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Cell Wall Biology: Perspectives from Cell Wall Imaging

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ABSTRACT Polysaccharide-rich plant cell walls are important biomaterials that underpin plant growth, are major repositories for photosynthetically accumulated carbon, and, in addition, impact greatly on the human use of plants. Land plant cell walls contain in the region of a dozen major polysaccharide structures that are mostly encompassed by cellulose, hemicelluloses, and pectic polysaccharides. During the evolution of land plants, polysaccharide diversification appears to have largely involved structural elaboration and diversification within these polysaccharide groups. Cell wall chemistry is well advanced and a current phase of cell wall science is aimed at placing the complex polysaccharide chemistry in cellular contexts and developing a detailed understanding of cell wall biology. Imaging cell wall glycomes is a challenging area but recent developments in the establishment of cell wall molecular probe panels and their use in high throughput procedures are leading to rapid advances in the molecular understanding of the spatial heterogeneity of individual cell walls and also cell wall differences at taxonomic levels. The challenge now is to integrate this knowledge of cell wall heterogeneity with an understanding of the molecular and physiological mechanisms that underpin cell wall properties and functions.

Key words: Cell structure; cell walls; fluorescence imaging; development; cell wall imaging; polysaccharides.

INTRODUCTION

Growing plant organs have the strength and flexibility to resist the impacts of rain and intense gusts of winds and also the capability of penetrating compacted soils. The mechanical robustness of plant organs is due to the presence of tough cell walls at cell surfaces. Cell walls do not only impart cell shapes and mechanical properties for extension growth in variable environments, but are also responsible for cell-to-cell adhesion that is a core attribute of the mechanical robustness of growing plants. The importance of plant cell walls extends into many areas of human activity and endeavor. Cell walls are an important set of biomaterials in that they are not only crucial for the properties of plant organs and hence crop growth, but they are in addition the major repository for the Earth's photosynthetically fixed carbon and a crucial resource in carbon recycling. Cell walls are therefore critical to plant and microbial growth, herbivore nutrition, and to the maintenance of terrestrial and marine ecosystems. Moreover, cell walls are widely exploited in diverse human activities relating to food, food additives, industrial enzymology, fibers, textiles, paper, lumber, and biofuels. This wide reach of issues pertaining to cell walls and their components places them with a central importance in biology (Albersheim et al., 2010).

Cell walls have long been classed as primary or secondary, depending upon whether they are, respectively, extendable or non-extendable during organ growth. Primary cell walls generate turgor pressure (thus resisting tensile forces), accommodate cell expansion, mediate cell adhesion, and occur at the surface of most plant cells. Secondary cell walls are restricted to specific sets of differentiated cells, tend to be thicker than primary walls, and resist compressive forces. As we learn about the microstructures of cell walls, we can see that there is a great variety of both primary and secondary cell walls in molecular terms and also that there may not be clear boundaries between the architectures and properties of the two types of wall, but more of a continuum (Albersheim et al., 2010; Knox, 2008).

FROM CELL WALL CHEMISTRY TO CELL WALL BIOLOGY

The cumulative achievement in the characterization of the major polysaccharide components that are found in cell walls

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(predominantly from seed plants) from the earliest chemistry to today's sophisticated biochemical analyses is considerable. This work indicates that cell wall polysaccharides are of both great structural complexity and with significant capacity for structural modulation within classes. The road to this achievement of cell wall chemistry is ably set out elsewhere (McNeil et al., 1984; Bacic et al., 1988; Carpita and Gibeaut, 1993; O'Neill and York, 2003; Harris, 2005; Caffall and Mohnen, 2009; Voragen et al., 2009). We now have a clear view of the biochemical structure of cellulose, of the sets of polysaccharides that are classed as hemicelluloses, and of the complex pectic polysaccharide group that contains polymers rich in galacturonic acid. The occurrence of polyphenolic lignin in cells with secondary cell walls is also a key facet of cell wall properties and land plant evolution (Weng and Chapple, 2010).

We can list around a dozen major polysaccharide structural features that exist in plant cell walls. These display wide ranges in abundance and occurrence. Examples include the β-1,4-glucan of microfibrillar cellulose, which is common to most cell walls and is present as 30–90% of wall polysaccharides; a class based on a backbone of β-1,4-xylan that shows strong taxonomic variation both in structure and in occurrence in primary and secondary cell walls; and β-1,4-galactan, a polysaccharide motif that often occurs in the context of a hypervariable rhamnogalacturonan-I (RGI) pectic polysaccharide that appears to be most predominant in the vascular plants. The hemicellulose polysaccharide grouping (that can be defined in terms of β-1,4-linked backbones with an equatorial configuration or as sets of polymers that have an ability to cross-link cellulose microfibrils and have been classed as cross-linking glycans (McCann and Roberts, 1991; Scheller and Ulvskov, 2010) consists of several polysaccharide groups that include xyloglucans, xylans, mannans, and mixed-linkage glucans. The first three of these hemicellulosic polymers display wide structural diversity in terms of substitutions and elaborations resulting in, for example, fucogalactoxyloglucan, glucuronoxylan, arabinoxylan, glucomannan, and galactomannan subsets that often have clear taxonomic or functional distinctions. Homogalacturonan (HG) is the major structural domain of the pectic polysaccharides and component of a matrix network that is co-extensive with and can also be linked to the cellulose–hemicellulose network. HG is found in conjunction with rhamnogalacturonan-II (RGII), RGI, xylogalacturonan, and arabinan and (arabino)galactan pectic domains. HG has a considerable capacity for enzymatic structural modulation *in muro* and RGI polymer structures appear to vary with developmental contexts and this may also involve *in muro* modulation. Most of the polysaccharides mentioned above, when present, are widespread throughout individual cell walls. Structural variants of these polysaccharides, however, can be present in cell and taxonomic patterns of occurrence and these are not always easily discerned or, as yet, understood. In contrast, callose, a 1,3-glucan, is an interesting and unique cell wall polysaccharide with a specific and restricted occurrence in cell plates, pollen tubes, plasmodesmata, and wounded cells, and is not found widely distributed within individual cell walls (Bacic et al., 2009).

The cell walls of terrestrial plants are unique in biochemical terms in that most of the macromolecular components, other than cellulose, do not occur in other biomaterials, with the exception of some algal cell walls (Popper and Tuohy, 2010; Sørensen et al., 2010). The range of polysaccharides outlined above is limited in comparison to those found across algal taxa—a factor that is supportive of the idea of the monophyletic origin of land plants from a charophycean green alga (CGA) ancestor (Graham et al., 2000). Innovation of new polysaccharides, with a few possible exceptions, such as that of borate cross-linked RGII and possibly other pectic polysaccharides, does not appear to have been a feature of land plant cell wall diversification. However, structural innovation within polysaccharide classes and modulation of occurrence, and possibly roles, appears to have been extensive (O'Neill et al., 2004; Harris, 2005; Peña et al., 2008; Burton and Fincher, 2009; Hsieh and Harris, 2009; Popper and Tuohy, 2010; Sørensen et al., 2010). A schematic overview of the occurrence of the major cell wall polymers in relation to the evolution of land plants is shown in Figure 1. How cell wall components have functioned during the transition to land and the subsequent evolution of complex plant bodies is currently an area for intense research activity. An important point here is that the CGA are a highly diverse group, with species living in a range of environments. They display a great range and diversity of cell wall structures that include many of the polymers known to be present in advanced land plants (Domozych et al., 2009; Popper and Tuohy, 2010; Sørensen et al., 2010). It is likely that it is among the pectic polysaccharides (RGII, RGI, and xylogalacturonan) that the most substantial innovation in

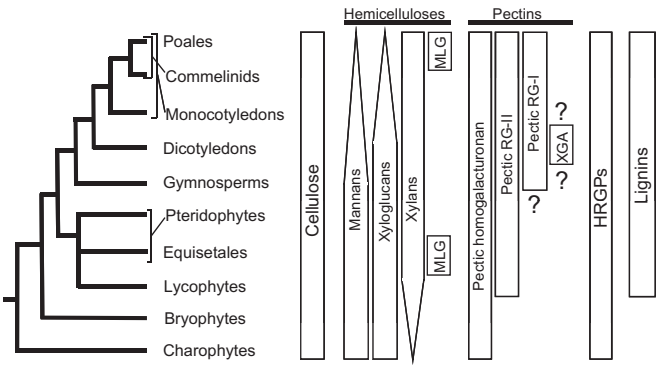

Figure 1. Schematic Outline of the Occurrence of the Major Cell Wall Polymers in Relation to the Major Extant Groups of Land Plants and Charophycean Green Algae.

Tapering boxes indicate that polymers are present at relatively reduced levels. Question marks indicate where little is known outside groups that have been examined. The plant phylogeny is highly simplified and does not take into account the distinction between eudicots and basal angiosperms, as little cell wall analysis has been conducted for the latter group. MLG, mixed-linkage glucan; XGA, xylogalacturonan; HRGPs, hydroxyproline-rich glycoproteins.

polysaccharide structures and functions have taken place in response to new requirements for cell wall materials in complex plant bodies in terrestrial environments. These pectic polysaccharides will be an important focus for future study in terms of both their taxonomic occurrence as well as their cellular functions.

How can all this detailed chemical information on wall polymers be understood in biological terms? Steps towards understanding the genetic basis of the biosynthesis of cell wall polysaccharides and their cell-based and physiological functions are advancing (Lerouxel et al., 2006; Sandhu et al., 2009; Liepman et al., 2010). Precise and excellent methods are in place for the analysis of aspects of the dynamics of cellulose deposition and the interaction of its synthesizing machinery with cytoskeletal systems (Crowell et al., 2009). Equivalent methodologies are assembling data on the impact of phytohormones and protein signaling systems on cell walls (Hématy et al., 2007; Ringli, 2010; Sánchez-Rodríguez et al., 2010) and the identification of networks of transcriptional regulators that control cell wall thickening and the deposition of secondary cell walls (Zhong et al., 2008). In relation to all of this activity, a significant challenge remains. This is the generation of equally robust, sophisticated and tractable methods to study non-cellulosic polysaccharides *in situ* and to generate detailed inventories of polysaccharides at the level of tissues, cell types, cell status, and individual cell wall architectures. In short, we are currently lacking comprehensive and detailed molecular descriptions of cell wall microstructures for many systems. We need to know this in relation to diverse cell wall origins (cell plate assembly, diffusive growth infill, or tip growth extension), cell wall architectures (wall thickenings, cell adhesion, pit fields), cell expansion, cell adhesion, cell type specifications, and also in relation to organ and species diversity. It is increasingly clear that cell walls within an organ appear to be mosaics of complex heterogeneous architectures and the goal is to describe and to understand this macromolecular patterning and what the structural features of specific polysaccharides impart to each cell wall or cell wall region. Other questions that arise from such a cell biological perspective on cell walls include questions such as how land plant evolution, discussed above, has impacted upon polymer presence, interactions, and functions. Furthermore, an important goal of cell wall biology is to understand how the heterogeneous cell wall structures are responsive to environmental and mechanical impacts during cell development and organ growth.

The deepening of chemical knowledge of cell wall polysaccharides has led to useful models and popular schematics of how cell wall components fit together (Keegstra et al., 1973; McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Somerville et al., 2004; Baba, 2006). These are now being refined in the light of the increasing appreciation of cell wall diversity. The most common of these views have at their core cellulose microfibrils cross-linked by hemicelluloses and these being co-extensive with a matrix of pectic polysaccharides. Both hemicellulose and pectic polysaccharides are structurally complex and likely to contain distinct subsets of polymers that can contribute to microfibril cross-linking or to diverse aspects of matrix properties. Before we have a clear understanding of cell wall mechanisms, we need to know what polysaccharides are present, how they are integrated with other polymers, and how structural modulations impact on cell wall properties. A major element of the shift of focus from cell wall chemistry to cell wall biology has been the development of imaging tools to define the spatial heterogeneities of cell walls.

IMAGING THE GLYCOME: THE CHALLENGES OF DETECTION AND BEYOND

A goal in imaging the glycans of cell walls is a methodology that allows the concurrent visualization of several components of polysaccharide configurations, their modulation, and their dynamics in a single cell—and ideally a living one. This is not yet possible and the *in vivo* imaging of glycans is only in early stages of development (Laughlin and Bertozzi, 2009). Currently, an exploration of cell wall chemistry in cellular contexts requires the use of specific stains or molecular probes on plant materials with the challenging and restrictive issues of molecular access through cell walls with low porosity. In spite of these persistent challenges, the available probe technologies have led to many insights into cell wall complexity, heterogeneity, and dynamics.

Currently, the best methods are arguably the use of molecular probes such as monoclonal antibodies and carbohydrate-binding modules (CBMs) that can bind to specific polysaccharide structures in context with high resolution and sensitivity (Moller et al., 2008; Knox, 2008; Pattathil et al., 2010; Ralet et al., 2010). Ideally, these probes should have defined epitope/ligand structures that will generally be of the order of three to eight sugars. However, this can be a challenge due to the restricted oligosaccharide availability for both immunogen preparation and probe characterization. The glycomics field and associated technologies are developing (Turnbull and Field, 2007) but procedures for the systematic and facile synthesis of oligosaccharides found in plant cell wall polysaccharides, to underpin probe generation and oligosaccharide microarray construction, are not yet available. Despite these technical challenges, several panels of monoclonal antibodies and recombinant CBMs have now been developed and are continually being added to (see online resources at www.biosupplies.com.au, www.carbosource.net, and www.plantprobes.net). Cell wall probes are powerful tools that have indicated previously unimagined complexity and diversity to cell wall structures.

In vitro and *in situ* analyses of probe binding can present challenges and a careful characterization of antibody specificities is required, in both of these experimental contexts, ideally with defined oligosaccharide haptens and the use of specific enzymes (e.g. Verhertbruggen et al., 2009; Marcus et al., 2010; Ralet et al., 2010). Such complementary analytical tools

are crucial, as recognition of polysaccharide preparations alone can be misleading—but also insightful when combined with other protocols. For example, as shown in Figure 2, the LM21 mannan (Marcus et al., 2010) and LM5 galactan (Jones et al., 1997) monoclonal antibody probes both offer effective recognition of a commercial sample of carob galactomannan. This could indicate that the LM5 1,4-galactan-directed antibody cross-reacts with a structural feature found in the galactomannan polysaccharide but, in fact, enzyme deconstruction analysis of the galactomannan indicates that the two probes recognize distinct polysaccharide domains in the sample. The observation is likely to reflect a close association of galactomannan and 1,4-galactan that has survived polymer isolation and may also reflect their similar location in certain cell walls that has been observed in, for example, *Pinus radiata* compression wood (Mast et al., 2009; Marcus et al., 2010).

Recent discoveries concerning polysaccharide detection in cell walls, which some researchers have perhaps met with an initial sense of dismay (as they can evoke doubts over observations made by cell wall immunohistochemistry), include the demonstration of clear cases of polysaccharide masking and blocked access of probes to their epitopes. However, these observations have in fact led to an advance in understanding of cell wall structures, as the effective masking or cloaking of cell wall polysaccharide epitopes is often developmentally regulated, indicating that it is a controlled facet of cell wall microstructures. In short, such studies indicate the existence of diverse microenvironments within cell walls that are likely to influence the access of proteins or enzymes to specific targets. It is now clear that xyloglucan, xylan, and mannan epitopes can be effectively masked in primary cell walls by the presence of pectic HG (Marcus et al., 2008; Hervé et al., 2009; Marcus et al., 2010). The demonstration that the access of a fluorescent mannan-directed CBM, a small-molecular-weight protein, is also restricted in its access to its ligand indicates that polymer masking is not an artifact due to the use of large antibody molecules as detection tools (Marcus et al., 2010). This phenomenon has currently been most characterized in cell walls of a range of dicotyledon stems, as large numbers of equivalent sections are required for the parallel and comparative enzyme treatments. The ability of pectic HG to block access to probes and enzymes has consequences for the understanding of cell wall deconstruction as much as for cell wall biology (Lionetti et al., 2010; Hervé et al., 2010). It will be important to explore the extent of this phenomenon in meristems and growing regions, as the regulated masking of polysaccharides has implications for the access of proteins such as expansins and their roles in controlling cell extension. It will also be of interest to determine whether there are also equivalent cryptic epitopes in grass cell walls with relatively low levels of pectic HG.

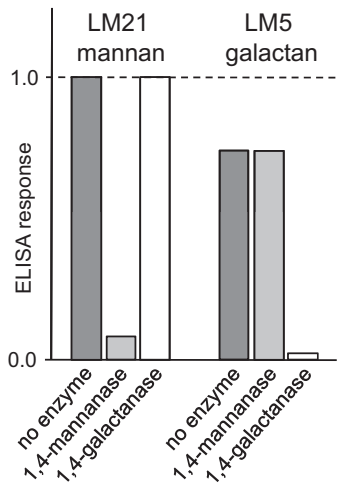

Figure 2. Enzyme Dissection of Antigens Can Be Essential to Determine Cell Wall Probe Recognition Properties.

Diagram showing binding of the LM21 mannan and LM5 galactan monoclonal antibodies to a sample of carob galactomannan. Although both antibodies bind effectively, as evidenced by ELISA at 100-fold dilution of hybridoma cell culture supernatants, the binding of LM21 is specifically sensitive to mannanase and not galactanase enzymes and the converse for LM5 binding. Enzymes were used at 10 μg ml^{-1}. This indicates that 1,4-galactan is a structural feature isolated in association with the galactomannan polymer.

Glycan microarray procedures using the cell wall probe panels are also being developed to generate information on cell wall glycomes (Moller et al., 2007). Development of microscale methodologies (involving the use of antibody/CBM probes as detection tools in association with chromatographic separations) to define the antigenic contexts of epitopes that are being tracked in particular systems will be a powerful approach to integrate with, and underpin, immunohistochemical and glycan microarray procedures. Such approaches also have the advantage that the methods will unmask any cryptic epitopes and will be useful, with enzyme use, to explore epitope/polymer associations. Such semi-quantitative analyses using panels of molecular probes aligned with high-throughput glycan mapping approaches will lead to an integrated analysis of the specific structural features of polysaccharides that can be directly related to cell wall contexts.

Defining in detail the molecular features of cell wall regions in terms of specific configurations of polysaccharides may not be easy. However, we suggest that we are on the verge of a new and detailed phase of the imaging of cell wall glycomes that will arise from the use of extended sets of molecular imaging probes in conjunction with systematic enzymatic deconstructions.

REVEALING POLYSACCHARIDE FUNCTION

There would appear to be much to learn about the cell biology of non-cellulosic polysaccharides. It is clear that the pectic polysaccharides are a set of complex glycans with considerable potential for structural modulation and that the pectic HG subset is involved in complex loops of regulation of its structure/properties involving methyl esterification and acetylation

influencing cell wall assembly, plant defense, cell adhesion, leaf primordia initiation, and cell extension (Lionetti et al., 2007; Peaucelle et al., 2008; Pelloux et al., 2007). The large classes of HG modifying enzymes (polygalacturonases, pectate lyases, pectin methyl esterases, and associated inhibitors) act to modify HG structure and wall properties and the individual genes/proteins of these classes are gradually being placed in development contexts (Pelloux et al., 2007; Wolf et al., 2009). Of the other major pectic domains, RGI, with a currently uncertain evolutionary origin, would appear to have the potential to be present in varied forms and is implicated in the regulation of mechanical properties in angiosperms (Caffall and Mohnen, 2009). The understanding of how the specific forms of RGI are generated or act is far from being understood. In terms of glycan imaging, RGI is currently assessed with the LM5 galactan and LM6/LM13 arabinan antibody probes for neutral side chains and the recent generation of probes for the RGI backbone (Ralet et al., 2010) will be an important addition to antibody panels. In angiosperms, modulated domains of galactan- and arabinan-rich RGI can be detected at some meristems (McCartney et al., 2003). An extended understanding of the regulation of RGI structures within physiological/hormonal networks that control meristem functions is an important goal. Major questions will concern how intimately the varied RGI structures are linked to the structures and functions of meristems and how RGI structure and function have evolved in the context of the evolution of the complex stratified meristems of angiosperms.

For the hemicellulose polysaccharides, there are also considerable gaps in our functional understanding of the polymers and their structural variants. Recent studies have revealed several aspects of cell wall heterogeneity in relation to hemicelluloses. These include patterns of hemicellulose epitopes in relation to cell adhesion in tomato fruit parenchyma (Ordaz-Ortiz et al., 2009) and unmasked mannan epitopes in regions of pit fields at the inner face of primary cell walls of tobacco stem pith parenchyma (Marcus et al., 2010) and as shown in Figure 3A and 3B. The possible *in muro* enzymatic remodeling of hemicelluloses, as, for example, by the action of fucosidases and galactosidases on xyloglucan (Obel et al., 2006), could contribute to diverse polymer structures to meet local needs within cell walls. Such studies are indicative that polymers currently defined as hemicelluloses and/or cross-linking glycans are likely to have diverse functions and that these may not always relate directly to the cross-linking of cellulose microfibrils controlling expansive growth. Mannans are a particularly interesting case, as these widespread polymers with (galacto)(gluco)mannan variants are implicated in a range of events, including oligosaccharide signaling, embryogenesis, and seed germination (Beňová-Kákošová et al., 2006; Schröder et al., 2009; Goubet et al., 2009). Mannans are abundant in lower plants (Liepman et al., 2007) and may have evolved new functions during land plant evolution. At the surface of the model, bryophyte *Physcomitrella patens* mannan epitopes can have very restricted patterns of occurrence as shown, for example, in Figure 3C–3E. In this case, mannan epitopes are detected at the base of side branches of caulonemal filaments by both a mannan-directed antibody and a CBM. We have virtually no understanding of the functional significance of this regulated pattern of occurrence that does not appear to be due to masking by HG. Defining the specific functions of polysaccharides in systems such as these may allow the implementation of a more refined nomenclature for hemicellulose polysaccharides than the current structurally based one or the rather loose term cross-linking glycans (Scheller and Ulvskov, 2010). Few methods currently compete with the sensitivity and subtlety of antibody or CBM binding at cell surfaces to gain insights into spatial heterogeneity across individual cell walls. The application of fluorescent tags to polysaccharides at the surfaces of living accessible cells such of those of *P. patens*, root hairs, pollen tubes, or the tractable desmid *Penium margaritaceum* (Domozych et al., 2009) are powerful experimental approaches.

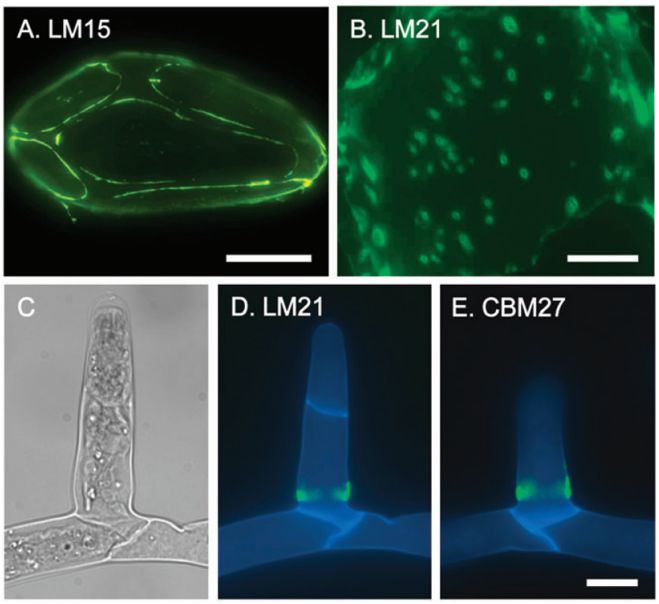

Figure 3. Immunofluorescence Imaging of Cell Walls Showing Examples of Chemical Heterogeneity at the Level of Individual Cell Walls.

(A) The LM15 xyloglucan epitope is specifically located at the edge of cell adhesion planes at the surface of an intact cell isolated from a tomato fruit by the action of a pectic HG-degrading enzyme. For details, see Ordaz-Ortiz et al. (2009). Bar = 100 μm.

(B) Inner face of transverse wall of tobacco pith parenchyma cell immunolabeled with LM21mannan after enzymatic removal of pectic HG shows 'unmasked' mannan epitopes in regions of pit fields. For details, see Marcus et al. (2010). Bar = 20 μm.

(C–E) Restricted occurrence of mannan at the surface of intact cells of a *Physcomitrella patens* gametophyte. (C) Bright field image of a side branch growing from a caulonemal filament. (D) Same cells as (C) immunolabeled with LM21 mannan antibody. (E) Equivalent cells labeled with CBM27, a mannan-specific probe. Mannan probe binding detected with FITC (green) is restricted in each case to the base of the side branches. Calcofluor White staining (blue) shows all cell walls. Bar = 20 μm.

The advent of technologies allowing the analysis of genomes and proteomes has led to increased understanding of the genes and proteins required for the synthesis and modification of cell wall polysaccharides (Chivasa et al., 2002; Egelund et al., 2004; Kwon et al., 2005; Albenne et al., 2009; Mutwil et al., 2009). It is in relation to such studies that cell wall imaging procedures have a very important role to play. Genetic interventions often lead to gross phenotypes where cells and cell walls can often remain as unexplored, or even unexplorable, black boxes—unless methodologies to define detailed molecular cell wall structures are available. The dissection of gene function has provided considerable insight into cell wall plasticity and in the striking case of the disruption of two xylosyltransferases has led to plants with no detectable xyloglucan (Cavalier et al., 2008). The observation that these plants have only relatively subtle changes in growth under standard conditions is important. Such studies offer real insights into cell wall plasticity and the possibility of redundancy at the level of polymers in which the loss of one polymer can lead to a structurally distinct polymer fulfilling its role. Cell wall plasticity has, of course, been well established from studies of cellulose-synthesis inhibitors. This then leads to somewhat of a paradox in our current understanding in that sets of polymers can be redundant to some extent, and perhaps interchangeable, and yet the more we study cell wall structures *in situ*, the more we see specific locations and occurrences for specific polymers that are suggestive of distinct and diverged functions. Untangling polymer functions and the mechanisms of cell wall plasticities will be an exciting phase of cell wall biology.

WHERE CELL WALL BIOLOGY MEETS MECHANICS AND PHYSICS

Gaps in our cell biological understanding of cell walls that are currently being filled concern the when and where of polysaccharides (and their structural variants) within single cells and growing organs. This is an essential prerequisite before the genetic basis of the intermolecular assembly and molecular remodeling of cell walls can be truly analyzed and understood in mechanistic terms. As we generate a more detailed understanding of the diverse polysaccharide configurations of cell wall materials, the questions that arise are of the specific functions of individual polymers within wall composites. A key here, maybe, is to consider cell walls from the perspective of smart materials—constantly being optimized on the microscale in terms of functions and physical/mechanical properties in response to intrinsic and extrinsic signals. It is in this context that there would appear to be much to reveal about the sets of polymers within the hemicellulose and pectic groups. Modeling of wall structures and studies of the physics of intact walls and synthetic gel systems are underway (Chanliaud et al., 2002; Thompson, 2005; Ulvskov et al., 2005; Wei and Lintilhac, 2007; Vincent et al., 2009; Kha et al., 2010; Winship et al., 2010). An understanding of the contribution of the full range of non-cellulosic polymers to the physical/mechanical properties of cellulosic walls will be an important goal. Complex signaling networks must be in place to effect local structural remodeling within individual cell walls and cell wall domains. At the core of these signaling systems—in terms of both detection and responses—will be the cell walls that confront the mechanical loads and the impacts that are faced by growing plants. The insights gained from the increasingly detailed cell wall imaging procedures and the assessments and the modeling of the physical attributes of intact cell walls are likely to be central to achieving the goal of integrated knowledge of cell wall biology.

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