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Building a plant cell wall at a glance

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ABSTRACT

Plant cells are surrounded by a strong polysaccharide-rich cell wall that aids in determining the overall form, growth and development of the plant body. Indeed, the unique shapes of the 40-odd cell types in plants are determined by their walls, as removal of the cell wall results in spherical protoplasts that are amorphic. Hence, assembly and remodeling of the wall is essential in plant development. Most plant cell walls are composed of a framework of cellulose microfibrils that are cross-linked to each other by heteropolysaccharides. The cell walls are highly dynamic and adapt to the changing requirements of the plant during growth. However, despite the importance of plant cell walls for plant growth and for applications that we use in our daily life such as food, feed and fuel, comparatively little is known about how they are synthesized and modified. In this Cell Science at a Glance article and accompanying poster, we aim to illustrate the underpinning cell biology of the synthesis of wall carbohydrates, and their incorporation into the wall, in the model plant *Arabidopsis*.

KEY WORDS: *Arabidopsis*, Cell wall, Cellulose, Microtubules, Pectin, Xyloglucan, Glycosyltransferase

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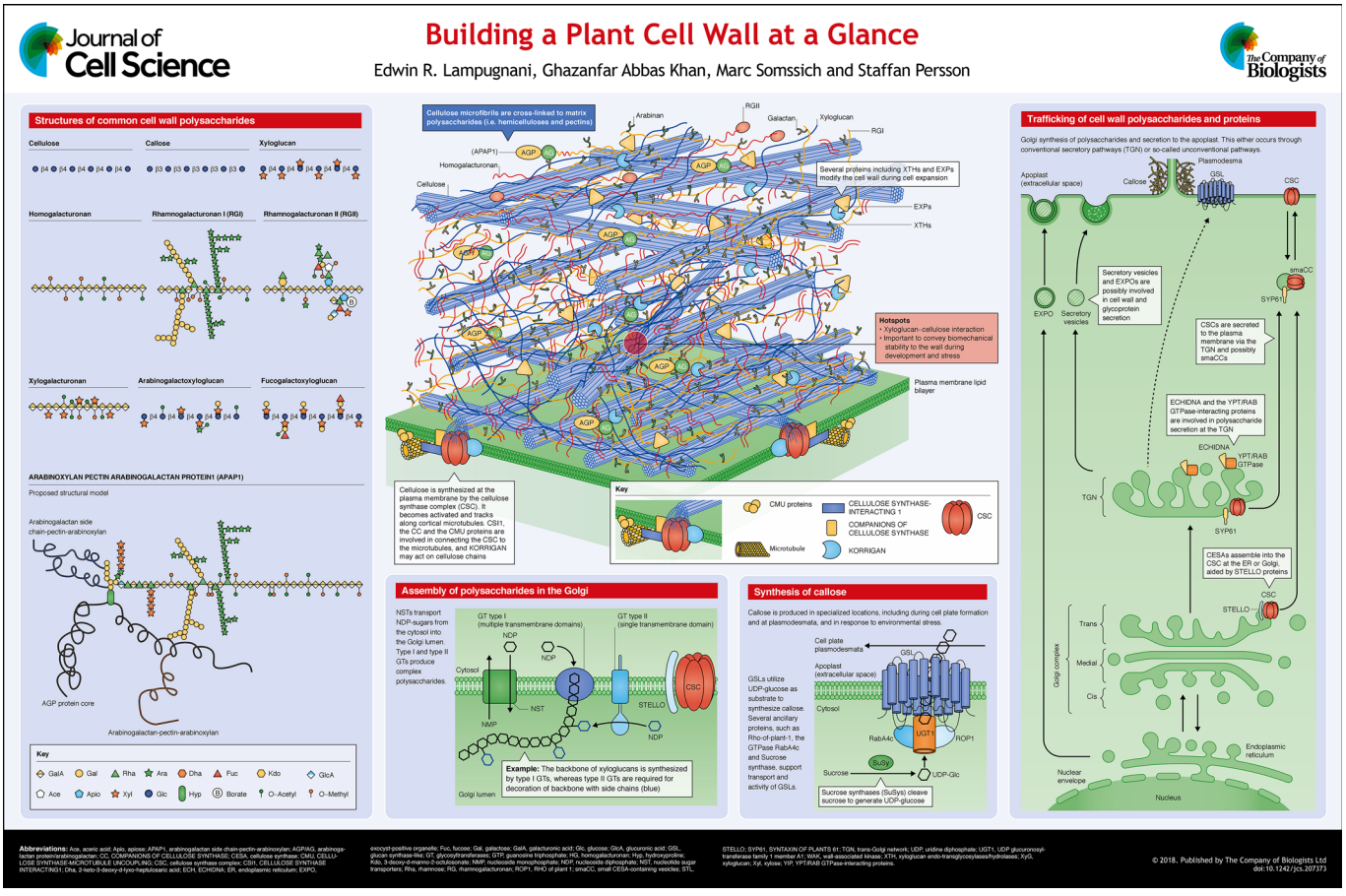
Introduction

The invention of the microscope in the late 1600s was critical to fuel interest in plant biology. Plant scientists such as Malpighi (1628–1694) and Grew (1641–1712) exploited this tool to observe structures during plant development. Notably, Hooke (1635–1703) used thin slices of cork in his 1664 book *Micrographia* and remarked that empty spaces were contained by walls. He coined these spaces pores, or cells. Since these remarks, increasingly sophisticated scientific tools have led to an ever-more refined picture of the plant cell wall. The cell walls comprise a hydrostatic polysaccharide-based skeleton. These polysaccharides constitute the major carbon reservoir in plants and they are ultimately sustained by the ability of the plant to fix carbon dioxide through photosynthesis. As plant cell growth is driven by internal turgor,



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cell walls need to be strong enough to prevent the cells from bursting, yet sufficiently flexible to steer plant cell expansion. Whereas the contents of plant cell walls differ from those of fungi and animals, certain functional aspects are similar. This includes roles in cell protection, cell-to-cell communication, and cell adhesion and proliferation (Free, 2013; Hynes, 2009; Tsang et al., 2010).

All growing plant cells are surrounded by a thin, highly hydrated and flexible primary wall. In dicotyledons such as *Arabidopsis thaliana*, this wall type typically consists of a framework of cellulose microfibrils that are cross-linked to each other by branched polysaccharides, which are referred to as hemicelluloses and pectins (Ivakov and Persson, 2012) (see poster). In some plant cells, such as those found in the tracheary elements and fibers in the xylem, a secondary cell wall is deposited inside of the primary wall. The major constituent of the secondary cell wall is cellulose, which is cross-linked by hemicellulosic polymers (Thornber and Northcote, 1962). Hydrophobic lignin is also deposited throughout the secondary walls, which results in dehydration of the wall compartment (Cesarino et al., 2016; Marriott et al., 2016). This composition increases the strength of the walls and reduces their flexibility, allowing the resulting tube-like structure to serve as water conduits and as the mechanical or structural support for the plant. Here, we highlight how cell wall components are synthesized and their subsequent integration into the plant primary cell wall.

An inventory of the plant cell wall polysaccharides

Cellulose and callose

Cellulose is a linear homopolysaccharide that is composed of repeating glucose residues linked by β(1,4)-bonds that make up long and rigid microfibrils and thus are the load-bearing structures in the walls. These microfibrils form the scaffold of the cell wall, and become interconnected by hemicelluloses and pectins (Nishiyama, 2009; Wang et al., 2012). Callose too is a linear homopolysaccharide composed of glucose residues; however, rather than being linked through β(1,4)-bonds, callose is made up of β (1,3)-linkages. Under normal growth conditions, callose is generally only found in specialized cells, such as tip growing cells, pollen tubes or stomata, and at specific cell compartments, such as the plasmodesmata, or as a transient component of cell plates in dividing cells (Schneider et al., 2016). Callose synthesis may also occur at the wall in response to abiotic or biotic stress (Nielsen et al., 2012). Callose typically functions as a regional wall stabilizer (e.g. at pathogen entrance points) and modulates plasmodesmata pore size (De Storme and Geelen, 2014).

Pectins

Pectins are a diverse and highly complex class of polysaccharides that are enriched in galacturonic acid (GalA) (Harholt et al., 2010). For example, homogalacturonan (HG) and rhamnogalacturonan (RG)II have backbones consisting of α(1,4)-GalA, whereas the backbone of the other RG polymer, RG-I, consists of a repeated motif of α(1,4)-GalA and α(1,2)-rhamnose. These backbones can be decorated with an array of oligosaccharides, such as α(l,5)-arabinans and β(1,4)-galactans, and complex heteropolysaccharides, as well as with methyl and acetyl groups. HGs are transported to the wall in highly esterified forms but become selectively de-esterified, which mediates polymer cross-linking through Ca^{2+}. RG-II may also be cross-linked by boron ions (B^{3+}); together, these links might rigidify the wall matrix. Pectic polysaccharides can also be linked to glycoproteins and other cell wall carbohydrates that may further stabilize the wall structure (Tan et al., 2013). Hence, pectins can control cell wall flexibility, and are thus important for cell proliferation and plant growth (Peaucelle et al., 2012).

Hemicelluloses

The most common hemicelluloses in cell walls of the plant model organism *Arabidopsis* are xyloglucans (XyGs) and xylans. The XyG backbone is composed of β(1,4)-linked glucose residues that have α(1,6)-linked xylosyl side chains. These side chains in turn can be further decorated with galactose – and sometimes fucose – residues to create complex levels of branches and patterns. The backbone of xylan is composed of β(1,4)-linked xylose residues which can be decorated with, for example, glucuronic acid to produce glucuroxylan (Pauly et al., 2013). Both XyGs and xylans may also be modified by acetylation, which affects their capacity to cross-link to other cell wall components (Zhang et al., 2017). XyG is the main hemicellulose in dicot primary walls, and most likely functions to cross-link cellulose microfibrils (Park and Cosgrove, 2015). While the initial assumption was that the cellulose microfibrils were coated with xyloglucans, which would then interact to cross-link the microfibrils, more recent data indicate that the xyloglucan–cellulose interaction is limited to distinct regions along the microfibril, referred to as hotspots, that are important to convey biomechanical stability to the wall (Park and Cosgrove, 2015). Although xylans are major hemicelluloses in secondary cell walls, they are also prominent in primary walls of monocots and some algae. Similar to XyGs in primary walls, xylans can also cross-link cellulose microfibrils (Simmons et al., 2016).

Assembly of the cell wall polysaccharides

Cell wall polysaccharides are composed of monosaccharide residues linked through an array of glycosidic linkages. Assembly of these subunits requires the repeated addition of single sugar residues that are provided in an activated form of nucleoside diphosphate (NDP)-sugars. Whereas most of these activated sugars are produced in the cytosol, the synthesis of pectins and hemicelluloses occurs in the Golgi; furthermore, the active sites of the glycosyltransferases (GTs) often face the Golgi lumen. Some activated sugars must therefore be transported into the lumen of the Golgi or endoplasmic reticulum (ER), where they are added to specific polysaccharide acceptors by the corresponding GTs. This transport is facilitated by nucleotide sugar transporters (NSTs), which are antiporters that exchange nucleoside monophosphate for specific NDP-sugars (Temple et al., 2016) (see poster). NSTs are generally highly substrate specific and, therefore, the *Arabidopsis* NST family has more than 40 putative members. The designation of NST function has seen a major leap forwards in recent years, and specific NSTs have been assigned for GDP-mannose, UDP-galactose, UDP-glucose, UDP-arabinose and other NDP-sugars (Orellana et al., 2016; Rautengarten et al., 2017).

The enzymes that act on carbohydrates are known as carbohydrate active enzymes (CAZYs; http://www.cazy.org). CAZYs are clustered into four main groups: GTs, glycosyl hydrolases, polysaccharide lyases and carbohydrate esterases, which is based on genomic, structural and/or biochemical information (Cantarel et al., 2009). Here, the largest group by far comprises the GTs, of which there are more than 100 families. Homology within and between GT families is intrinsically low, as most GTs produce specific types of glycosidic linkages. Plant cell wall-related GTs are broadly grouped into two types. The first (type I) consists of enzymes that catalyze the processive addition of glycosyl residues such that they do not release the polymer product,

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which allows for very high polymerization efficiencies. These are typically integral membrane proteins that synthesize homo-polysaccharides, such as β(1,4)-D-glucan (cellulose) or β(1,3)-D-glucan (callose). In contrast, type II GTs catalyze only a single transfer after which the enzyme–product complex dissociates. The type II GTs generally share a common topology that consists of a short N-terminal cytoplasmic domain, a single transmembrane domain and a large catalytic domain facing the Golgi lumen. As an example, biosynthesis of XyG chains requires the activity of one type I GT, cellulose synthase-like C4, to create the β(1,4)-glucose backbone, whereas a variety of type II GTs act as xylosyltransferases, galactosyltransferases and fucosyltransferases to add the different side chains (Schultink et al., 2014; Pauly and Keegstra, 2016) (see poster).

In contrast to the aforementioned polymers, callose is not synthesized in the Golgi but rather at the plasma membrane by callose synthases or glucan synthase-like (GSL) proteins. The *Arabidopsis* genome encodes 12 GSL genes (Verma and Hong, 2001). Consistent with callose production occurring only in specialized cells and in response to environmental stimuli, GSL genes are expressed in a tissue-specific fashion. Here, GSL1, GSL2, GSL6, GSL8 and GSL10 contribute to fertility through pollen development and cell division, and GSL5, GSL7 and GSL12 provide structural reinforcement to the cell wall (Verma and Hong, 2001). The GSL proteins can interact with a number of ancillary proteins that include those that are involved in potential substrate supply, for example sucrose synthase, and those that control secretion and localization, such as Rho-of-plant 1 and the GTPase RabA4c (Ellinger and Voigt, 2014; Nedukha, 2015).

Cellulose is also synthesized at the plasma membrane by a symmetrical cluster or 'rosette' of six particles called the cellulose synthase (CESA) complex (CSC; Somerville et al., 2004) (see poster). First detected through freeze fracture microscopy in maize, the current view is that each of the six subunits of the rosette contain 12 to 36 CESA proteins (Nixon et al., 2016; Guerriero et al., 2010). *Arabidopsis* has ten structurally similar CESA isoforms (CESA 1 to 10). Current evidence supports the view that both the CSCs of the primary and secondary wall contain three CESA isoforms: the CSC of the primary wall contains CESA 1, CESA 3 and CESA 6-like proteins, and the CSC of the secondary wall contains the CESAs 4, 7 and 8 (Desprez et al., 2007; Persson et al., 2007). Both show equimolar stoichiometry of the CESA subunits (Gonneau et al., 2014; Hill et al., 2014), and the GSLs and the CESAs utilize UDP-glucose as substrate. A major route of UDP-glucose production is through the cleavage of sucrose by sucrose synthases or by UDP-glucose pyrophosphorylase and cytosolic invertases (Fujii et al., 2010; Rende et al., 2017; Verbančič et al., 2017). Sucrose synthases that are associated with the plasma membrane might channel UDP-glucose to the CESAs, although it does not seem likely that this occurs in *Arabidopsis* (Barratt et al., 2009; Chourey et al., 1998; Coleman et al., 2009). Mutations in two cytosolic invertases in *Arabidopsis* lead to dwarfism, which is thought to be a consequence of defects in cell wall synthesis (Barratt et al., 2009), a hypothesis that is supported by analyses in poplar (Rende et al., 2017). Hence, the substrate supply pathway for callose and cellulose synthesis remains to be firmly established.

From the inside to the outside – polysaccharide secretion

Both the Golgi-assembled polysaccharides and the protein complexes that are to become active at the plasma membrane need to be secreted to the cell surface. Indeed, the secretion of polysaccharides has been illustrated in experiments using an alkynylated fucose analog that was incorporated into pectins during their synthesis in the Golgi, and subsequently could be detected in the cell wall through click-chemistry (Anderson et al., 2012). Polysaccharide secretion may occur either through conventional or unconventional routes. Here, we refer to conventional protein secretion as vesicles that are generated through coats (i.e. clathrin and the COPs), which progress via the trans-Golgi network (TGN) and fuse to target membranes through traditional tethering factors (Kanazawa and Ueda, 2017). Whereas the mechanisms underlying the secretion of cell wall polymers remain largely unknown, some of it has been shown to be controlled by a protein complex composed of ECHIDNA (ECH) and the YPT/RAB GTPase-interacting proteins, YIP4a and YIP4b. This pathway appears to be crucial for both secretion of XyGs and pectins, and occurs through the TGN (Gendre et al., 2011, 2013) (see poster). Although many polysaccharides are believed to be produced by large enzyme complexes, the configurations and assembly mechanisms of these remain largely elusive. Nevertheless, some recent studies have demonstrated that several enzymes that are involved in the production of XyGs, HGs or xylans can interact and have proposed that these may constitute catalytically active multiprotein complexes in the Golgi (Atmodjo et al., 2013; Chou et al., 2015; Zeng et al., 2016).

Several so-called unconventional secretory pathways have also been implicated in polysaccharide secretion, such as a pathway involving the exocyst-positive organelle (EXPO), which is hypothesized to function as a large organelle that is encased by a double membrane (Wang et al., 2010) (see poster). EXPO is thought to be formed in the cytosol, possibly emanating from the ER, where it can sequester proteins and perhaps other material destined for the apoplast. The outer EXPO membrane is then thought to fuse with the plasma membrane and a compartment with a single membrane is then delivered to the apoplast (Ding et al., 2012). The EXPO has been suggested to play a role in pathogen defense, where it might transport cell wall material to the apoplast to reinforce the wall in response to an attack (Pečenková et al., 2011). Nevertheless, more evidence to support the function and formation of EXPOs is needed.

Callose has been detected in multivesicular bodies (MVBs), which are specialized endosomes; this suggests that GSLs could be targeted to the plasma membrane through a pathway that occurs via this organelle (Cui and Lee, 2016; Xu and Mendgen, 1994). Another type of vesicles that appears to be specific to the transport of CESAs are a population of small cytosolic CESA compartments that are referred to as small CESA-containing vesicles (smaCCs; Crowell et al., 2009; Gutierrez et al., 2009). These compartments interact with the cytoskeleton and are thought to be involved in the cycling of the CSC to and from the plasma membrane (see poster).

The CSC is assembled in the endomembrane system and this process is aided by Golgi-localized STELLO (STL) proteins. STLs can interact with and regulate the distribution of CESAs in the Golgi, and so control the secretion of CSCs (Zhang et al., 2016) (see poster). The secretion of the CSCs is presumably facilitated through secretory vesicles that are associated with the tethering factor SYP61, as this SYP associates with vesicles that also contain primary wall CESAs (Drakakaki et al., 2012). Efficient secretion of CSCs also appears to depend on the pH of the endomembranes, the actin cytoskeleton, phosphoinositide levels and kinesin-related activities (Fujimoto et al., 2015; Luo et al., 2015; Sampathkumar et al., 2013; Zhu et al., 2015). Interestingly, the sugar status of the plant may also regulate the trafficking of CSCs (Ivakov et al., 2017). Similar to CSCs, it is likely that GSL complexes are also assembled in the ER or Golgi, and trafficked to the plasma membrane through

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the TGN (Cai et al., 2011). Indeed, GSLs are secreted to infection sites at the plasma membrane through a RabA4c-related pathway during pathogen infection (Ellinger et al., 2013).

Wall incorporation and modification of the polysaccharides

Activation of the CSCs and GSLs is thought to occur after their integration into the plasma membrane, although the exact mechanism is unknown. One possibility is that the activation is achieved through phosphorylation, as this affects the catalytic activity of CSCs (Chen et al., 2010; Sánchez-Rodríguez et al., 2017). It is assumed that the cellulose microfibrils become entangled in the wall structure and the catalytic activity of the CESAs therefore drives the CSCs forward through the membrane. Fluorescently tagged CESAs have been observed to move along linear trajectories at the plasma membrane (Paredez et al., 2006). This movement is typically directed by cortical microtubules, both during primary and secondary wall synthesis, with these microtubules serving as intracellular 'rails' on which the enzyme complexes can track along (Paredez et al., 2006; Watanabe et al., 2015). Several proteins have been implicated in connecting CSCs to the cortical microtubules: these include the CELLULOSE SYNTHASE-INTERACTING 1, COMPANIONS OF CELLULOSE SYNTHASE and CELLULOSE SYNTHASE–MICROTUBULE UNCOUPLING (CMU) proteins (Bringmann et al., 2012; Endler et al., 2015, 2016; Gu et al., 2010; Li et al., 2012; Liu et al., 2016) (see poster). These proteins appear to either aid in maintaining the CSC link to microtubules or to sustain microtubule positions during cellulose synthesis. Although not as well studied, GSLs may also colocalize with cortical microtubules; however, it is unclear whether the GSLs move and if any such movement is also guided by cortical microtubules (Cai et al., 2011).

The cellulose microfibrils are the load-bearing structures in the wall and the organization of these therefore determines the direction of the turgor-driven cell expansion. Hence, transversely organized cortical microtubules, and thus cellulose fibers, would lead to a predominantly longitudinal cell expansion (Baskin, 2005; Sugimoto et al., 2000). Newly secreted pectic polymers have been observed to be incorporated at sites that co-occur with cellulose microfibrils, which suggests that cellulose might indeed provide a scaffold for the addition of other cell wall polysaccharides (Anderson et al., 2012).

Several protein families can modify cell wall polymers, or the links between them, after they have been incorporated into the cell wall. Perhaps the best studied is the family of expansins (EXPs). Although no clear enzymatic function has been attributed to EXPs, they have been suggested to modify the interactions between XyGs and cellulose in a pH-dependent manner, which presumably is the mechanism underlying the acid-growth hypothesis, which postulates that cell walls are able to expand at an acidic pH (Cosgrove, 2005). Thus, low pH activates EXPs, which results in wall relaxation, thereby allowing wall creep and access to the wall structure for other wall-modifying proteins (Cosgrove, 2005). One such group of proteins is the xyloglucan endo-transglycosylases/hydrolases (XTHs), which can cleave and re-ligate XyG backbones, thereby possibly incorporating new XyG fragments into the wall (Eklöf and Brumer, 2010). It is hypothesized that the sites of XTH activity are determined by the cellulose framework, as some XTHs can act specifically on XyG–cellulose contact sites (Vissenberg et al., 2005) (see poster).

The pectin matrix can also be modified by a combination of several activities, including its cleavage by pectate lyases and/or through enzyme-independent loosening by reactive oxygen radicals that occur in the apoplastic space; this aids in re-arranging the matrix (Chen and Schopfer, 1999; Domingo et al., 1998; Marín-Rodríguez et al., 2002). Other proteins that contribute to pectin modifications are the polygalacturonases and pectinmethylesterases (Caffall and Mohnen, 2009). Polygalacturonases also influence wall strength by degrading HGs (Atkinson et al., 2002). Consequently, overexpression of different polygalacturonases typically results in reduced levels of HG and a reduction in wall stability (Atkinson et al., 2002; Capodicasa et al., 2004). Pectin methylesterases can demethylesterify HGs; this impacts on cell wall elasticity as this modification is one of the prerequisites for the cross-linking of HGs to other pectic polysaccharides and cell wall proteins (Caffall and Mohnen, 2009). Pectin methylesterases are counteracted by pectin methylesterase inhibitors, which promote pectin methylesterification and thus wall loosening (Caffall and Mohnen, 2009). However, it has been demonstrated that decreased wall elasticity correlates with demethylesterification, which would indicate that pectinmethylesterase activity can also promote wall loosening (Peaucelle et al., 2011). This complex interplay between different enzymes of opposing functions works to fine-tune wall expansion and cell growth, while at the same time providing structural support and mechanical stability – a common theme in plant cell wall construction and regulation.

The status of the cell wall is thought to be monitored by proteins that sense wall integrity (Wolf, 2017). These include receptor-like kinase proteins, such as FERONIA, and its close homolog THESEUS1, and several wall-associated kinases (WAKs) that respond to changes in the cell wall architecture (Hématy et al., 2007; Li et al., 2016; Yeats et al., 2016). The receptor kinase MALE DISCOVERER 1-INTERACTING RECEPTOR LIKE KINASE 2 may also have a similar function as it is important to convey cellulose deficiencies in the wall to the cell (Van der Does et al., 2017). A direct link to cell wall components has been demonstrated for the WAKs that can bind to pectins and it is plausible that they therefore recognize and respond to changes in the pectin polymers (Decreux and Messiaen, 2005; Kohorn and Kohorn, 2012). Another protein involved in sensing changes to the pectic network is the receptor-like protein 44 that, in concert with BRI1-ASSOCIATED KINASE 1, triggers responses to impaired pectin demethylesterification (Wolf et al., 2014). Whereas several components that sense wall integrity have therefore been identified, it is clear that a plethora of internal and external cues that affect the cell wall need to be communicated to the cell to ensure precise responses. Hence, this is an emerging field that holds high future potential in plant cell wall biology.

Conclusions and perspectives

Although the past decades have seen a major boost in cell wall research, many specific areas remain ill defined. For example, much of our understanding relies on data from tissues rather than specific cell types, and there is therefore an underappreciation for cell-type-specific synthesis and modification of cell wall structures. In addition, a growing number of protein complexes – or perhaps super-complexes – have been found to be involved in cell wall synthesis. Further analyses of these complexes and their sub-compartmentalization will certainly aid in our understanding of how and where cell wall carbohydrates are made. Finally, the coordination of synthesis and secretion of polysaccharides and/or plasma-membrane and apoplastic enzymes is an important and growing topic that should reveal how compartments communicate with each other to modulate the overall architecture of the cell wall.

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Cell science at a glance
A high-resolution version of the poster and individual poster panels are available for downloading at http://jcs.biologists.org/lookup/doi/10.1242/jcs.207373.supplemental

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