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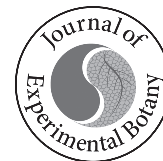

REVIEW PAPER

We be jammin': an update on pectin biosynthesis, trafficking and dynamics

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Abstract

Pectins are complex polysaccharides that contain acidic sugars and are major determinants of the cohesion, adhesion, extensibility, porosity and electrostatic potential of plant cell walls. Recent evidence has solidified their positions as key regulators of cellular growth and tissue morphogenesis, although important details of how they achieve this regulation are still missing. Pectins are also hypothesized to function as ligands for wall integrity sensors that enable plant cells to respond to intrinsic defects in wall biomechanics and to wall degradation by attacking pathogens. This update highlights recent advances in our understanding of the biosynthesis of pectins, how they are delivered to the cell surface and become incorporated into the cell wall matrix and how pectins are modified over time in the apoplast. It also poses unanswered questions for further research into this enigmatic but essential class of carbohydrate polymers.

Key words: Cell expansion, cell wall biosynthesis, glycosyltransferase complexes, pectate lyase, pectin methylesterase, polygalacturonase, vesicle trafficking.

Introduction

The walls of plant cells must be both flexible and strong to enable and constrain cellular, tissue and organ growth. These specialized extracellular matrices, which are largely built of carbohydrate polymers that are synthesized using sugars derived from photosynthesis, serve as dynamic support structures for plants to enable the development of a wide array of morphologies (Cosgrove, 2005). Cell walls also serve as protective barriers that buffer the interiors of plant cells against abiotic and biotic stresses. The so-called primary walls that surround actively growing plant cells are composed of cellulose, hemicelluloses, pectins and structural glycoproteins, and also contain a wide array of enzymes and other proteins that can modify wall structure or trigger cellular responses to intrinsic and environmental stimuli (Somerville *et al.*, 2004; Keegstra, 2010). The cell wall is often described as a distinct extracellular compartment, but in fact it is intimately connected to the cell surface by components of the wall biosynthetic machinery itself, as well as structural proteins that maintain plasma membrane-wall contacts and sensory proteins that can bind to wall components to monitor wall integrity (Liu *et al.*, 2015).

Pectins have long been used by human societies as gelling agents in foods, and more recently, their influence on wall

Abbreviations: APAP1, ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN1;ARAD, ARABINAN DEFICIENT;CESA, CELLULOSE SYNTHASE; FRA1, FRAGILE FIBER 1 kinesin;GalA, galacturonic acid;GAUT, GALACTURONOSYLTRANSFERASE;GT, glycosyltransferase;HG, homogalacturonan;PG, polygalacturonase;PL, pectate lyase;PME, pectin methylesterase;RG-I, rhamnogalacturonan-I;RG-II, rhamnogalacturonan-II;RGXT, RHAMNOGALACTURONAN XYLOSYLTRANSFERASE;RLP, RECEPTOR-LIKE PROTEIN;SCAMP, SECRETORY CARRIER MEMBRANE PROTEIN;ssNMR, solid-state Nuclear Magnetic Resonance;SVC, secretory vesicle compartment;TBL, TRICHOME BIREFRINGENCE-LIKE;WAK, WALL-ASSOCIATED KINASE.

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charge and porosity has made them targets for studies aimed at optimizing cell wall deconstruction, which is one of the most expensive stages in bioenergy production from plant-based biomass (Xiao and Anderson, 2013). Additionally, pectins are often used as biodegradable carriers for time-released drug delivery, and limited studies have indicated that pectins themselves can influence the gut microbiome (Koropatkin *et al.*, 2012) and, in modified forms, act as antitumor agents in some types of cancers (Leclere *et al.*, 2013). Thus, the utility of pectins is widespread, and is poised to expand rapidly as biorefineries become more sophisticated in producing a wide array of biomass-derived, value-added products (Edwards and Doran-Peterson, 2012).

The unique biomechanical properties of plant cell walls, which exhibit both elastic and plastic deformation under stress (Burgert and Dunlop, 2011), have long fascinated biologists. Although cellulose-hemicellulose networks are thought to act as the main load-bearing components of plant cell walls and cellulose-hemicellulose interfaces are the targets of a major class of wall-loosening proteins called expansins (Wang *et al.*, 2013), pectins also play critical functions in wall architecture, cellular growth and tissue morphogenesis (Palin and Geitmann, 2012; Wolf and Greiner, 2012). However, many details of how pectins are generated, delivered to the wall and interact with other wall components to achieve their functions either remain completely mysterious or are only beginning to be understood. This update will focus on synthesizing connections between specific aspects of pectin dynamics, some of which have been covered in further detail in a series of excellent recent reviews (Palin and Geitmann, 2012; Wolf and Greiner, 2012; Atmodjo *et al.*, 2013; Senechal *et al.*, 2014b; Levesque-Tremblay *et al.*, 2015), and will also pose some key questions for future research.

Evolving views of pectin abundance and structure

Pectins are highly abundant in the primary walls of eudicot plant species and can comprise up to one third of the non-water mass of the wall (Somerville *et al.*, 2004). However, their much lower abundance in secondary walls, which are deposited after plant cells cease growth, and the walls of commelinid monocots, which include bioenergy crops and other grasses (Vogel, 2008), has led to speculation that the functions of pectins can be supplanted by other wall matrix polymers such as glucuronoarabinoxylans, which despite their classification as hemicelluloses also contain large numbers of glucuronic acid residues (Rennie and Scheller, 2014) that make them somewhat pectin-like. However, many monocot genomes contain significant numbers of putative pectin-synthesizing and -modifying genes (Tyler *et al.*, 2010; McCarthy *et al.*, 2014; Senechal *et al.*, 2014*b*), implying that pectins function in at least some aspects of monocot growth and development. For example, pectins are among the earliest detectable wall elements of the cell plate during cytokinesis in plants and their presence in the cell plates of both dicots (Samuels *et al.*, 1995) and monocots (Baluska *et al.*, 2005) suggests that a function for pectins in cytokinesis might be conserved among both groups. Additionally, pectin composition and abundance changes in some monocot species in response to environmental stimuli such as drought (Leucci *et al.*, 2008), suggesting that pectins might be involved in facultative adaptations to environmental perturbation across diverse plant taxa. Thus, the perception that pectins are limited to functioning in eudicot primary walls is likely incorrect.

Conventionally, pectins have been classified into so-called ‘domains’ that include homogalacturonan (HG), xylogalacturonan, apiogalacturonan, rhamnogalacturonan-I (RG-I) and rhamnogalacturonan-II (RG-II) (Atmodjo *et al.*, 2013; Mohnen, 2008). HG is the most abundant form of pectin in the primary walls of eudicot plants, comprising 50–70% of recoverable pectins in *Arabidopsis thaliana* primary walls (Zablackis *et al.*, 1995). It is composed of unbranched α-1,4-linked galacturonic acid (GalA) chains that can be decorated with xylose to form xylogalacturonan, apiose to form apiogalacturonan, and acetyl groups at the O2 and O3 positions of GalA (Ishii, 1997). The carboxyl groups of GalA residues in HG can be methyl-esterified, and when de-methyl-esterified can be negatively charged, with apparent pKa values ranging from 3.55 to 4.10 depending on the overall degree of methyl-esterification of the polymer (Plaschina *et al.*, 1978); thus, methyl-esterification status can considerably alter the charge, chemistry and rheology of HG (Levesque-Tremblay *et al.*, 2015). RG-I is the second-most abundant form of pectin and possesses a backbone of alternating rhamnose and GalA subunits that are decorated with galactan, arabinan and arabinogalactan side chains. The most structurally complex pectin domain is RG-II, which has an HG-like GalA-containing backbone that is modified with four different side chains; these side chains include 12 different monosaccharide constituents, and side chain A contains apiose residues that can form borate diester linkages to crosslink RG-II molecules, generating RG-II dimers (Funakawa and Miwa, 2015).

These classifications are based on monosaccharide composition and linkage analyses of pectins that have been solubilized from cell wall preparations and purified to varying degrees, but they do not provide a complete picture of pectin structure. The extents to which different pectin domains themselves are linked have not been fully established (Vincken *et al.*, 2003), and although degree of polymerization can be measured in some cases and has been estimated to be fairly homogeneous (Yapo *et al.*, 2007), the precise size distribution for most pectins remains poorly defined. In addition, some pectins are more difficult to extract from the insoluble portions of cell wall preparations (Immerzeel *et al.*, 2006), and their chemical compositions and linkage structures are therefore less certain. Whether these insoluble pectins are molecules that are topologically entangled with other wall polymers such as cellulose or hemicelluloses, are covalently linked to these other polymers (Thompson and Fry, 2000; Popper and Fry, 2005), are themselves highly insoluble, or comprise some combination of these populations, remains unclear. One intriguing possibility is that pectin domains might be linked to polypeptides that serve as nuclei for their biosynthesis. Crosslinks between RG-I and extensins, which are hydroxyproline-rich

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glycoproteins that localize to cell walls, have been reported (Qi *et al.*, 1995), and in 2013, Tan and coworkers reported a peptidoglycan structure in which HG, RG-I, galactan and xylan domains were linked to ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN1 (APAP1) (Tan *et al.*, 2013). Mutants lacking the APAP1 protein displayed alterations in wall composition and extractability, and the authors estimated that up to 95% of extractable RG-I from suspension-cultured cell walls is linked to an arabinogalactan protein (Tan *et al.*, 2013). Whether all pectin domains are attached to polypeptides remains to be fully established. It is possible that APAP1-like peptidoglycans represent a differentially soluble sub-population of pectins, or that these are core 'remnants' of pectin biosynthetic products from which pectin macromolecules are cleaved after synthesis. It will be interesting to learn to what extent APAP1-like proteins are involved in pectin biosynthesis, whether their glycans are common or distinct from those of other types of pectins and exactly how attachment to a polypeptide affects pectin behavior in the wall.

Pectin biosynthesis: a complex subject

Given the structural complexity of pectins, over 65 distinct enzyme activities have been hypothesized to be required for their synthesis (Mohnen, 2008; Caffall and Mohnen, 2009; Harholt *et al.*, 2010). However, genetic and biochemical experiments are making progress toward identifying the genes and enzymes that generate pectin linkages. The site of pectin synthesis is thought to be the Golgi lumen (Sterling *et al.*, 2001), wherein nucleotide-sugar substrates, S-adenyl-L-methionine and acetyl-CoA or some other acetylated carrier molecule (Schultink *et al.*, 2015) are imported from the cytoplasm and used by a variety of glycosyltransferases (GTs), methyltransferases and acetyltransferases to construct pectin macromolecules. One of the best-characterized pectin biosynthetic enzymes is GALACTURONOSYLTRANSFERASE1 (GAUT1), which catalyzes the transfer of GalA from UDP-GalA to an oligo-GalA acceptor (Sterling *et al.*, 2006). GAUT1 is cleaved at its N-terminus during maturation and thus lacks a membrane anchor itself, but it has been reported to be anchored to the Golgi membrane in a complex with GAUT7; GAUT7 does not possess detectable GalA transferase activity, but retains an N-terminal membrane anchor (Atmodjo *et al.*, 2011). HG can also be xylosylated to form xylogalacturonan by the membrane-associated protein XYLOGALACTURONAN DEFICIENT1 (Jensen *et al.*, 2008). For RG-I, genetic evidence indicates that ARABINAN DEFICIENT1 and ARABINAN DEFICIENT 2 function non-redundantly to synthesize arabinan side chains, and these proteins can form both homo- and heterodimers when expressed transiently (Harholt *et al.*, 2012; Lund *et al.*, 2015). Likewise GALACTAN SYNTHASE1 has been confirmed biochemically as a β-1,4-galactan galactosyltransferase (Liwanag *et al.*, 2012). In the case of RG-II, three RHAMNOGALACTURONAN XYLOSYLTRANSFERASE (RGXT) proteins have been proposed to transfer xylose residues to RG-II (Egelund *et al.*, 2006, 2008., and two sialyltransferase-like proteins are hypothesized to add 2-keto-3-deoxy-D-lyxo-heptulosaric acid (Dha) and/or 2-keto-3-deoxy-D-manno-octulosonic acid (Kdo) to its side chains (Dumont *et al.*, 2014).

The acetylation of pectin is putatively carried out by pectin acetyltransferases, possibly in the TRICHOME BIREFRINGENCE-LIKE (TBL) protein family (Schultink *et al.*, 2015). However, no specific protein has thus far been demonstrated as a bona fide acetyltransferase for pectin. Methyl-esterification of GalA residues in HG is thought to be achieved by the putative methyltransferases QUASIMODO2 (QUA2), QUASIMODO3 (QUA3), COTTON GOLGI-RELATED2 (CGR2) and/or COTTON GOLGI-RELATED3 (CGR3) (Mouille *et al.*, 2007; Held *et al.*, 2011; Miao *et al.*, 2011; Kim *et al.*, 2015), resulting in HG chains that have a high degree of methyl-esterification (DM) soon after synthesis. In *A. thaliana*, *qua2* mutants display lower HG methyl-esterification, are dwarfed and have less HG in their cell walls (Mouille *et al.*, 2007). Whether the reduction in HG is a compensatory response to lowered methyltransferase activity or is due to increased pectin degradation is not entirely clear, although the degree of polymerization for HG is not substantially altered in the mutant (Ralet *et al.*, 2008), suggesting that a compensatory decrease in HG synthesis occurs. Likewise, *cgr2 cgr3* double mutant plants are dwarf, have HG with a lower degree of methyl-esterification, and display less HG methyltransferase activity in microsomes (Kim et al., 2015). The precise contribution of each of these enzymes to HG methyl-esterification remains to be determined.

The coordination of so many enzyme activities during pectin biosynthesis might be achieved, at least in part, by the formation of multi-subunit protein complexes that can 'pass' acceptor substrates to one another. The presence of several types of such complexes might result in a 'domain synthesis' mechanism for pectin biosynthesis, in which individual domains are built and then linked together to form final pectin macromolecules (Atmodjo *et al.*, 2013). The fact that QUA3 and a related protein have been detected as interactors of the GAUT1:GAUT7 complex (Atmodjo *et al.*, 2011) suggests that HG methyl-esterification might occur coordinately with its polymerization, and the interaction of ARAD1 with both itself and ARAD2 implies that these proteins might work together to construct arabinan side chains. However, 'oligoglycosyltransferases' that can link multimeric pectin chains together have not yet been reported. Further analyses of interactions between pectin biosynthetic enzymes, facilitated by newly developed protein-protein interaction reporters that reversibly reconstitute fluorescent or luminescent proteins (Gookin and Assmann, 2014; Lund *et al.*, 2015) and are thus less prone to false positive results than previous generations of reporters, should shed more light on the extent to which pectin biosynthesis involves orchestration between physically linked enzymes.

Transport and delivery of pectins to the apoplast

Although pectins are synthesized in the Golgi, there is considerable uncertainty about how they are then trafficked from

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the Golgi to the plasma membrane. Pioneering immunoelectron microscopy experiments using antibodies raised against isolated cell wall components have indicated that in some cell types, pectins might exit from the cis/medial Golgi in vesicles that are delivered directly to the plasma membrane, but in other cell types, both pectins and hemicelluloses are detectable in later Golgi compartments (Moore *et al.*, 1991; Lynch and Staehelin, 1992; Zhang and Staehelin, 1992). However, these data are not conclusive, in that biophysical or chemical changes in pectins in later Golgi compartments might prevent recognition by antibodies in the former cell types. Uncertainty also stems in part from the complexity of post-Golgi trafficking, in which cargo transits from the Golgi to the trans-Golgi network (TGN) before being targeted along exocytic or post-endocytic pathways. The post-Golgi trafficking mediators ECHIDNA, YPT/RAB GTPase Interacting Protein 4a, and YPT/RAB GTPase Interacting Protein 4b are likely to function in the proper targeting of pectins to the plasma membrane, since mutants lacking these genes mislocalize wall matrix polymers to the vacuole (Gendre *et al.*, 2011, 2013; McFarlane *et al.*, 2013). Mobile Secretory Vesicle Compartments (SVCs) marked by the late trafficking protein SECRETORY CARRIER MEMBRANE PROTEIN 2 (SCAMP2) have been demonstrated to contain pectins (Toyooka *et al.*, 2009) and have therefore been proposed to perform bulk delivery of pectins to the cell surface, and members of the exocyst complex, which mediates secretion at the plasma membrane in plant cells (Hala *et al.*, 2008), have been implicated in the final delivery of pectins to the apoplast, albeit in highly specialized seed coat cells that produce large amounts of pectin-rich mucilage (Kulich *et al.*, 2010).

One predicted consequence of pectin transit through the secretory apparatus is a decrease in the pH of the environment from ~6.6 in the Golgi to a more acidic pH that ranges ~5 in growing cell walls (Felle, 2001; Martiniere *et al.*, 2013). This change in pH might be expected to alter the physical properties of pectins, possibly resulting in changes in their solubility and/or interactions with other wall matrix polymers, which are synthesized in the same Golgi compartments. These changing physical properties, in turn, might also influence the size, shape and dynamics of vesicular compartments along the secretory pathway: for example, seed coat and root cap cells, both of which secrete large amounts of pectins, have Golgi that appear distinct from those of other cell types (Staehelin *et al.*, 1990; Young *et al.*, 2008). The development of techniques for measuring local pH and the precise synthesis status and nanomechanical properties of pectins in different secretory compartments will be required to test these ideas.

The timing of when HG is methyl-esterified relative to its initial synthesis (see above) is also relevant to its stability. Pectin methyl-esterases (PMEs), which remove methyl-ester groups from HG, releasing methanol and a proton, polygalacturonases (PGs), which hydrolyze HG backbones and pectate lyases (PLs), which cleave HG backbones via a β-elimination mechanism, would be expected to co-exist with nascent pectin molecules in Golgi and post-Golgi compartments before their secretion to the apoplast. Inhibitory and autoinhibitory proteins and domains exist that might prevent PMEs, PGs and PLs from acting prematurely on their substrates, and some of these can be removed by subtilisin-like proteases in the wall (Rautengarten *et al.*, 2008; Senechal *et al.*, 2014*a*). Additionally, the aforementioned pH gradient along the secretory pathway might prevent PMEs, PGs and PLs from operating until they reach the relatively acidic environment of the apoplast; indeed, the activity of many PMEs, PGs and PLs is highly pH-dependent (Senechal *et al.*, 2014*b*). Whether these or other regulatory mechanisms are responsible for stabilizing pectins before their delivery to the apoplast, or if in fact pectin degradation begins during transit, remains to be determined.

The question of how the spatial distribution of pectin delivery sites at the cell surface is regulated has not yet been answered, although tantalizing hints have emerged. In tip-growing pollen tubes, pectin subtypes are distributed heterogeneously along the length of the cell (Rounds *et al.*, 2011; Chebli *et al.*, 2012), which may be a consequence of oscillations in wall component delivery and/or growth (Li *et al.*, 1994; McKenna *et al.*, 2009) and/or variations in the timing and/or location of PME activity that result in differential epitope localization. Golgi bodies move through the cytoplasm in diffusely growing cells via actin-myosin-driven cytoplasmic streaming, and this mobility would be expected to spread the delivery of pectins to the apoplast relatively evenly across the cell surface, but recent data have also implicated the microtubule cytoskeleton in pectin transport and delivery. Genetic and metabolic labeling experiments using click chemistry-based detection of a taggable sugar analog, fucose-alkyne, have shown that pectins are delivered to discrete sites in root epidermal cells (Anderson *et al.*, 2012) and that mutations in the FRAGILE FIBER 1 kinesin result in reduced delivery of fucose-alkyne-labeled pectins to the cell wall (Zhu *et al.*, 2015). The relatively normal distribution and density of CELLULOSE SYNTHASE3 (CESA3) in *fra1* mutants suggests that CESAs and pectins might be delivered to the cell wall in distinct compartments, providing differential control over the sites and densities of matrix polymer delivery and cellulose extrusion into the wall, although further experiments will be required to fully support this hypothesis.

Pectin dynamics in the wall

The cell walls of plants first form during cytokinesis, when the secretory apparatus of the cell is reorganized to direct the delivery and/or synthesis of wall polymers to the growing cell plate, which coalesces from post-Golgi compartments at the midline of the phragmoplast and requires massive membrane rearrangements for its formation, expansion and remodeling into a nascent cell wall (Jurgens, 2005). Pectins are detectable early in the formation of the cell plate (Samuels *et al.*, 1995) and their flexibility might facilitate cell plate deformation and expansion. It is also possible that pectins, through interactions with extensins and/or other structural proteins (Cannon *et al.*, 2008), might provide the proper chemical or mechanical environment for the initiation of cellulose biosynthesis in newly forming cell walls (Miart *et al.*, 2014), perhaps by forming nanoscale patches of wall with differential stiffness that cause nascent cellulose chains to coalesce into microfibrils.

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Indeed, pectins have recently been reported to bind extensively to bacterial cellulose during its synthesis, although they do not appear to dramatically affect cellulose crystallinity (Lin *et al.*, 2015, 2016). Pectins have also been detected in close proximity to cellulose in intact plant cell walls using solid-state Nuclear Magnetic Resonance (ssNMR) experiments (Dick-Perez *et al.*, 2012; Wang *et al.*, 2015), suggesting intimate interactions with the cellulose-hemicellulose network. Upon the completion of cytokinesis, the new wall fuses with the parental wall and distinct wall layers become apparent as wall deposition continues in both daughter cells. The layer that lies between the primary walls of each cell is the so-called middle lamella, which is highly enriched in pectins and is hypothesized to function in cell-cell adhesion, a topic that has recently been reviewed (Daher and Braybrook, 2015) and will not be discussed in detail here.

Recently, pectin de-methyl-esterification in the wall has been proposed as a key event in the symmetry breaking that initiates anisotropic cellular expansion during the elongation of epidermal cells in *Arabidopsis* hypocotyls (Peaucelle *et al.*, 2015), a process that heretofore has largely been attributed to constraints placed on radial expansion by the strength of transversely oriented cellulose microfibrils. As the wall expands during cell growth, the middle lamella might be expected to thin to the point of disappearance, but this does not appear to be the case. Are additional pectins 'squeezed' into the middle lamella as the cell grows? Intriguing observations in the unicellular algae *Penium margaritaceum* suggest that this might occur, since in these cells, pectins are enriched in a reticulated outer wall layer that is distal to an underlying cellulosic layer, suggesting that as cell growth and wall deformation occurs, pectins are extruded from the primary wall (Domozych *et al.*, 2014). In the case of continuous pectin delivery in growing plant tissues, an equilibrium might exist in which the steady state fraction of pectin in the wall is constant, but as wall biosynthesis diminishes and growth continues, as has been shown to be possible in the case of cellulose for elongating hypocotyls (Refregier *et al.*, 2004), the amount of pectin in the primary wall might decrease and/or become reorganized (Anderson *et al.*, 2012). This successive physical de-pectination might have important implications for wall biomechanics and cellular adhesion.

The ultimate fate of much HG that is delivered to the wall appears to be de-methyl-esterification by PMEs, potentially followed by degradation. Depending on the cell type and the specific enzyme, HG degradation by PGs can facilitate either cellular expansion (Xiao *et al.*, 2014) or controlled cell separation events that occur during plant development and maturation (Ogawa *et al.*, 2009). Whether this degradation is regulated by transcriptional networks that turn different pectin-modifying genes on and off in diverse spatiotemporal patterns (Kim *et al.*, 2006), by post-translational control of enzyme activities (Senechal *et al.*, 2014*b*), or by both of these factors in combination has yet to be fully determined.

Pectins and wall integrity signaling

As degradable wall components, pectins are ideally situated to act as molecular sentinels in the apoplast for the detection of wall damage or degradation, either as a result of intrinsic growth processes or by invading pathogens, and have therefore been proposed to act as key players in wall integrity signaling networks (Engelsdorf and Hamann, 2014). Pectins have been identified as binding interactors with cell wall integrity-sensing WALL-ASSOCIATED KINASES (WAKs) (Wagner and Kohorn, 2001; Decreux and Messiaen, 2005), inducing MAP kinase signaling cascades that result in changes in gene expression (Kohorn *et al.*, 2009). Although pectin-derived oligogalacturonides have been proposed to act as ligands for WAKs (Brutus *et al.*, 2010), the exact nature of the pectic epitope(s) that bind to WAKs have not been precisely defined, and the question of whether pectins act in concert with other molecules, such as secreted peptides, to activate WAKs and/or other receptor-like kinases has not been fully addressed. Another open question is exactly how bound pectin molecules transmit 'integrity' signals: are these signals based on size-dependent interactions between pectin fragments and receptor binding pockets, mechanical tension (or lack thereof) between the receptor and ligand, which is in turn connected to one or more supermolecular wall networks, or some combination thereof? Genetic evidence has also recently implicated RECEPTOR-LIKE PROTEIN 44 (RLP44) in sensing changes in the status of pectins in the cell wall and transmitting this information through the brassinosteroid hormone signaling network (Wolf *et al.*, 2014). However, the ligand for RLP44 has not yet been isolated. Reductive dissection of the wall integrity sensing machinery by testing combinations of heterologously expressed receptors and purified or synthesized candidate ligands might be required to fully understand how pectins, alone or in combination with other wall components, activate wall integrity signaling.

New methods for detecting pectin structure and dynamics

Given their complex structures, pectins have resisted complete characterization. A large number of antibodies has been generated that can detect specific pectin epitopes in intact plant tissues or wall extracts (Pattathil *et al.*, 2010); despite their broad utility, these probes should be used with the knowledge that the epitopes for many of these antibodies have not been fully defined (due to the difficulty of purifying or synthesizing the appropriate oligo- or polysaccharides for binding studies). Additionally, certain pectin epitopes might be masked in intact walls by other wall polymers (Marcus *et al.*, 2008, 2010), and antibodies are large in comparison with the average pore sizes of cell walls, raising questions about penetration and accessibility.

Fortunately, progress is being made in the development of sensitive and comprehensive methods for detecting pectin structure and degradation. So-called 'epitope detection chromatography' can be used to separate subpopulations of pectins and probe their structures using the above-mentioned antibodies (Cornuault *et al.*, 2014). Alternatively, after printing specialized glycan arrays containing a wide variety of pectin epitopes and probing them with sets of monoclonal

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antibodies, multivariate regression analysis can be used to identify trends in antibody-epitope binding patterns (Sousa *et al.*, 2015). However, the complexity and apparent heterogeneity of pectins makes these methods imperfect for determining complete structural information. PME activity and pectin degradation can be detected using the cationic dye Ruthenium Red on agar plate-based assays in combination with chromatographic separation of pectin fragments (Lionetti, 2015), and the oligogalacturonides produced by pectin-degrading enzymes in intact plants can be measured using MALDI-TOF mass spectrometry (Korner *et al.*, 1998; Pontiggia *et al.*, 2015). Ideally, the community will be able to develop the ability to 'sequence' pectin structures to more completely understand structure-function relationships.

Another promising avenue for detecting pectins involves the development and application of small molecule-based probes. One promising study along these lines involves chitosan oligosaccharides, which molecularly 'dock' with stretches of de-methyl-esterified HG; when covalently linked to a fluorophore, these probe molecules can be used to label de-methyl-esterified HG *in situ* (Mravec *et al.*, 2014). Additionally, as mentioned earlier, the time-resolved behavior of populations of synchronously synthesized pectins can be followed using metabolic labeling and click chemistry (Anderson *et al.*, 2012), although this approach does not currently allow for *in vivo* fluorescent labeling and requires the use of pulse-chase experiments to follow pectin dynamics.

In summary, our understanding of pectin synthesis, trafficking and behavior is far from complete, but the future looks bright. As functional genomics and advances in biochemistry and cell biology allow us to identify the full contingent of pectin biosynthetic and modifying genes and the life histories of their products, we will become better able to manipulate pectin structure and study its effects on wall organization and behavior, plant development and the production of renewable food, materials and energy resources.

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