS IN THE EXTRACELLULAR MATRIX OF LEGUME ROOT NODULES, Kathryn A. VandenBosch, Sandra G. McLain, and Gail S. Taylor, Department of Biology, Texas A&M University, College Station, TX 77843

Legume roots show several distinct early responses to *Rhizobium* inoculation, including root hair curling, induction of cell division in the root cortex, and infection thread formation. All three of these responses are accompanied by new wall synthesis, and differentiation of the affected cells is accompanied by the expression of some putative wall protein genes, ENOD2 and ENOD12. The predicted amino acid sequence of these two early nodulins indicates that they encode proline-rich proteins (PRPs), but the proteins themselves have not been isolated or shown to be targeted to cell walls. We have used polyclonal antibodies against PRP2 from soybean (1,2) to identify proline-rich proteins and to localize them in pea nodules. On Western blots, both antibodies recognize prominent nodule proteins at approximately 110, 70, and 55 kDa, and several abundant polypeptides between 20 and 32 kDa. Immunocytochemical analysis reveals expression of PRPs in multiple cell types; the proteins are targeted to distinct wall layers which varies with cell type. In newly infected cells and the nodule inner cortex, tissues shown to express ENOD2 or ENOD12, PRPs are found within the intercellular space or its analog, the infection thread matrix. Several immunoreactive proteins are also made by *Rhizobium* These are found at the cell surface of mature bacteroids, and can be extracted from cell fragments by a high salt buffer. Work is underway to isolate these proteins to verify that they are PRPs.

1. Bradley, D.J., et al. 1992. Cell 70, 21-30.
2. Marcus, A., et al. 1991. Physiol. Plant. 81, 273-279.

A 223 FORMATION OF *FUCUS* CELL WALL ADHESIVE, Valerie Vreeland,[1] Eva Grotkopp,[1] Susan Espinosa[2], Daniel Quiroz[2], Watson M. Laetsch[2] and John West[2], [1]Department of Plant Biology, University of California, Berkeley, CA, USA 94720, and [2]Department of Acuicultura, Faculty of Marine Sciences, University of Antofagasta, Antofagasta, Chile.

Adhesion between plant cells is critical for morphogenesis and wall properties. The *Fucus* zygote provides a unique system for investigating developmentally-regulated wall adhesive formation in a single plant cell. This zygote adheres to intertidal substrates a few hours after fertilization triggers wall assembly. Binding of colored microspheres to the zygote surface enabled localization and quantitative measurement of adhesion.

The adhesive process begins with patchy secretion of sticky fibers which remain attached to the wall. An adhesive mucilage coating forms as more fibers are produced. This wall glue binds nonspecifically to materials as different as glass and teflon. Carbohydrate and phenolic compounds were secreted into the medium during initial adhesion, and clumps of sticky material were detected in culture medium.

We propose a mechanism in which vanadate-requiring haloperoxidase catalyzes the formation of phenolic crosslinks between wall carbohydrates and positions phenolic compounds along wall polymers as multiple nonspecific adhesive sites. Alginate and fucans provide soluble wall carbohydrate fibers, and phloroglucinol-based phenolics provide both crosslinks and adhesive sites. Localization and experimental perturbation results support roles for vanadate peroxidase, phenolic compounds and alginate in adhesion. *In vitro* reconstitution results show that carbohydrate crosslinking and adhesiveness can occur by a fiber-phenolic-catalyst mechanism.

A 224 VITRONECTIN-LIKE PROTEINS AND mRNAS IN PLANTS II, Linda L. Walling and Elizabeth M. Lord, Department of Botany and Plant Sciences, University of California, Riverside, CA 92521-0124

Substrate adhesion molecules, SAMs, are extracellular matrix glycoproteins which confer dynamic capabilities to animal matrices. One such SAM, vitronectin (VN), is involved in a number of processes including cell movement in animals. We have reported the presence of a vitronectin-like molecule in all organs of the plant using human VN antibodies and cDNA probes (Sanders *et al.*, 1991). Hybridizations with a human vitronectin cDNA probe and genomic DNA from *Vicia faba*, *Glycine max*, and *Lycopersicon esculentum* revealed vitronectin-like sequences. Human vitronectin antibodies detected vitronectin-like proteins in leaf and root protein extracts from *Lilium longiflorum*, *V. faba*, *G. max*, and *L. esculentum*. In addition, immunocytochemical staining of frozen sections of *L. longiflorum* leaf and *V. faba* gynoecium demonstrated that vitronectin-like proteins were localized to the ECM on the cell surface. A model is provided, describing how a plant SAM could function to facilitate pollen tube extension in the style. Here, we show that in lily, two VN-like proteins are heterogeneous. A 55-kD and a 41-kD protein consist of three isoforms in roots and leaves with an additional two isoforms of the 41-kD protein occuring in the roots alone. Heterogeneity in these VN-like proteins is also demonstrated by the identification of two species of VN-like mRNA, both in lily leaves and soybean roots. We have used different preparations of human VN antisera to identify these two proteins. Goat anti-human VN serum strongly recognizes the 41-kD protein, while rabbit anti-human VN serum recognizes the 55-kD protein. Monospecific antibodies to either 41-kD or 55-kD root proteins cross react with human VN, but the reciprocal is the case only for the 55-kD protein. We are continuing our efforts to establish that the 41-kD protein is recognized by human VN antibodies, and we are in the process of sequencing both proteins. Preliminary data show that the 55-kD protein is highly hydrophobic. We are also producing antibodies from both plant proteins for immunogold labeling of tissues at the TEM level and for the screening of expression libraries.

A 225 REGULATED PROLINE-RICH PROTEIN GENE EXPRESSION DURING NODULATION OF *Medicago truncatula*. Robert Wilson and Jim Cooper, Dept. of Biological Sciences, University of California, Santa Barbara, CA 93106

Proline-rich proteins (PRPs) represent a class of cell wall structural proteins which show tissue-specific expression during plant development. Two members of the PRP gene family are specifically expressed during symbiotic root nodule development in legumes. ENOD12 transcripts have been localised to cells nearest the infection thread structures during early nodule development. ENOD2 is expressed somewhat later in the cortical cells adjacent to vascular tissue surrounding the cortex of infected, symbiotically active cells. We have isolated two cDNA clones, in addition to ENOD2, encoding proline-rich proteins from the legume *Medicago truncatula*. One of the clones contains an ORF encoding decameric amino acid repeats consisting of PPVEKPPVYK identifying it as a *Medicago* homolog of Soybean PRP1. The ORF of the second cDNA clone encodes a short stretch of ENOD2-like pentameric repeats (PPHEK/N) at the 5' end and the remainder of the ORF is rich in proline but does not contain repeating peptide motifs. Preliminary Northern Blot analyses show both clones hybridizing to transcripts of sizes 1.35 and 1.15kb from both roots and root nodules. For both clones, the transcript levels are greatly diminished in nodules compared with roots. A developmental Northern Blot is being probed to show when this down-regulation occurs. The spatial expression patterns of the PRP clones are being determined by *in situ* hybridization of root and nodule tissue. To determine whether PRP gene down-regulation is a response to plant-microbe interactions in general or specifically to legume-*Rhizobia* symbioses, PRP gene expression during infection of *Medicago* roots with mycorrhizal fungi is being examined using Northern Blot analyses.

A 226 CHLAMYDOMONAS AS A MODEL SYSTEM FOR STUDYING DITYROSINE CROSSLINKING OF STRUCTURAL HRGPs, Jeffrey P. Woessner and Sabine Waffenschmidt, Dept. of Biology, Washington Univ., St. Louis, MO 63130, Institut für Biochemie der Universität Köln, D-5000 Köln 1, Germany

The unicellular green alga Chlamydomonas reinhardtii, with its easily isolated HRGPs and lack of matrix polysaccharides, has long been recognized as an excellent system for studying cell wall assembly. Early work focused on only one of the two structurally and biochemically distinct cell walls elaborated during the Chlamydomonas life cycle, the vegetative wall. Chaotropes were found to release several extensin-like HRGPs from the crystalline outer layer, and these HRGPs were shown to be capable of self-assembly in vitro.

Now, we have broadened our focus to examine traits that are common to both the zygote and vegetative cell wall. DNA sequencing and immunological studies have revealed that, like higher plant cell wall proteins, repetitive amino acid sequences (e.g., XP_3 and $(SP)_X$) are found in both zygote and vegetative structural wall proteins (Plant Sci. 83 (1992) 65-76). Recently, we have isolated a gene that we believe encodes a component of the insoluble inner layer of the vegetative wall. Repeating units of YGG comprise one domain of this protein, which suggested the possibility of dityrosine crosslinks being involved in insolubilization. Correspondingly, we find a burst of H_2O_2 production and peroxidase activity at the time of vegetative and zygote wall insolubilization. Insolubilization of each wall is retarded by ascorbate or tyrosine, providing further evidence that a Chlamydomonas peroxidase is responsible for dityrosine crosslinking. Ultraviolet and fluorescence spectral analyses of hydrolyzed vegetative inner wall components which have been subjected to cellulose phosphate chromatography indicate that, as in higher plants, the crosslinks are isodityrosine. GC/MS is being done to confirm this identification.

A 227 ROOT GROWTH MAINTENANCE AT LOW WATER POTENTIALS: INCREASED ACTIVITY OF XYLOGLUCAN ENDOTRANSGLYCOSYLASE, Yajun Wu[1], William G. Spollen[1], Robert E. Sharp[1], Stephen C. Fry[2] and Richard Hetherington[2], [1]Agronomy Dept., Univ. Missouri, Columbia, MO 65211; [2]Center for Plant Science, Univ. Edinburgh, Edinburgh, EH9 3JH, U.K.

Root growth is often less inhibited than shoot growth at low water potentials (ψ_w). In previous work with maize primary roots, rates of cell elongation close to the apex were unaffected by ψ_w as low as -1.6 MPa (1). Pressure probe measurements showed that turgor in this region was around 0.7 MPa at high ψ_w, but decreased to 0.3 MPa at a ψ_w of -1.6 MPa (2). Xyloglucan endotransglycosylase (XET) is believed to cleave and re-form bonds between xyloglucan molecules which link cellulose microfibrils, thereby contributing to cell wall yielding under the influence of turgor (3). In this study we found that XET activity: 1) generally correlated with the spatial growth distribution in well-watered roots, and 2) increased greatly in the root growth zone at low ψ_w compared to high ψ_w. The increased XET activity at low ψ_w was evident whether expressed per unit root length, fresh weight, total soluble protein, or cell wall dry weight. Increase in XET activity may thus play an important role in increasing cell wall yielding and allowing continued root elongation at low ψ_w despite incomplete turgor maintenance.

Previous work showed that increased endogenous ABA was required for the maintenance of maize primary root growth at low ψ_w (4). In this study we found that fluridone (an inhibitor of ABA synthesis) largely prevented the increase in XET activity in the root growth zone at low ψ_w. Thus, ABA may play a role in regulating XET activity.

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3. Fry SC, RC Smith, KF Renwick, DJ Martin, SK Hodge, KJ Matthews 1992 Biochem. J *283*:821-828.
4. Saab IN, RE Sharp, J Pritchard, GS Voetberg 1990 Plant Physiol. *93*:1329-1336.

A 228 ROOT MORPHOGENESIS: CORTICAL MICROTUBULES AND CELLULOSE MICROFIBRILS, Tobias I. Baskin[1] and Richard E. Williamson[2], 1: Division of Biological Science, University of Missouri, Columbia, MO 65211, and 2: Plant Science Center, Research School of Biological Sciences, Australian National University, Canberra, ACT 2601, Australia.

Morphogenesis has been studied in shoot internodes where a link between alignments of cortical microtubules, cellulose microfibrils and cell shape has been generally established. However, there are many specific details of this link that remain obscure, for example the mechanism whereby cortical microtubules affect the alignment of microfibrils. As a model system to study morphogenesis, we are using root growth in Arabidopsis because roots grow rapidly, have radial symmetry, are readily accessible to experimentation, have growing cell walls that are not crossed-polylamellate, and because of the possibility of isolating mutants with altered root morphogenesis. We have isolated 25 mutants whose phenotype is to cause extensive radial swelling of the root. These phenotypes are temperature sensitive, appearing fully wild-type at the permissive T, but mutant at the restrictive T. These phenotypes result from single gene, recessive mutations at different loci. The mutants follow different kinetics of radial swelling following induction, and each phenotype has a specific morphology and histology. The mutations do not affect the root cap or root hairs. We are attempting to define the ultrastructural basis of each phenotype. To do so, we are using immunocytochemistry to view the alignment among microtubule arrays; quantitative polarized-light to analyze the alignment of cellulose microfibrils; and cell wall fractionation of pulse-labeled material to measure rates of incorporation into cellulosic and polysaccharide wall components. Results on the mutants have been complemented with experiments where wild-type plants are treated with compounds that disrupt microtubules or microfibrils. Some results from these inhibitor experiments have also indicated that the control of cell shape in the meristem may differ from that exerted in the zone of pure elongation. To date, one mutant appears to result from a reduced synthesis of cellulose but not of other wall polysaccharide fractions. Whether some of the other mutants involve disorganization of either microtubules or microfibrils is now being addressed, and results are expected by the time of the meeting.

Biochemistry of Extracellular Matrix Components

A 300 cDNA CLONING OF AN α-FUCOSIDASE THAT INACTIVATES XYLOGLUCAN-DERIVED OLIGOSACCHARINS, Christopher Augur, Peter Albersheim and Alan G. Darvill, Complex Carbohydrate Research Center and the Department of Biochemistry, University of Georgia, 220 Riverbend Rd, Athens, Ga, 30602-4712 USA

Oligosaccharide fragments released from plant and microbial polysaccharides regulate various biological functions in plants [1]. The demonstrated activities of these oligosaccharins include the ability of xyloglucan oligosaccharides to inhibit auxin-induced elongation of pea stem segments. A xyloglucan oligosaccharide (XG9) has been shown to inhibit auxin-induced growth [2]. Structurally related heptasaccharide XG7 and octasaccharide XG8 (XG9 minus fucose) do not inhibit the elongation. Since the terminal fucosyl residue of XG9 has been shown to be essential for its biological activity, we looked for, detected and purified an α-fucosidase from pea stems. The N-terminal region and several proteinase-released internal peptides of the α-fucosidase were sequenced. The amino acid sequences of the N-terminal region and one internal peptide were used to design redundant oligonucleotides that were utilized as primers in a polymerase chain reaction (PCR). A specific PCR amplification product containing 357 base pairs was isolated and cloned. Sequence analysis of the PCR product demonstrated that it consisted of a reading frame coding for a total of 119 amino acids that encode at least 65% of the mature enzyme. The deduced amino acid sequence included the two peptides used to design the primers for the PCR as well as two other peptides obtained by proteinase digestion of α-fucosidase. No sequence homology to other α-fucosidases was apparent, although the N-terminal region of the α-fucosidase is strongly homologous to Kunitz-type trypsin inhibitors. The PCR product is presently being used to screen pea cDNA and genomic libraries. Characterization of these clones will help in the elucidation of the initial events that regulate oligosaccharin activities.

The work was supported in part by Department of Energy grant DE-FG09-85ER13425, and by the DOE-funded Center for Plant and Microbe Complex Carbohydrates (DE-FG09-87ER13810).

[1] Darvill et al. 1992 Glycobiology, 2, 181-198. [2] Augur et al. 1992 Plant Physiol. 99, 180-185.

A 301 **ACCUMULATION OF POLYGALACTURONASE-INHIBITING PROTEIN (PGIP) IN *Phaseolus vulgaris* L. IS INDUCED BY WOUNDING, ELICITORS, AND INFECTION WITH *Colletotrichum lindemuthianum*.** Carl W. Bergmann[1], Yuki Ito[1], Darrell Singer[1], Peter Albersheim[1], Alan G. Darvill[1], Laurence Nuss[2], Giovanni Salvi[2], Felice Cervone[2], Giulia De Lorenzo[2], and Nicole Benhamou[3], [1]Complex Carbohydrate Research Center and Department of Biochemistry, The University of Georgia, 220 Riverbend Road, Athens, GA 30602-4712, [2]Dipartimento di Biologia Vegetale, Universita di Roma "La Sapienza," Rome, Italy, [3]Département de Phytologie, Université Laval, Québec, Canada.

Fungal *endo*polygalacturonases hydrolyze polygalacturonic acid to mono-, di-, tri- and tetragalacturonic acid. All dicotyledons examined contain a cell wall-associated protein (PGIP) that specifically inhibits fungal *endo*polygalacturonases. The inhibition of fungal *endo*polygalacturonases by PGIP results in an increased half-life of α-1,4-linked oligogalacturonides with degrees of polymerization of 8-20, which function as elicitors of phytoalexins and other plant defense responses[a]. Thus, it has been proposed that PGIP plays an important role in plant resistance to fungal pathogens by optimizing the formation of elicitor-active oligogalacturonides.

The gene encoding *Phaseolus vulgaris* PGIP has recently been cloned and characterized[b]. Using the cloned PGIP gene as a probe, we have demonstrated that the transcription of the gene is induced in suspension-cultured bean cells following addition of elicitor-active oligogalacturonides to the medium. Rabbit polyclonal antibodies (Abs) specific for PGIP have been generated against a synthetic *N*-terminal peptide coupled to the carrier protein KLH. Using the antibodies and the cloned PGIP gene, we have shown that the synthesis of PGIP and its mRNA is induced in *P. vulgaris* hypocotyls in response to wounding or treatment with salicylic acid. We have also demonstrated, using gold-labeled goat anti-rabbit secondary Abs in EM studies, that, in bean hypocotyls infected with *C. lindemuthianum*, the level of PGIP increases in the cells surrounding the infection site. Our data suggest that synthesis of PGIP represents an active defense mechanism of plants regulated by signal molecules (elicitors) known to induce the defense genes in plants. [This work is supported in part by the U.S. Department of Energy (DOE)-funded Center for Plant and Microbial Complex Carbohydrates (DE-FG09-87ER13810, to PA and AD); by DOE grant DE-FG09-85ER13425 (to PA); by the National Research Council of Italy; and by European Community grant BIOT-CT90-0163 (to FC).] [a]Darvill et al., 1992, *Glycobiology* 2:181-198. [b]Toubart et al., 1992, *The Plant J.* 2:367-373.

A 302 ANALYSIS OF TRANSGENIC TOMATO FRUIT WITH ALTERED LEVELS OF CELL-WALL HYDROLYTIC ENZYMES, Jeremy M. Boniwell, Giles Hyder, Jonathon D. Fletcher, Colin R. Bird, and Wolfgang Shuch. ICI Seeds, Jealotts Hill Research Station, Bracknell, Berkshire, RG12 6EY, UK.

Enzymes acting on cell wall components can have a major effect on tomato processing quality, field holding, handling and transport characteristics. Reduction in activity of some enzymes, specifically polygalacturonase(PG) and pectin methyl-esterase(PE), by genetic modification has been shown to have marked beneficial influence on several of these properties. Although the effect of such manipulations on the degree of polymerisation (by PG antisense) and esterification (by PE antisense) of pectic components is profound, and clearly manifested by improvements in the viscosity parameters of processed fruit, effects on whole fruit physiology is so subtle as to be not readily apparent externally. Rapid, high throughput biochemical assays for PG and PE have been developed to screen large numbers of transformed plants for individuals with optimal reductions in enzyme activities. A novel enzyme-linked, stopped colorimetric assay for PE suitable for the rapid handling of large numbers of samples with activities ranging over two orders of magnitude is presented. Transformant plants in which both PG and PE have been simultaneously reduced using a single construct have been generated, and data is presented on the linkage of reduction of the activities of the two enzymes by this means.

A 303 MICROSCALE ANALYSIS OF LIGNIFIED AND CUTINISED PLANT TISSUES BY PYROLYSIS MASS SPECTROMETRY. Jaap J.Boon. FOM Institute for Atomic and Molecular Physics, Kruislaan 407, 1098 SJ Amsterdam, The Netherlands. telefax 31-20-6684106.

Plant polymers in plant tissues can be fragmented into structurally significant oligomeric and monomeric constituents by flash pyrolysis methods using lasers or hot inert surfaces. Temperature resolved analysis of micrograms of plant tissue inside the ionisation chamber of a mass spectrometer *i.e. in-source PYMS* gives a complete inventory of the tissue constituents. Volatile components such as oils and waxes appear in the low temperature ranges. At higher temperatures the various polymeric constituents of the tissues are observed. Pectins, arabinogalactans, lignins, xyloglucans, cellulose and cutins appear in different temperature windows. The mass spectra are further deconvoluted with multivariate data analysis techniques.

Lignin and lignified tissues: Lignin isolates and lignin in the cell wall matrix give similar phenolic compound distributions. Guaiacyl and syringyl lignins are easily discriminated. Coumaryl lignins are rarely observed and only seem to occur in compression wood of conifers. Lignin in young xylem tissue of angiosperms is of the guaiacyl type whereas in the more mature tissue a mixed guaiacyl/syringyl lignin is observed. Syringyl units are absent in lignin formed in elicited suspension cells of angiosperms. Changes in lignin composition as a result of genetic manipulation can be traced and identified.

Cutin and cutinised tissues: Waxes, pectins, a pentosan-cellulose complex, and cutin appear in different temperature windows of the temperature resolved chemical ionisation PYMS data. Pectin polysaccharides associated with the cuticle are found to be remarkably highly methylated (DM of over 80 % determined by quantitative PYMS). The various cutin acids of the C_{16} and C_{18} hydroxy fatty acid families and phenolic acids associated with the cuticles can be identified directly or by reactive transesterification using PYMS and PYGCMS. Ester and mid-chain ether bonded fatty acyl groups are observed. Oligomers give information on the sequence of the fatty acyl groups. The effects of chemical and enzymatic depolymerisation are investigated by examination of the solid phase residues.

A 304 CINNAMYL ALCOHOL DEHYDROGENASE FROM EUCALYPTUS : MOLECULAR CLONING, EXPRESSION AND GENE CHARACTERIZATION

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Cinnamyl alcohols are the monomeric precursors which are polymerised at the cell wall to give rise to lignins. Cinnamyl alcohol dehydrogenase (CAD) catalyzes the final step in the biosynthesis of these monomers. In this poster we report the cloning of a CAD cDNA (pEuCAD2) isolated by screening a λGT 11 library generated from cell suspension cultures of *Eucalyptus gunnii*. The identity of this clone was unambiguously demonstrated (1) by comparison with peptide sequence data obtained from the corresponding CAD isoenzyme purified from Eucalyptus and (2) by functional expression of the recombinant enzyme in *E. coli*. The recombinant CAD exhibited the same biochemical and immunological properties as the purified Eucalyptus CAD. Preliminary site-directed mutagenesis experiments have allowed us to define some structure/function relationships of the enzyme. Finally, a CAD gene was isolated from a Eucalyptus genomic library and its promoter and coding regions have been characterized.

A 305 HYDROXYCINNAMOYL-CoA REDUCTASE FROM *EUCALYPTUS* : MOLECULAR ANALYSIS OF A KEY CONTROL POINT OF LIGNIFICATION

Malcolm M. Campbell and Alain M. Boudet, Centre de Biologie and Physiologie Végétale, URA CNRS N° 1457, Université Paul Sabatier, Toulouse 31062, France

Lignins may represent up to 30% of the biomass of the plant cell wall and are crucial determinants of the physical support, defence and solute transport of all terrestrial plants. Despite this fact, very little is known about the molecular mechanisms which underlie the developmental and stress-induced control of lignification. In order to identify potential control points of lignification in a woody angiosperm, *Eucalyptus gunnii*, we have conducted studies both at the whole plant level using heterologous polyclonal antibodies and with a model system using elicitor-treated cell cultures. Results obtained with these systems demonstrated that both developmental and stress-related lignification are tightly correlated with the spatial and temporal expression of the enzyme hydroxycinnamoyl-CoA reductase (CCR). CCR catalyses the conversion of hydroxycinnamoyl-CoA esters to their corresponding aldehydes and, as such, represents the step at which phenylpropanoid metabolites are channelled into lignin biosynthesis. In order to develop tools to gain a better understanding of the role of the molecular regulation of CCR with respect to lignification, *Eucalyptus* CCR was purified to homogeneity and characterized. Purified *Eucalyptus* CCR was used to obtain amino acid sequence data and homologous antibodies. Oligonucleotide probes developed from the amino acid sequence data have been used in both cDNA cloning experiments and expression studies. In addition to the results outlined above we will also report on the progress made in the cloning of this enzyme and how the expression of the enzyme is integrated into the control of developmental and stress-induced lignification in *Eucalyptus*. These studies mark the first time that CCR has been analyzed at the molecular genetic level in any plant species.

A 306 XYLOGLUCAN-OLIGOSACCHARIDE-SPECIFIC α-D-XYLOSIDASE: MOLECULAR MODE OF ACTION AND CLONING OF A cDNA FROM GERMINATED NASTURTIUM (*Tropaeolum majus* L.) SEEDS, Sumant Chengappa, Carl Jarman, Cristina Fanutti* and J.S. Grant Reid*, Unilever Research Labs, Sharnbrook, Bedford, MK44 1LQ, U.K, *Department of Biol. & Mol. Sci., University of Stirling, Scotland FK9 4LA, U.K.

The post-germinative mobilization of storage xyloglucan in nasturtium cotyledonary cell walls is brought about by the concerted action of many enzymes[1,2,3,4] including a xyloglucan-oligosaccharide specific α-D-xylosidase, which has been purified to homogeneity[4]. The enzyme removes only the xylosyl residue attached to the backbone glucose at the non-reducing end of the xyloglucan chain (underlined in fig). The smallest structure acting as a substrate for the enzyme is the tri-saccharide highlighted in the fig. The enzyme is a glycoprotein (approx M_r=85000on SDS gels) and shows microheterogeneity on isoelectric focusing. The purified protein has been Lys C digested, and the peptides sequenced. An oligonucleotide primer designed from the amino acid sequence has been used in a polymerase chain reaction to amplify a 5' cDNA fragment from a nasturtium seed cDNA library. The amplified fragment was used to isolate a 1500bp α-xylosidase partial cDNA whose deduced aminoacid sequence contains some of the peptides identified by protein sequencing. Biochemical analysis of the pure protein, and sequence analysis of the partial cDNA indicate that the glycosyl substituents are predominantly O-linked. Efforts are ongoing to isolate a full length cDNA molecule and to determine the size of the protein core of the glycoprotein.

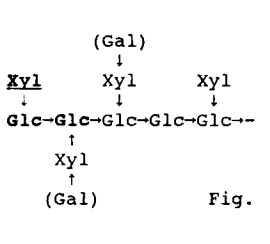


Fig.

[1]Edwards,M. *et al.*, 1985, *Planta* 163:133, [2]Edwards,M. *et al.*, 1986, *J. Biol. Chem.* 261:9489, [3]Edwards,M. *et al.*, 1988, *J. Biol. Chem.* 263: 4333, [4]Fanutti *et al.*,1991,*Planta* 184:137.

A 307 CHANGES IN CELL WALL PROTEINS ASSOCIATED WITH CELL ELONGATION IN DICOTYLEDONOUS PLANT SPECIES. Fiona M.K. Corke and Keith Roberts, Cell Biology Department, John Innes Institute, Colney Lane, Norwich, UK, NR4 7UH.

Ordered growth in higher plants is dependent upon a balance between the number of cells and the manner in which the cells grow, since, unlike animal cells their position within a tissue is relatively fixed. Plant cells show very defined patterns of growth, including the ability to expand rapidly in a particular direction (ie. elongation). It is known that both the orientation and composition of cell wall polysaccharides alter during cell elongation, but changes in the protein components of the cell wall are less well defined.

We have chosen to catalogue the cell wall proteins of two dicotyledonous species (mung bean and Arabidopsis) both before and during the phase of rapid hypocotyl elongation. The high resolution of two dimensional electrophoresis has been used to analyse subtle changes (and revealed the large number of cell wall proteins in dicot cell walls). Certain proteins increase, whilst, others are reduced in level in elongating tissues. Pulse labelling experiments have shown that the range of proteins being synthesised *de novo* before and during elongation, changes. Immunoblotting of 2D gels using antibodies to known proteins has allowed certain proteins to be identified. These descriptive data will be used to select proteins for further analysis by molecular methods.

Acknowledgement: F.C. is funded by a joint AFRC/Unilever Research initiative, K.R. is funded by AFRC.

A 308 MOLECULAR CHARACTERISATION OF NASTURTIUM SEED XYLOGLUCAN ENDO-TRANSGLYCOSYLASE, Jacquie de Silva, Dave Arrowsmith and Carl Jarman, Unilever Research, Sharnbrook, Bedford, U.K.

Dicot primary cell walls derive strength from a network of cellulose microfibrils interconnected by xyloglucan (XG) bridges. Enzymes which catalyse the reversible cleavage of XG, xyloglucan endo-transglycosylases (XET), have been purified from nasturtium seed (31kDa) [1,2] and from *Vignia* stems (33kDa) [3] and detected in a wide variety of different plants [4]. In the nasturtium seed, XET is one of a number of enzymes required for the post-germinative mobilisation of XG storage reserves. In non-seed tissue XET activity has been correlated with tissue elongation [5] and may have a role in reversible wall-loosening.

XET is localised exclusively within the thickened cell walls of 12 day nasturtium cotyledons. It is synthesised as a 33.5kDa precursor detected by immunoprecipitation of translated 12 day nasturtium RNA. We have isolated a 1.3kb DNA copy of the nasturtium XET transcript (single gene) from a 12 day cDNA library, encoding the 295 amino acid precursor polypeptide. An N-terminal core of hydrophobic amino acids (signal peptide) and a putative signal peptide cleavage sequence have been identified. The deduced mature protein sequence (31kDa) exhibits no homology with other plant endo 1,4 ß glucanases in keeping with its unique substrate specificity (XG) and transglycosylase activity. A search of the protein sequence data base with the nasturtium XET protein sequence has revealed identity with an *Arabidopsis* protein, Meri-5, of unknown function, which is abundantly expressed in growing shoot tips [6]. Experiments involving the transfer of a xyloglucanase cDNA fragment, under the control of a constitutive promoter, (sense and antisense orientation) are ongoing. By examining the consequences of manipulating XET levels *in vivo* we hope to gain information about the role of the enzyme.

[1]Edwards *et al.*, 1986, J. Biol. Chem. **261**:9489. [2]Fanutti *et al.*, 1991, Scottish Cell Wall Meeting. [3]Nishitani, 1992, 6th Cell Wall Meeting. [4]Fry *et al.*, 1992, Biochem J., **282**:821 [5]Pritchard *et al.*, 1992, 6th Cell Wall Meeting. [6]Medford *et al.*, The Plant Cell, 3:359.

A 309 CELL WALL METABOLISM IN INTACT FLAX PLANTS, Tatyana A.Gorshkova, David M.Gibeaut*, Marsel R. Ibragimov, Nicholas C.Carpita*, and Vera V.Lozovaya, Laboratory of Cytophysiology and Cell Engineering, Institute of Biology, Russian Academy of Sciences,420503, Kazan, Russia; *Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, USA

We investigated cell wall metabolism during development of intact plants under natural conditions. One-month-old flax plants grown in open air were exposed to a 40 minute pulse with $^{14}CO_2$ (0.03%) followed by various periods of chase with ambient CO_2. Our approach has many advantages: 1) the native way of carbon assimilation permits evaluation of unperturbed cell wall metabolism, 2) all plant organs are labeled effectively, 3) the short time of the pulse enables examination of the initial radioactivity incorporated into cell wall polymers, and 4) prolonged chases allow examination of long-term redistribution of carbon from cell wall polymers. Total radioactivity of cell walls steadily increased at least up to 24 hours chase mostly due to considerable turnover of starch. Pectic substances and alkali-soluble fractions from various plant organs were separated, and gas chromatography proportional counting was used to analyze both mass and radioactivity of sugar constituents. The major monosaccharide of the alkali-soluble fraction was xylose, which accounted for 70% of the mass of this fraction in roots and nearly 50% of that in fibrous stems. Immediately after the pulse, the proportion of radioactivity in xylose was much lower, but increased substantially during subsequent chase periods. Other data indicate a differential turnover of Ara and Gal(A). Our results show extensive redistribution of radioactivity in many of the cell wall polymers during development.

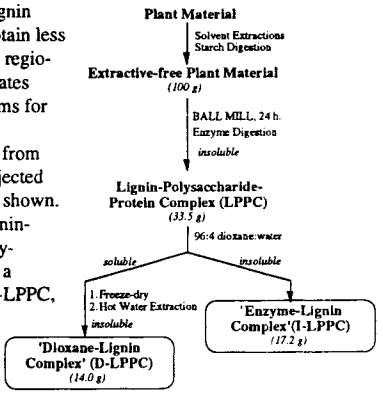
A 310 MANIPULATION OF LIGNIN BIOSYNTHESIS IN TRANSGENIC PLANTS EXPRESSING CINNAMYL ALCOHOL DEHYDROGENASE ANTISENSE RNA, Claire Halpin, Mary E. Knight, Wolfgang Schuch, Malcolm M. Campbell* and Geoffrey A. Foxon, ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell, Berks RG12 6EY, U.K. * Centre de Physiologie Vegetale, Universite Paul Sabatier, 118 route de Narbonne, 31062 Toulouse-Cedex, France.

The biosynthesis of lignin, one of the most abundant organic polymers in the biosphere, is attracting increasing interest. Cinnamyl alcohol dehydrogenase (CAD), catalysing the production of the direct monomeric precursors of the lignin polymer, is one of the few enzymes truly specific to lignin and lignan biosynthesis. We have recently purified and cloned CAD from tobacco stems and are currently investigating the role of this enzyme in lignification. A 1kb fragment of the CAD cDNA cloned in reverse orientation into a transformation vector with the CaMV-35S promotor and nos terminator, was introduced into tobacco via Agrobacterium. Many regenerated plants exhibited CAD enzyme levels lower than control values. Northern analysis of these plants demonstrated the presence of CAD antisense RNA and the disappearance of CAD mRNA. Plants exhibiting particularly low levels of CAD enzyme activity were analysed to determine transgene number. Those with only a single site of insertion were backcrossed to controls to generate populations of control and antisense plants with a uniform genetic background. These populations have been extensively characterised. The results show that CAD activity can by inhibited by over 90% without interfering with the development of an outwardly normal plant phenotype. However a range of analytical techniques show clear differences in lignin composition between antisense and control plants. The precise nature of these differences is currently being investigated and should yield interesting insights into how lignin quality may be modified by genetic engineering.

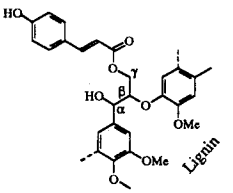
A 311 STRUCTURAL CHARACTERIZATION OF ISOLATED CORN LIGNINS.

Ronald D. Hatfield, John Ralph, John Grabber and Hans J. Jung, U.S. Dairy Forage Research center, USDA-Agricultural Research Center, 1925 Linden Drive West, Madison, WI 53706.

Grass lignins contain substantial amounts of *p*-coumaric acid esterified to lignin. Although there is some evidence supporting its presence at both the α- and γ-positions on lignin sidechains, we sought to obtain less ambiguous evidence of this regiochemistry. Each site implicates entirely different mechanisms for the esterification process.



The rind tissue isolated from mature corn stems was subjected to the fractionation scheme shown. The two major resultant lignin-rich fractions (containing hydroxycinnamic acids) were a solvent-soluble fraction (D-LPPC, 45%) suitable for NMR and an insoluble fraction (I-LPPC, 55%). Each fraction was extensively characterized by pyrolysis-GC-MS, sugar analysis, thioacidolysis, hydroxycinnamoyl ester and hydroxylcinnamyl ether measurements, and lignin determination. The solvent soluble fraction was also extensively analyzed by NMR, leading to the unambiguous determination of *p*-coumaric acid regiochemistry. For this soluble D-LPPC fraction, the *p*-coumaryl residues are predominantly attached at the γ-positions of lignin sidechains.



A 312 CELL WALL COMPOSITION OF MARINE YEASTS, Daniel Hernández-Saavedra, José Luis Ochoa, F. López-Gutiérrez, Centro de Investigaciones Biológicas, La Paz, B.C.S. 23000 México.

Yeasts play an important role in the marine food chain because of their ability to assimilate a great variety of carbon compounds. Current knowledge of the composition, structure and metabolism of yeast cell walls is based on data derived from Sacch. cerevisiae (terrestrial), but little is known of marine yeast cell wall composition. To determine the major cell wall components of marine yeasts, 100 mg of lyophilized cells of Debaryomyces hansenii, Rhodotorula sp. were disrupted by Braun MSK homogenizer for 5 min to prepare the cell wall. Lyophilized cell walls were separated into the 4 classical fractions according to Leal-Morales and Ruiz-Herrera (1985) Exp. Mycol. 9: 28-38. These fractions were analyzed for neutral sugars, and 20-25 mg of total cell walls were analyzed for soluble proteins, lipids, chitin and inorganic material. The preliminary data from these analyses are shown below. In general terms, the marine yeasts were higher in inorganic material and chitin. These differences undoubtedly reflect the different environments of the two organisms. The chemical nature of the lipids, carbohydrates and inorganic material is under investigation.

Table 1. Major cell wall components of two marine yeasts as compared to Sacch. cerevisiae.

Genera	Sugars	Proteins	Chitin	Lipids	Ash
	(% of cell wall dry wt.)				
Rh.	33.9	27.0	10.2	11.0	10.8
Deb.	82.7	8.0	5.2	3.6	4.1
Sacch.	69.8	25.2	2.2	4.7	ND

A 313 FERULOYL OLIGOSACCHARIDES FROM *SPINACIA OLERACEA* L., Tadashi Ishii [1] and Tetsuya Tobita[2] [1]Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, 305, Japan, [2]Japan Tobacco Co., Yokohama, 227 Japan

Plant cell walls contain a small amount of phenolic side-chains esterified to polysaccharides. Interest in these compounds arises from the fact that these side-chains appear to undergo *in vivo* oxidative coupling to yield cross-linked polysaccharides; such coupling may contribute not only to the control of cell wall extensibility and cell growth, but also to decreased digestibility by ruminant-secreted enzymes. We have isolated and characterized oligosaccharides containing cinnamic acids derivatives from monocots. We now report on structural characterization of feruloyl oligosaccharides from cell walls of spinach (*Spinacia oleracea* L.) and sugar beet (*Beta vulgaris* L.). These are *O*-(2-*O*-*trans*-feruloyl-α-L-Ara*f*-(1→5)-L-Ara*f*, *O*-(6-*O*-*trans*-feruloyl-β-D-Gal*p*)-(1→4)-D-Gal, and *O*-α-L-Ara*f*-(1→3)-*O*-(2-*O*-*trans*-feruloyl-α-L-Ara*f*)-(1→5)-L-Ara*f*, on the basis of NMR spectroscopy, methylation analysis, and FAB-MS.

A 314 CHELATION OF METAL IONS BY DIFFERENT COMPONENTS OF THE PLANT CELL WALL, Paul J. Jackson[1], Huei-Yang D. Ke[2], Edward R. Birnbaum[2], Dennis W. Darnall, Cheryl R. Kuske[1], and Gary D. Rayson[2], [1]Life Sciences Division, Los Alamos National Laboratory, Los Alamos, NM 87545, and Department of Chemistry, New Mexico State University, Las Cruces, NM 88003.

Different mineral nutrients and toxic metal ions are reversibly chelated to different components of the plant cell wall. Metal binding was studied using *Datura innoxia* plant suspension cells. The accumulation of many metal ions does not require cell viability. Binding is often reversible, but results suggest that other factors besides simple ion exchange play a role in metal chelation and release. A pulsed tunable dye laser was used to obtain excitation spectra and fluorescence decay curves of solid metal ion-*Datura innoxia* complexes. The excitation spectra generated were used to study the electronic structure factors contributing to the interaction between metal ions and the binding sites on the cell walls. Binding of Ba, Cd, Cu, Eu, Gd, Pu and U were studied. Carboxyl and sulfate groups are the dominant functional groups for forming binding sites at high (≥4) and low (≤3) pH, respectively. However, the noticeable broadened and asymmetric excitation spectra obtained at high pH are ascribed to multiple binding sites for different metal ions. Competitive binding of different metal ions was also studied. The information generated is useful for a better understanding of the role that the cell wall plays in sequestering and accumulating necessary mineral nutrients and selected toxic metal ions.

A 315 PURIFICATION AND CHARACTERIZATION OF ACETYL- XYLANESTERASE FROM ASPERGILLUS NIGER, James C. Linden, Meropi Samara, Miklos Pecs, Ellen Thomas, Michelle Joy, Scott Grieshaber, *William Adney and *Michael Himmel, Department of Microbiology, Colorado State University, Fort Collins, CO 80523 and *Biochemical Conversion Division at the National Renewable Energy Laboratory, Golden, CO 80401

Combined action of hemicellulase and acetylxylan esterase (AXE) enzymes are required to partially degrade the plant cell wall matrix and make cellulose microfibrils accessible to hydrolysis by cellulases. Optimized AXE production conditions using Aspergillus niger ATCC 10864 in 14 L fermentation jars were determined to be 33°, 1.5 vvm aeration and 300 rpm agitation without pH control.

The AXE was purified by precipitation in 60 to 80 percent saturation in ammonium sulfate fraction. The pellet was applied directly to a Pharmacia high load Phenyl Sepharose column for hydrophobic interaction chromatography and purified to homogeniety in two steps.

The pH and temperature stability and kinetic characteristics of AXE were determined over a pH range of 4.0 to 7.5 and from 4° to 37°. At temperatures greater than 25°, stability was superior at lower pH values (<pH 5.0) than at higher pH values. These properties make this enzyme source suitable for an ensiling additive.

A 316 PEROXIDASE ACTIVITY AND TERMINATION OF CELL ELONGATION IN TALL FESCUE LEAF BLADES, Jennifer W. MacAdam, Department of Plants, Soils, and Biometeorology, Utah State University, Logan, UT 84322-4820

An increase in apoplastic peroxidase activity occurs in the region of tall fescue (*Festuca arundinacea* Schreb.) leaf blades where leaf elongation slows and stops (*Plant Physiology* 99:879). Apoplastic peroxidase activity could contribute to the termination of cell growth in numerous ways, such as by cross-linking cell wall polysaccharides via formation of diferulic acid. Recent studies have demonstrated that localization of ferulic acid is similar to that of peroxidase in the elongation zone. Lignification occurs after cessation of cell elongation, and peroxidase is known to catalyze the last step of this process. Numerous isoforms of peroxidase exist in tall fescue leaf blades, and it is likely that the isozymes of peroxidase that participate in processes such as lignification and formation of diferulic acid would differ from one another. Only cationic isoforms of peroxidase were identified in the elongation zone of tall fescue, where critical cell wall cross-linking occurs, and anionic isoforms first appeared in the region of secondary cell wall deposition, where lignification occurs; anionic isozymes of peroxidase have also been associated with lignification by other researchers. The objective of this study was to further assess the developmental differences in peroxidase isoform activity in tall fescue leaf blades. The results suggest that, although several isoforms of peroxidase are common to tall fescue leaf blade tissue at virtually every stage of development, two cationic isoforms are uniquely prevalent in elongating or recently elongated tissue.

A 317 PHOTOREACTIVE ACTIVATORS OF CALLOSE SYNTHASE AS POTENTIAL PHOTOAFFINITY PROBES FOR THE ACTIVATOR-BINDING SUBUNIT SITE, K. Ng and B. A. Stone, Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia.

A UDP-glucose: (1→3)-β-glucan synthase (callose synthase) located on the plasma membrane of higher plants appears to be a complex of several polypeptides which may bear, in addition to a UDP-Glc-binding site, sites for the binding of non-covalent activators. We have recently identified a 31 kDa polypeptide as the catalytic subunit of an immunoprecipitated callose synthase complex from *Lolium multiflorum* endosperm by photoaffinity labelling with a photoreactive UDP-Glc analogue (Meikle *et al.*, J. Biol. Chem., 266, 22569-22581, 1991) and have now sought to identify the β-D-glucoside-binding (activator) site using high-affinity, photoreactive derivatives of β-D-glucoside activators. To this end we have synthesized a number of photoreactive β-D-glucosides, each bearing a photoreactive aglycon: (a) N-[4-Azidosalicoyl]-β-D-glucosylamine (Kd 0.19 mM; *FS 68); (b) N-[Azidosalicoyl]-β-D-cellobiosylamine (Kd 0.47 mM; FS 54); (c) 2'-[4-Azidosalicylamino]ethyl-1-thio-β-D-glucose (Kd 0.033 mM; FS 85); (d) N-[4-(Benzoyl)Benzoyl]-β-D-glucosylamine (Kd 0.03 mM; FS 40). *In vitro* assays show that all compounds except (b) are relatively high affinity activators of callose synthase compared with the commonly used cellobiose (Kd 1.2 mM; FS 72). Activators of callose synthase, however, have an absolute requirement for the glucose C_6-OH since 6-O-[4-azidobenzoyl]-methyl-β-D-glucose, 6-O-[4-azidobenzoyl]-*p*-nitrophenyl-β-D-glucose, *o*-nitrophenyl-β-D-xylopyranoside and *p*-nitrophenyl-β-D-glucuronide all failed to stimulate activity above basal level. These results may be compared with the stimulation of callose synthase by methyl-β-D-glucose (Kd 1.1 mm; FS 70) and *p*-nitrophenyl-β-D-glucose (Kd 0.56 mM; FS 66).

*FS 68: 68-Fold stimulation of activity above basal level measured in the absence of activator.

A 318 THE DISTRIBUTIONS OF NON-METHYL ESTERIFIED GALACTURONIC ACID IN PECTIN OF THE CULTURE COTTON CELL WALL, Feng Qiu and Andrew J. Mort, Department of Biochemistry and Molecular Biology, Oklahoma State University, Stillwater, OK 74078

Pectin is one of the main components of cell walls (over 30%) and it is therefore important to completely identify its structural features. Two types of pectin were extracted from cell walls isolated from cotton suspension culture, c.v. acala 44. Cell walls were treated with HF at -23°C (30 min) to solublize homogalacturonans. Homogalacturonic acid sample (pectin A) was obtained by extraction with water followed by precipitation in 80% ethanol. Another homogalacturonan fraction (pectin B) was obtained by subsequent extraction with 0.5M imidazole-HCl buffer (pH 7.0). Pectin A has high methyl esterification (about 40%), while pectin B has a degree of methyl esterification of only 10-15%. The distribution of non-methyl esterified galacuronic acid in the homogalacturonan polymers were determined by a newly developed method. The methyl esterified galacturonic acids were reduced to galactose by $NaBH_4$. These polymers were then treated with HF (1% H_2O v/v) at -15°C to cleave at carbohydrate linkages after galactose and obtain $GalA_xGal$ (x=1,2,3···) oligomers. These oligomers were separated by ion-exchange chromatography using a Dionex HPLC system fitted with a Dionex PA1 column (4x250 mm). A sodium acetate buffer (pH 5.2) gradient with PAD detector was used for separating the oligomers of pectin A. After calibrating the peak areas, we quantitatively measured the mole ratios of $GalA_xGal$ in pectin A. A potassium oxalate buffer (pH 6.0) gradient with fluorescence detector was used for eluting the oligomers of pectin B after derivatized by 2-amino pyridine. We successfully determined the distributions of GalA in pectin A and B. They are not randomly distributed. The concentration of $GalA_1Gal$ is about 10 times higher than other oligomers produced from pectin A, suggesting that there are clusters of GalA-GalAMe.

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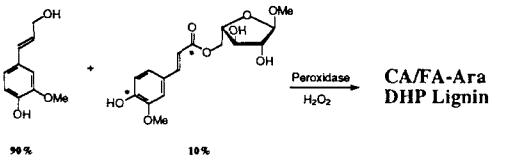
A 319 DETERMINATION OF THE REGIOCHEMISTRY OF INCORPORATION OF HYDROXYCINNAMOYL ESTERS INTO SYNTHETIC LIGNINS.

John Ralph, Ronald D. Hatfield, Richard F. Helm, Stéphane Quideau and Hans J. Jung, U.S. Dairy Forage Research center, USDA-Agricultural Research Center, 1925 Linden Drive West, Madison, WI 53706.

Feruloyl esters on arabinoxylans in grass cell walls are known to become attached to lignins forming critical cross-links. This attachment is frequently considered to be at the α-position via opportunistic attack of the phenol on intermediate quinone methides during lignification. However, this site and mechanism overlooks the potential for feruloyl esters to become actively involved in the free radical process, adding to the complexity of structures produced. Free-radical participation can potentially produce a variety of structures, only some of which would be subsequently identifiable as arising from feruloyl moieties by current analytical procedures.

In order to establish what structures are likely in free radical reactions that are presumed to constitute lignification, strategically labeled FA-Ara (methyl-5-*O*-*trans*-feruloyl-α-L-arabinofuranoside) was co-polymerized into coniferyl alcohol DHP lignin. Analysis of the resultant product by two-dimensional NMR experiments, particularly the HMBC experiment and selective-pulse variants, provides conclusive proof that the feruloyl moiety incorporates efficiently into β-5′ (phenylcoumaran), β-β′ (pinoresinolide), and β-O-4′ (β-ether) products as well as the anticipated 4-O-α′ and 4-O-β′ ethers. As anticipated, only a fraction (ca. 10%) can be recovered as ferulic acid by solvolytic techniques currently used to quantitate hydroxycinnamic acids in plant tissues.



A 320 RIPENING ASSOCIATED CHANGES IN CELL WALL ARCHITECTURE OF TOMATOES. Nancy M. Steele, M.C. McCann, F.M.K. Corke and K. Roberts, Department of Cell Biology, John Innes Institute, Colney Lane, Norwich, NR4 7UH, U.K.

Ripening associated softening of the tomato fruit is achieved by modifications of the cell wall matrix, although how these changes are accomplished is not fully understood.

We have been using a variety of techniques, including immunocytochemistry, electron microscopy, sequential extraction of cell wall material and SDS PAGE in order to study these ripening-associated modifications.

Immunolocalisation studies show that the pectins of the central region of the cell wall are demethylesterified during ripening. During sequential extraction of the cell wall material, calcium chelation releases carbohydrate fractions rich in middle lamella pectins associated with the wall by calcium cross-links, treatment with Na_2CO_3 releases fractions rich in primary cell wall pectins, presumably by breaking ester-linkages, while progressively stronger alkali treatment disrupts hydrogen bonding to solubilise the hemicellulosic molecules. Replica shadowing of fractions of the sequentially extracted molecules suggest that specific classes of pectins and hemicelluloses are partially depolymerised during the cell wall modifications associated with fruit softening. In addition to visualising these polysaccharide components, the proteins present in each of the fractions have been separated by SDS PAGE, and comparisons between the fractions from green and red fruit made.

Acknowledgement: N.M.S. is funded by a John Innes Foundation CASE award studentship, F.C. and M.M. are funded by a joint AFRC/Unilever Research Initiative and K.R. by AFRC.

A 321 HYDROXYCINNAMIC ACID BRIDGES IN CELL WALLS OF GRASSES, B.A. Stone, T.B.T. Lam and K. Iiyama, Department of Biochemistry, La Trobe University, Bundoora, Victoria, 3083, Australia.

Hydroxycinnamic acid ester-ether bridges between polymers in lignified walls of grasses have been proposed by Scalbert *et al.* (Phytochemistry, 24, 1345, 1985) and circumstantial evidence for their occurrence presented by Scalbert *et al.* (Holzforschung, 40, 191, 1986) and Iiyama *et al.* (Phytochemistry, 29, 733, 1990).

Using a procedure based on the different reactivities of free carboxylic acids and their esters toward borohydride reductants we have directly measured the content of ester-ether bridge hydroxycinnamic acids in dioxane-H_2O soluble fractions in wheat (*Triticum aestivum*) and phalaris (*Phalaris aquatica*) internode walls (Lam *et al.*, Phytochemistry, 31, 1179, 1992). Hydrogenation of the hydroxycinnamic acids prior to borohydride reduction is necessary to achieve quantitative reduction and, since a Pd/C catalyst is used, the procedure is restricted to dioxane-water soluble, lignin-carbohydrate fractions. These fractions represent only ~5% of the lignin in the walls.

A modification of the procedure has now been devised to allow quantitation of hydroxycinnamic acids in ester-ether bridges in whole walls. In this procedure an homogenous hydrogenation catalyst is used and the $LiBH_4$ reductant is replaced with the more powerful and organic solvent-soluble lithium triethylborohydride. The preliminary results indicate that about half of the etherified ferulic acid in whole internode walls of wheat is also ester-linked whereas *p*-coumaric acid is not involved in ester-ether bridges.

A 322 IDENTIFICATION OF A MUTANT OF *ARABIDOPSIS THALINA* BLOCKED IN THE CONVERSION OF HIGH MANNOSE TO COMPLEX ASPARAGINE-LINKED GLYCANS, [1]Arnd Sturm, [2]Antje von Schaewen and [3]Maarten J. Chrispeels, [1]Friedrich Miescher-Institut, Basel, Switzerland, [2]Universität Osnabrück, Osnabrück, Germany, [3]University of California, San Diego, CA.
The biosynthesis of many secretory plant proteins and their movement along the secretory pathway is accompanied by the attachment of high mannose glycans to specific asparagine residues, and the subsequent modification of these glycans in the endoplasmic reticulum and the Golgi apparatus. Glycosidases and glycosyltransferases in the Golgi convert typical $Man_9GlcNAc_2$ high mannose glycans into complex glycans that have less mannose residues and additional sugars such as α(1-3)fucose, ß(1-4)galactose, and ß(1-2)xylose.
In the course of our work on the function of N-glycosylation in plants we generated an antiserum that is specific for ß(1-2)xylose residues. With this antiserum we isolated a mutant in *Arabidopsis thaliana* that is unable to complete the conversion of high mannose to complex glycans and which accumulates $Man_5GlcNAc_2$ glycans. In the presence of deoxymannojirimycin, an inhibitor of mannosidase I, the mutant cells synthesize $Man_9GlcNAc_2$ and $Man_8GlcNAc_2$ glycans, suggesting that the biochemical lesion in the mutant is not in the biosynthesis of high mannose glycans in the ER, but in their modification in the Golgi. These data a consistent with the absence of GlcNAc transferase I activity from the mutant. The mutant plants are able to complete their development normally, suggesting that the complex glycans are not essential for normal developmental processes under optimal growth conditions. We must, therefore, consider the possibility that complex glycans are needed only on a small subset of glycoproteins which are synthesized when plants are grown under non-laboratory conditions, and subjected to particular biotic or abiotic stresses.

A 323 A NEW PREPARATION OF DHP (DEHYROGENATIVE POLYMER OF LIGNOL) FROM ITS GLUCOSIDE, N. Terashima*, M. Nakao** and R.H. Atalla*, USDA Forest Products Laboratory*, Madison, WI 53705, and Faculty of Agriculture**, Nagoya University, Nagoya, Japan

Dehydrogenatve polymers of monolignols (DHP) have been widely used as models of lignin in the cell wall. Most DHPs have been prepared by polymerizing monolignols in the presence of hydrogen peroxide and peroxidase or of laccase and oxygen. These DHPs, however, have structures which differ significantly from that of milled wood lignin as reflected in the results of degradative analysis and in UV and NMR spectra.

The possible participation of monolignol glucosides in the biogenesis of lignified plant cell walls suggests a method which may more closely approximate the natural process for the formation of lignin. In this preparation of DHPs, the glucosides are used as precursors, β-glucosidase is used to liberate the monolignol, the resulting glucose is used to generate equimolar quantities of hydrogen peroxide through action of a glucose oxidase, and this in turn, in concert with peroxidase, initiates the polymerization of the monolignol.

In this work, coniferin was dissolved in water, together with β-glucosidase, glucose-oxidase and peroxidase. Air was then bubbled into the solution, and the preparation was carried out both with and without added cell wall matrix polysaccharides. DHPs were formed in about 50% yield. Comparison of UV and NMR spectra and analysis of dimer composition on thioacidolysis showed the DHPs to have structures which resemble that of milled wood lignin more closely than do the structures of DHPs prepared from coniferyl alcohol by the conventional method.

A 324 EXISTENCE OF REPEATING UNITS IN PECTINS?

Jean-François THIBAULT and Catherine M.G.C. RENARD, Laboratoire de Biochimie et Technologie des Glucides, INRA, Centre de Recherches de Nantes, B.P. 527, 44026 NANTES CEDEX 03, FRANCE

Pectins have been shown to consist of "hairy" regions, where the neutral sugars are concentrated, and "smooth", homogalacturonic regions. We have used mild acid hydrolysis to investigate their size and disposition.

Deesterified pectins from apple, beet and citrus were submitted to hydrolysis (0.1M HCl, 80°C, up to 72h). Their hydrolysis, monitored by measurements of intrinsic viscosities, followed a (pseudo-first order) two stages kinetics. There was first a rapid decrease corresponding to rupture of the more susceptible linkages between rhamnose and galacturonic acid residues, followed by a slower decrease corresponding to the cleavage of the linkages between galacturonic acid residues. During the course of the hydrolysis, some galacturonic acid and most of the neutral sugars were solubilised. They gave comparable fractions (composition, K_{av} values) on Sepharose CL-6B for all pectins. After 72h, the insoluble material consisted of almost pure polygalacturonic acids; their length was estimated, by H.P.S.E.C.- M.A.L.L.S. to be of about 72-100 galacturonic acid residues.

The pectins from apple, beet or citrus are therefore composed of repeating units, of alternating "smooth" and "hairy" regions. The length of the "smooth" regions is independent of the origin of the pectins and is at least 72-100 residues. The beet pectins have a higher proportion of "hairy" regions than the apple pectins, and than the citrus pectins. Some repeating features may also be present in these regions.

A 325 **AFFINITY PURIFICATION OF BIOTINYLATED COTTON FIBER CELL SURFACE COMPONENTS**, Barbara A. Triplett, Fiber Physics & Biochemistry Research, USDA, ARS, Southern Regional Research Center, P.O. Box 19687, New Orleans, Louisiana 70179
INTRODUCTION: The importance of plant cell walls as structures influencing many cellular processes is well-appreciated. Cell growth depends on the coordinated synthesis and integration of carbohydrate, protein, and lipid components of the cell wall, plasma membrane, and cuticle. While it is recognized that plant cell surface components are not static, it has been difficult to examine directly developmental changes. Cotton fiber cells represent a particularly well-suited system to examine plant cell wall dynamics. Fiber cells initiate growth nearly synchronously from the seed epidermis. The cells elongate for a period of approximately three weeks and deposit a highly-ordered cellulosic secondary wall.
METHODS: Cell surface components of developing cotton fiber cells have been covalently modified with short exposures to sulfosuccinimidyl 2-(biotinamido)ethyl-1,3-dithiopropionate or NHS-SS-biotin (Pierce, Rockford, IL), a reagent that reacts with free amino groups. Ionically-associated cell wall proteins were released from fiber cells by washing with a buffer containing 0.25 M $CaCl_2$. Biotinylated wall proteins were affinity purified with streptavidin conjugated paramagnetic particles (MagnaSphere, Promega Corporation, Madison, WI). Addition of a sulfhydryl reducing agent, dithiothreitol, released the affinity purified material from the paramagnetic particles in a form suitable for SDS-polyacrylamide gel electrophoresis.
RESULTS: Ionically-associated cell wall proteins from developing fiber cells and other cotton tissues were purified using this method. The procedure is simple, rapid, and sensitive enough to detect subtle developmental differences in cell wall protein profiles. Sufficient material can be generated by affinity purification for immunological analysis or protein micro-sequencing. Digitonin-soluble microsomes isolated from biotinylated cotton fiber cells contained proteins that were biotin labelled. Modification of plasma membrane proteins in the presence of an intact primary cell wall suggests that this procedure will be useful in identifying relationships between the cell wall and plasma membrane. Progress in characterizing ionically-associated proteins from cotton fiber cell will be presented.

A 326 ENZYMIC DEGRADATION OF XYLOGLUCANS, J.-P. Vincken, G. Beldman and A.G.J. Voragen, Department of Food Science, Wageningen Agricultural University, P.O. Box 8129, 6700 EV Wageningen, The Netherlands

Xyloglucans generally comprise as much as 20-25% of the primary cell walls of dicotyledons. They consist of a ß-(1,4)-glucan backbone with α-(1,6)-xylosyl residues attached to the 6-position of ß-glucosyl residues. Species-specific differences occur as to the distribution of additional branching of fucosyl-galactosyl residues. Xyloglucans are hydrogen bonded to cellulose[1] as well as linked to pectic material[2]. Renard *et al.*[3] indicated that degradation of a fucogalactoxyloglucan enhances the solubilisation of other cell wall material. The aim of this study is to investigate the degradation of xyloglucans from different tissues.

Extensive washing of apple, pear and potato tissue yielded a residue (Water-Unextractable Solids) which was subjected to sequential extractions with increasing strength of alkali. Xyloglucans were purified from the 4N KOH extract by anion exchange chromatography and selective degradation of co-extracted hemicellulosic material. Contrary to apple and pear xyloglucan, potato xyloglucan lacked fucose residues but contained some arabinose instead.

The degradation of these xyloglucans was further studied using purified endo-glucanases. Oligomeric products were prefractionated on Bio-Gel P2 followed by a final fractionation on semi-preparative CarboPac PA1. Fractions were analysed for sugar composition. Apple and pear xyloglucan showed similarity concerning the larger oligomers although they appeared in different ratios. Further, apple contained minor quantities of a diversity of smaller oligomers. Potato xyloglucan was composed of completely different building blocks which, on the average, had a lower degree of polymerisation. The oligomers will be further characterised with ^{1}H-NMR and mass spectroscopy.

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A 327 MOLECULAR GENETIC ANALYSIS OF MAIZE *GLOSSY* GENES: ROLE IN EPICUTICULAR WAX PRODUCTION Julie M. Vogel & Michael Freeling, Department of Plant Biology, University of California, Berkeley CA 94720

All aerial epidermal cell surfaces of plants are covered by a multilayered, nonliving cuticle, which serves as the primary physical barrier that protects the underlying living cells against mechanical injury from the environment. Comprising the outermost layer of the cuticle, the crystalline, hydrophobic epicuticular waxes are important for preventing transpiration through the leaves. Wax-deficient mutants or variants are common, and some of the enzymes, such as fatty acid elongases, involved in wax biosynthesis are now the focus of intense biochemical study. Nevertheless, little is known overall about the individual steps or regulation of the wax biosynthetic pathway, or about the mechanisms that govern intracellular wax synthesis, and transport and extracellular deposition of the wax products on the outer cuticular surface.

In maize, epicuticular wax deposition occurs in a leaf organ-specific and juvenile developmental stage-specific manner. We are using a transposon tagging approach as a means to isolate and characterize the expression of the *Glossy* genes involved in epicuticular wax biosynthesis specifically in maize juvenile leaves. Both directed and undirected tagging strategies involving three different maize transposable element systems, *Mutator*, *Ac-Ds* and *Spm*, are in progress, and so far have produced several new *glossy* mutants. Complementation analysis has identified mutants representing new alleles of *gl1*, *gl2*, *gl4*, *gl8*, and *gl15* loci. Inspection of leaf surfaces by scanning electron microscopy reveals that revertant sectors in the somatically unstable mutants produce normal epicuticular wax in a cell autonomous manner. Ongoing molecular, biochemical and cell biological analysis of these tagged *glossy* genes and their encoded biosynthetic and regulatory proteins should contribute to a better understanding of plant wax biosynthesis, transport and epicuticular deposition.

Role of Plant Cell Walls in Pathogenesis;

The Oligosaccharin Signalling Pathway of Plants

A 400 CHITOSAN AS AN INDUCER OF MYCELIAL GROWTH IN THE DIMORPHIC FUNGUS, *SPORISORIUM REILIANUM*, Shyamala Bhaskaran and Roberta H. Smith, Soil and Crop Sciences Department. TAES, Texas A&M University, College Station, Texas 77843

Sporisorium reilianum, the pathogen that causes head smut in *Sorghum bicolor*, is a dimorphic fungus whose parasitic mycelial phase of development occurs within the host plant. A glycoprotein fraction of the host plant extract induced mycelial growth in *S. reilianum in vitro*. Inducing ability was destroyed by pretreatment with Pronase E, whereas pretreatment with driselase seemed to enhance its activity. Since driselase is not known to have chitinase, or ß-N-acetyl glucosaminidase activities, diacetyl chitobiose units if present in the glycoprotein should still be linked to the peptide. In order to examine if N-acetyl glucosamine is the active inducer, commercial N-acetyl glucosamine and its naturally occurring polymer, chitin were tested and found to be inactive. On the other hand, chitosan which is deacetylated chitin, promoted rapid mycelial growth in *S. reilianum in vitro*. This implies that glucosamine units associated with a glycoprotein in the host plant may trigger parasitic growth of *S. reilianum in vivo*.

A 401 AN ACIDIC EXOPOLYSACCCHARIDE REQUIRED FOR NODULE INVASION BY *Rhizobium meliloti*. Juan Gonzalez, T. Lynne Reuber, Alexandra Glucksman, Andrea Marra, and Graham C. Walker, Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

We have shown that an acidic exopolysaccharide, termed succinoglycan or EPS I, is required for alfalfa nodule invasion by *Rhizobium meliloti* strain Rm1021. *R. meliloti* mutants that fail to synthesize succinoglycan elicit the formation of empty nodules that contain neither bacteria nor bacteroids. This polysaccharide is a polymer of octasaccharide subunits composed of one galactose and seven glucoses, with acetyl, pyruvyl, and succinyl substituents. Genetic analyses have led to the identification of a cluster of *exo* genes which are necessary for its synthesis. Like many complex bacterial polysaccharides, succinoglycan is synthesized on polyprenyl lipid carriers present in the cytoplasmic membrane of the cell. Recently we have been be able to assign biochemical functions to many of the *exo* gene products by isolating lipid-linked biosynthetic intermediates from *exo* mutant strains, hydrolyzing the oligosaccharides from the lipid carriers, and characterizing the oligosaccharides obtained to determine the biochemical block of each mutant. On the basis of this work, we have proposed a model of succinoglycan biosynthesis in which the products of the *exoY* and *exoF* genes function in the addition of the first sugar, galactose, to the lipid carrier, and the products of the *exoA*, *exoL*, *exoM*, and *exoU* genes function in subsequent sugar additions. The product of the *exoH* gene is needed for addition of succinate, and the product of the *exoZ* gene is needed for addition of acetate. *exoP*, *exoQ*, and *exoT* mutants appear to make complete succinoglycan subunits, but these mutants make no high molecular weight polysaccharide, and therefore, the products of these genes are postulated to affect polymerization of the octasaccharide subunits or transport of the completed polymer. Recent results have raised the possibility that a low molecular weight form of this polymer may function as a signal to the plant since the addition of oligosaccharides related to succinoglycan can suppress the symbiotic deficiencies of *exo* mutant strains, albeit at low efficiency. In light of these results, it is interesting that we have recently found that the *exoK* gene encode a β-glucanase that cleaves the succinylated form of succinoglycan. We are investigating possible roles that this exopolysaccharide might play in the process of nodule invasion.

A 402 EARLY MEMBRANE RESPONSES INDUCED BY FUNGAL ELICITORS IN SUSPENSION-CULTURED RICE CELLS,

Kazuyuki Kuchitsu and Naoto Shibuya, *Department of Cell Biology, National Institute of Agrobiological Resources, Kannondai, Tsukuba, Ibaraki 305, JAPAN*

Oligosaccharides derived from the cell surface of pathogenic microorganisms as well as host plants act as signal molecules that induce various defense responses. Although some possible factors for signaling pathways have been proposed from the experiments with crude preparations of cell wall components or the mixture of those fragments, little is known about the early responses and signal transduction mechanisms induced by specific oligosaccharide signals. We previously showed that the fragments of β-glucan from rice blast disease fungus, *Piricularia oryzae*, and N-acetylchitooligosaccharides act as elicitors for the production of phytoalexins such as momilactones and oryzalexins in suspension-cultured rice cells (1). We now have tried to identify early membrane responses induced by those specific oligosaccharide signals which might consist a part of signal transduction network. Treatment of rice cells with such oligosaccharides induced rapid efflux of K^+ and influx of H^+. *In vivo* ^{31}P-nuclear magnetic resonance spectroscopy revealed that cytoplasmic pH decreased, correspondingly. Only the N-acetylchitooligosaccharides with a degree of polymerization higher than 5 were active. Even less than 1 nM of $(GlcNAc)_7$ induced such rapid redistribution of ions. Deacetylated chitosan oligomers were inactive. Very rapid and transient generation of O_2^- (superoxide anion) and other active oxygen species induced by these oligosaccharides were also observed by chemiluminescence analyses. Such characteristics of early membrane responses were similar to those observed for the production of phytoalexins, suggesting their possible involvement in signal transduction sequences leading to the activation of genes required for the various defense responses.

Acknowledgment: The authors are grateful to Drs. Yoshiaki Yazaki and Katsuhiro Sakano for their help and discussion for NMR and microelectrode experiments.

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A 403 ROLE OF VIRAL MOVEMENT PROTEINS IN THE PATHOGENESIS OF SQUASH LEAF CURL VIRUS,

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The bipartite geminiviruses such as squash leaf curl virus (SqLCV) encode three proteins essential for systemic movement and disease development in the plant. While one of these proteins (AL2) has been implicated as a transactivator of viral gene expression, the role of the movement proteins BR1 and BL1 in pathogenesis remains to be elucidated. To investigate both the function and potential interactions of these proteins with host and viral proteins, we have constructed transgenic plants expressing these movement proteins in various combinations. Plants expressing AL2 and/or BR1 appear to be phenotypically normal. Rather unexpectedly, plants expressing BL1, either singly or in combination with other viral proteins, phenocopy the viral disease symptoms, which include leaf epinasty (curling under) and mosaicism. In addition to these characteristics, the BL1-expressing plants exhibit a "slow-grow" phenotype in which they are slow to shoot and slow to root, and exhibit decreased fertility, traits that appear to segregate in a Mendelian fashion. Thus, it appears that BL1 is at least in part responsible for disease symptoms in the infected plant. Cellular fractionation of transgenic plants and immunoblot analysis demonstrates that BL1 is localized to cell wall and cell membrane fractions. Electron microscopic analysis of thin sections to identify potential sub-cellular alterations and more precisely localize the viral movement proteins within the plant are currently in progress. These, as well as biochemical analyses to delineate interactions with other viral or host proteins and the role of the movement proteins in determining viral host range, will be presented.

A 404 CONSERVED PRIMARY STRUCTURE OF TWO TERPENOID PHYTOALEXIN BIOSYNTHETIC ENZYMES WHICH ARE INDUCED BY CELL WALL ELICITORS: CLONING OF CASBENE SYNTHETASE cDNA,

Christopher J. D. Mau[1] and Charles A. West[2], [1]Dept. of Biology and [2]Dept. of Chemistry and Biochemistry, UCLA, Los Angeles, CA 90024

Casbene synthetase catalyzes the conversion of geranylgeranyl pyrophosphate to casbene, a macrocyclic diterpenoid phytoalexin whose formation is elicited in castor bean seedlings by oligogalacturonides larger than the decamer. Earlier investigations using a partial cDNA clone suggested that expression of the casbene synthetase gene in response to oligogalacturonide treatment is under transcriptional control[1].

A near full-length cDNA clone has now been isolated and sequenced. The deduced polypeptide shows 44% identity with the deduced sequence for 5-*epi*-aristolochene synthase (EAS) from tobacco[2]. EAS forms the macrocyclic sesquiterpenoid precursor of the tobacco phytoalexin, capsidiol, after exposure of tobacco cells to cellulase.

The casbene synthetase gene appears to be present in a single copy. Comparison of the cDNA sequence to a genomic clone suggests that the gene contains 6 introns.

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2) Facchini, P. J. and Chappell, J. Submitted.

A 405 OLIGOGALACTURONIDE CONFORMATION AND ELICITATION OF DEFENCE GENES IN CARROT CELLS.

Johan Messiaen and Pierre Van Cutsem, Laboratoire de Biotechnologie théorique, FUNDP, rue de Bruxelles 61, B-5000 Namur, Belgium.

We investigated the ability of oligogalacturonides to depolarize cell membranes and mobilize cytoplamic calcium. Carrot protoplasts were exposed to alginates and polygalacturonic acid (PGA), pectic fragments with a degree of polymerization (DP) higher than 9, and pectic fragments with a DP<9. Single chain and dimeric "egg-box" conformations were induced using a combination of mono- and divalent cations as described by Liners et al. (1,2).

Carrot protoplasts underwent membrane depolarization following exposure to any pectic fraction independently of the size or conformation of the stimulating molecule. On the contrary, there was a completely different response in terms of cytosolic calcium mobilization. Only oligogalacturonides in a dimeric conformation were able to mobilize cytosolic calcium. DPs < 9 and DPs > 9 in a single chain conformation had no effect on cytosolic calcium. PGA and alginates slightly increased cytosolic calcium.

In collaboration with Dr. Somssich (MPI Cologne, FRG), we identified the defence genes induced by oligogalacturonides and the circumstances in which gene activation occured in terms of elicitor conformation. We have observed by *in vitro* nuclear run off transcription that oligomers of DP > 9, PGA, *Erwinia carotovora* and culture filtrates of *E. carotovora*, but not short DPs and single chain conformations, induce the specific transcription of defence-related genes (phenylalanin ammonia-lyase, 4-coumarate-CoA ligase, HRGP, PR1, PR2, chalcone synthase, peroxidase, tyrosin decarboxylase, S'-adenosyl-L-homocystein hydrolase and S'-adenosyl-L-methionin synthetase) in carrot suspension cultured cells and protoplasts. These results support the view that the pectic elicitors are active under the "egg box" conformation.

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A 406 OLIGOSACCHARINS FROM PLANT AND FUNGAL CELL WALLS RELEASED BY HF-SOLVOLYSIS, Bruno M. Moerschbacher, Barbara Graeßner, Peter Vander, Institut für Biologie III, RWTH Aachen, W-5100 Aachen, Germany

Puccinia graminis f. sp. tritici, the wheat stem rust fungus, is an obligately biotrophic parasite of wheat leaves. The fungus is growing in the intercellular spaces of the leaves, penetrating host cell walls only by minute haustoria. In resistant wheat plants, penetrated cells undergo hypersensitive cell death caused by the induction of an intracellular lignification. In the absence of the living fungus, hypersensitive lignification can also be induced by the application of elicitors derived from fungal cell walls and, possibly, endogenous elicitors, derived from wheat cell walls. Conversely, elicitor induced lignification can be suppressed by the simultaneous application of endogenous suppressors derived from host walls and, possibly, other suppressors derived from the rust cell wall. Suppressor active fractions were also able to suppress the hypersensitive reaction induced by the invading haustorium, thus making the resistant wheat leaf more susceptible. We have used sequential solvolyses in anhydrous hydrogen fluoride at increasing temperatures, extracting the cell walls after each step first with HF/ether, then water. The resulting water soluble fractions, containing monosaccharides in the HF/ether extracts, and oligo- and polysaccharides in the aqueous extracts, were then tested for elicitor and suppressor activity, both in liquid suspension cultures of wheat cells and in intact wheat leaves. Fractions were analyzed for their monosaccharide composition by GLC and for their oligosaccharide composition by HPLC. Active fractions were further fracitonated using MPLC. HF-solvolysis at -73 °C yielded an elicitor active aqueous extract presumably containing the well known glycoprotein elicitor of the stem rust fungus. At lower concentrations, the same fraction was active as a suppressor of elicitor induced reactions. Fractions containing chitin oligomers were inactive, altough oligomers of commercial crab chitin are potent elicitors in wheat. Uronic acid rich fractions of the wheat cell wall were active as suppressors, whereas the aqueous extracts after solvolyses at -23 °C and 0 °C were active as elicitors. These fractions contained mainly oligomers made up of glucosyl residues, presumably cellulo-oligosaccharides. The possible role of these oligosaccharins and of the enzymes releasing them from the cell walls are discussed.

A 407 IDENTIFICATION OF GENES AND PROTEINS INVOLVED WITH THE BIOSYNTHESIS OF CELL WALL COMPONENTS IN LOBLOLLY PINE XYLEM, D.M. O'Malley, W. Bao, W.W. Liu, C.A. Loopstra, J.J. MacKay, R.R. Sederoff, K.S. Voo, R. Whetten, Department of Forestry, North Carolina State University, Raleigh, NC 27695-8008

Wood consists of the cell walls of secondary xylem, which are composed primarily of cellulose (40-50%), hemicellulose (20-25%), and lignin (20-30%). The development of this tissue is specialized for the biosynthesis of the components of the secondary cell wall. This tissue can be obtained in kilogram quantities from mature trees. We have used differentiating xylem of loblolly pine for the isolation and identification of genes and proteins involved in cell wall formation. Our general strategy is to first obtain specific knowledge of the protein and its function, then to clone the corresponding gene. Our goal is to understand in one organism, the genetic regulation of the pathway involved in lignin biosynthesis and cell wall formation. To date, 4 lignin biosynthetic enzymes and a cell wall structural protein have been isolated and purified to homogeneity from this tissue: phenylalanine amonia-lyase, 4-coumarate coA ligase, cinnamyl alcohol dehydrogenase, laccase, and an extensin-like cell wall protein. cDNA clones have been isolated for Pal, Cad and 4CL, and genomic clones of Pal and Cad have been obtained. Two xylem specific genes of unknown function have been isolated to obtain xylem specific promoters. One of these genes, 3H6, shows features of a cell wall protein. Thus, by harvesting differentiating xylem from mature trees, we have obtained unambiguous identification of several genes involved in synthesis of secondary cell wall components

A 408 IMMUNOLOGICAL CHARACTERIZATION OF A CELL WALL PROTEIN CORRELATED WITH ELONGATION GROWTH AND AUXIN BINDING IN HIGHER PLANTS, Thomas Reinard, Stefanie Sprunck, Sandra Altherr, Hans-Jörg Jacobsen, LG Molekulargenetik der Universität Hannover, Herrenhäuser Straße 2, D-3000 Hannover 1, FRG.

A monoclonal antibody of IgM-type called TIM-11B2 has been screened from a hybridoma library. This antibody recognizes the probably O-linked glycosylic residue of a 40 kDa protein, p40, with a high specificity. This primary cell wall located protein was detected in all plant species examined and it is restricted to the epidermal layer of growing parts of seedlings. No or only very weak signals were found in tissues not undergoing elongation growth, like basal parts of etiolated seedlings, outgrown seedlings, green leaves, or apical parts of primary leaves from various graminaceae. This led us to conclude that the occurrence of p40 is restricted to tissues with the ability for elongation growth. Short term effects of elongation within 3h after exposure of 5d old seedlings to gravitropism had no effects on the abundance of p40.

Furthermore, purification of p40 resulted in copurification of auxin binding activity ($sABP_1$). Variations in purification procedures induced by incubation with 5mM ATP resulted in identical changes of both auxin binding and p40 occurrence during extraction. Nevertheless, analyzing the various data from binding tests revealed that p40 is not identical to $sABP_1$, but both polypeptides seem to be closely related.

A 409 CELL WALL MUTANTS OF *ARABIDOPSIS*. Wolf-Dieter Reiter, Clint Chapple, and Chris Somerville, MSU-DOE Plant Research Laboratory, East Lansing, MI 48824-1312

The primary cell wall of higher plants plays numerous roles during plant growth and development such as the determination of cell shapes and sizes; however, very little is known about biochemical and regulatory aspects of its synthesis. We have used a genetic approach to address these questions by isolating mutants of *Arabidopsis thaliana* with alterations in the composition of cell wall polysaccharides. More than 5000 EMS-mutagenized plants were screened for changes in the relative amounts of the cell wall-derived monosaccharides rhamnose, fucose, arabinose, xylose, mannose, and galactose, leading to the isolation of 38 mutant lines which are currently being analyzed by genetic and biochemical means. The observed alterations in monosaccharide composition encompass the complete absence of a monosaccharide, reductions or increases in the relative amounts of specific monosaccharides, or more complex changes in sugar composition data. Some, but not all, of the observed cell wall mutations correlate with morphological or physiological abnormalities. We obtained several alleles of a locus designated *fus1* which cause the virtual absence of L-fucose in the shoot apparently due to the inability of the plants to synthesize GDP-L-fucose by the *de novo* pathway. The fucose content in the roots of *fus1* plants is only slightly reduced, suggesting the presence of a second fucose-biosynthetic gene with root-specificity. The *fus1* mutation causes structural alterations in pectic and hemicellulosic polysaccharides, leads to a threefold decrease in tensile strength in elongating regions of inflorescence stems, and correlates with a slightly dwarfed growth habit. Overall, this genetic approach should help elucidate the roles of individual polysaccharide components for cell wall structure and function, and permit the isolation of cell wall-related genes.

A 410 THE MANIPULATION OF CELL WALL COMPONENTS IN TRANSGENIC PLANTS, Wolfgang Schuch, ICI Seeds, Plant Biotechnology Section, Jealott's Hill Research Station, Bracknell

The structure of cell walls plays an important role during plant development and the interaction of the plant with the environment. In addition, cell wall characteristics have major impacts on the industrial uses of crop plants. In order to determine the role of different cell wall polymers in these processes we have generated transgenic plants with structurally modified cell wall polymers. We have focussed our work initially on the modification of two of the major cell wall polymers: pectin and lignin. Pectin has been modified in tomato fruit through the inhibition of polygalacturonase and pectinesterase. Lignin has been modified in tobacco through the inhibition of cinnamyl alcohol dehydrogenase. Data will be presented on the physical modifications obtained and the consequence which these changes have on plant development. In addition, we have determined the industrial potential of plants with modified cell wall structure.

A 411 GROWTH-INHIBITING PUTATIVE OLIGOSACCHARINS FROM GRAPEVINE EXUDATE AND HYDROLYSATES OF GUM KARAYA, Stanley Strother, James A. Campbell, Anne M. Drake and Victor W.K. Lee. Department of Biological Sciences, Deakin University, Geelong, Victoria, 3217, Australia

Grapevines are known to exude considerable volumes of sap if canes are excised just prior to the breaking of winter dormancy. Given the dramatic physiological changes during budburst, it seems likely that this exudate would carry extremely active biological signals through the plant. This study utilised large volumes of exudate in an attempt to identify naturally-occurring oligosaccharins. Grapevine exudate was collected from year-old canes of a mature, field-grown *Vitis vinifera* var. Waltham Cross grapevine. Samples collected over 24 hours were pooled, and a 0.5 - 10 kD fraction obtained by ultrafiltration. The biological responses elicited by this fraction have been examined using a *Lemna minor* growth assay, and partial purification has been achieved using chromatographic techniques.

A further inhibitory putative oligosaccharin has been isolated from hydrolysates of gum karaya which significantly inhibit ($p < 0.05$) growth of *Lemna minor* in axenic culture. The hydrolysates were subject to dialysis using 1 kD cut-off tubing in order to remove low molecular weight material such as monosaccharides and small oligosaccharides. Controls using glucose subjected to the same hydrolysis procedure did not show any inhibition compared to untreated control flasks. The growth inhibition was associated with severe bleaching of the *Lemna* fronds. Dialysed hydrolysate was subjected to Sephadex G-15 chromatography using a column equilibrated with 50 mM potassium phosphate buffer (pH 7.0). This showed that an anthrone-positive peak was eluted from the column in a position consistent with its being an oligosaccharide or a mixture of oligosaccharides. Samples of the peak eluate were inhibitory to the growth of *L. minor* and caused bleaching of fronds. The hydrolysates react with Mejbaum's orcinol reagent to form a pigment absorbing at 660 nm, indicating that glucuronic acid is present.

These two lines of research both provide evidence for anthrone-positive inhibitors which have apparent molecular weights consistent with their being oligosaccharides.

A 412 INDUCTION OF ROOT GROWTH ON TCL EXPLANTS BY PECTIN OLIGOSACCHARIDES, V.V.Lozovaya, O.A.Zabotina, R.G.Malychov, M.V.Zihareva, *G.Beldman, A.G.J.Voragen, Institute of Biology Russian Academy of Sciences, P.O.30, Kazan 420503, Russia, *Dep.of Food Science, Wageningen Agricultural Univ., Bomenweg 2, 6703 HD Wageningen, The Netherlands.

Thin cell-layer (TCL) explants were obtained from hypocotyles of 5-6 days old buckwheat seedlings grown aceptically on MS medium (with half content of microsalts) in the dark. TCLs approximately 5mm long and 2-5mm wide were cut from hypocotyle tissues and consisted of 1 layer of epidermis cells, 2 layers of subepidermis cells and 2-3 layers of parenchyma cells. Explants were cultured individually in 2ml Petry dishes with liquid RX medium (in the absence of phytohormones). We tested root-inducing activity of oligosaccharides obtained by acid hydrolyses of pectic polysaccharides isolated from pea stem cell walls. Thee fractions were obtained after separation of the pectic hydrolysate on gel-permeation column (TSK-40H). The part of the peak with a degree of polymerization of about 20-25 was then applied onto a DEAE-cellulose column. Five fractions were obtained after elution with a NaCl gradient, the first fraction named 1PN was eluted from resin before the NaCl gradient was started. HPLC analysis (CarboPac PA 100) showed that the fraction 1PN contained a large amount (about 30%) of galacturonic acid oligomers with degree of polymerization 3-14, as well as neutral oligomers. Addition of this fraction (10 μg/ml) to the growth medium of buckwheat TCLs resulted in a rapid induction of root formation on the explants in numbers greater than in control variants. Also the number of roots formed on each explant of this variant was higher than on the control. Besides of those observations 1PN was shown to promote growth of roots in the length. Neutral part of this fraction consisted of galactose (65%), glucose, mannose, xylose (4-7%), arabinose (8%) and rhamnose (15%).

Late Abstract

A MODEL SYSTEM FOR THE ANALYSIS OF ERWINIA-PLANT INTERACTIONS, Jürgen Denecke, Sabina Vidal, K. Maria E. Hurtig and E. Tapio Palva, Dept. Molecular Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

Erwinia carotovora subsp. carotovora (Ecc) secretes a family of plant cell wall degrading enzymes, which constitute the main virulence factors of this plant pathogen. We are interested in analyzing the contribution of individual enzymes within the complex array of events that take place during the interaction of the bacterium with its host plant. To monitor events in planta, we use transgenic tobacco plants which are homozygous for a chimeric gene coding for the reporter enzyme GUS under control of the ß-1,3-glucanase promotor from Nicotiana plumbaginifolia. Individually produced enzymes from Escherichia coli strains carrying the plasmid borne genes from Ecc are applied to plants and the induced expression of the trans gene is monitored in function of the time. Using this model system, we could demonstrate that pectic enzymes can elicit plant defence reactions in contrast to isolated cellulase. We are currently analyzing the effect of combinations of different enzymes on the induction of the plant response.

Preliminary experiments indicated that salicylic acid treated plants contain enhanced levels of pectic enzyme inhibitors. The individually produced pectic enzymes are used to identify the specificity of the plant encoded inhibitors for different isoforms. We have also shown that salicylic acid induces the expression of the luminal binding protein (BiP), a chaperone which is located in the endoplasmic reticulum (ER). BiP induction by salicylic acid or treatment with pectic enzymes is compared to the induction of the plant homologue of calreticulin, which is the most abundant ER protein and can therefore be regarded as a measure for the amount of ER. By studying both the proliferation of the secretory apparatus and the induction of defence related proteins that pass through this system, we hope to elucidate the events in planta which are associated with pathogen attack.