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Dynamic Construction, Perception, and Remodeling of Plant Cell Walls

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Abstract

Plant cell walls are dynamic structures that are synthesized by plants to provide durable coverings for the delicate cells they encase. They are made of polysaccharides, proteins, and other biomolecules and have evolved to withstand large amounts of physical force and to resist external attack by herbivores and pathogens but can in many cases expand, contract, and undergo controlled degradation and reconstruction to facilitate developmental transitions and regulate plant physiology and reproduction. Recent advances in genetics, microscopy, biochemistry, structural biology, and physical characterization methods have revealed a diverse set of mechanisms by which plant cells dynamically monitor and regulate the composition and architecture of their cell walls, but much remains to be discovered about how the nanoscale assembly of these remarkable structures underpins the majestic forms and vital ecological functions achieved by plants.

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INTRODUCTION

Plants, which cover much of Earth's terrestrial surface, owe their growth and persistence to their ability to construct walls around their cells, spinning their bodies out of sunlight, air, water, and mineral nutrients. Plant cell walls are complex and dynamic biological networks composed of interacting polysaccharides, proteins, polyphenolics, small molecules, and water that provide support and protection to plants and determine both their morphology and mechanical characteristics over the course of development. As the most abundant repository of biomass on Earth, plant cell walls have been used for millennia by people as sources of food, animal feed, clothing, shelter, and energy. Over the 400 million years since plants emerged from the oceans onto land, plant cell walls have evolved to resist degradation while allowing for growth, making them both tough and flexible. Decrypting the codes by which plants construct and modify their walls promises to usher in a new era of renewable materials and bioenergy, but our current knowledge of how plant cell walls are built, monitored, and modified during plant growth, development, and interactions with biotic and abiotic environments is limited. The discovery and characterization of many of the genes responsible for these processes are beginning to peel back the layers of regulation governing cell wall dynamics in vivo, but how the products of most of these genes function and interact at the molecular level is still largely shrouded in mystery.

Primary cell wall: the cell wall layers that are deposited by a plant cell before and during its growth

This review focuses on the cell and molecular biology underlying the synthesis, assembly, integrity sensing, and remodeling of plant cell walls, highlighting recent advances in our efforts to understand these phenomena. Although we focus mainly on primary cell walls, it is important to realize that plant cell walls are heterogeneous in structure and function across cell types,

developmental stages, and species and are categorized into three main types: primary walls, which are deposited by actively growing plant cells and contain mainly polysaccharides and proteins; secondary cell walls, which are deposited in certain differentiating cell types, mostly after growth has ceased, and contain polysaccharides and lignin (122); and gelatinous layer walls, which are made only in some species, composed almost entirely of polysaccharides, and capable of generating contractile forces (76). Likewise, wall composition varies widely across different plant taxa (149): For example, type I primary walls are common in eudicots and nongrass monocots, whereas type II primary walls predominate in grasses (202). Due to its extensive genomic, biochemical, cell biological, and mechanical characterization, *Arabidopsis thaliana* (*Arabidopsis*) is the main focus of our discussion, but we also highlight a small portion of the biological diversity of cell wall architectures and dynamics across the plant kingdom. For a more extensive review of type II wall synthesis, see Reference 72. Excellent recent reviews cover cell wall structure and nanoscale mechanics (37, 38) as well as experimental approaches for monitoring cell wall dynamics (203), so those topics are not extensively covered here. We apologize to colleagues whose important contributions are not highlighted due to space constraints.

Secondary cell wall: the thickened layers of the cell wall that are deposited by some types of plant cells, often after cell expansion has ceased

Cellulose microfibril: a strong and abundant, partially crystalline biomolecule containing multiple β-1,4-linked chains of glucose held together by hydrogen bonds

Hemicelluloses: wall polysaccharides that have β-1,4-linked backbones with an equatorial configuration

FUNDAMENTALS OF PLANT CELL WALL STRUCTURE

Molecular Structures of Cell Wall Components

The polysaccharides that make up most of the plant cell wall range from linear polymers to highly complex biomolecules (**Figure 1**). Cellulose is the dominant mechanical component of all types of plant cell walls and has a tensile strength similar to that of steel. It is composed of multiple β-1,4-linked glucan chains that are hydrogen bonded together to form cable-like cellulose microfibrils, but despite decades of intense scrutiny, the precise molecular structure of cellulose remains mysterious and might consist of a heterogeneous mixture of highly crystalline regions interspersed with or surrounded by amorphous stretches or layers. Cellulose Iβ is most commonly associated with plant cell walls, but other allomorphs of cellulose exist naturally or can be generated industrially (156). Recent modeling (107) has proposed different potential cross sections of native plant cellulose based on evidence that cellulose microfibrils contain 18–24 chains (65, 109, 132, 191, 192, 225). Cellulose microfibrils in primary walls are approximately 3 nm in diameter, whereas cellulose in secondary walls can coalesce into macrofibrils that are more than 20 nm in diameter, and the degree of polymerization for the glucan chains in cellulose ranges from a few hundred glucose monomers to over 10,000.

Hemicelluloses comprise several different classes of polysaccharides with neutral sugar backbones linked by equatorial β-1,4 linkages, some of which are decorated with side chains (170). These include xyloglucan, the most abundant hemicellulose in type I primary walls, which has a β-1,4-linked glucan backbone decorated with side chains that can consist of sequentially linked xylose, galactose, and fucose residues in specific patterns as well as other sugars in some plant species (221). Xylans have β-1,4-linked xylosyl backbones with side chains consisting of arabinose and glucuronic acid, some of which are methylated or feruloylated, and are the predominant hemicellulose in type II primary walls and eudicot secondary walls but are also present in small amounts in type I primary walls (154). Mannans, which are polymers of β-1,4-linked mannose, are prevalent in gymnosperms but are also present in other plant taxa. Glucomannans contain repeating disaccharide subunits of glucose and mannose joined by β-1,4 linkages. Mixed-linkage glucan, which contains β-1,4-linked stretches of glucose interspersed with β-1,3 linkages, is common in rapidly growing grass tissues (170).

Callose, which is sometimes classified as a hemicellulose but does not contain the equatorial β-1,4 linkages that are shared by hemicelluloses (170), is a polymer of β-1,3-linked glucose subunits.

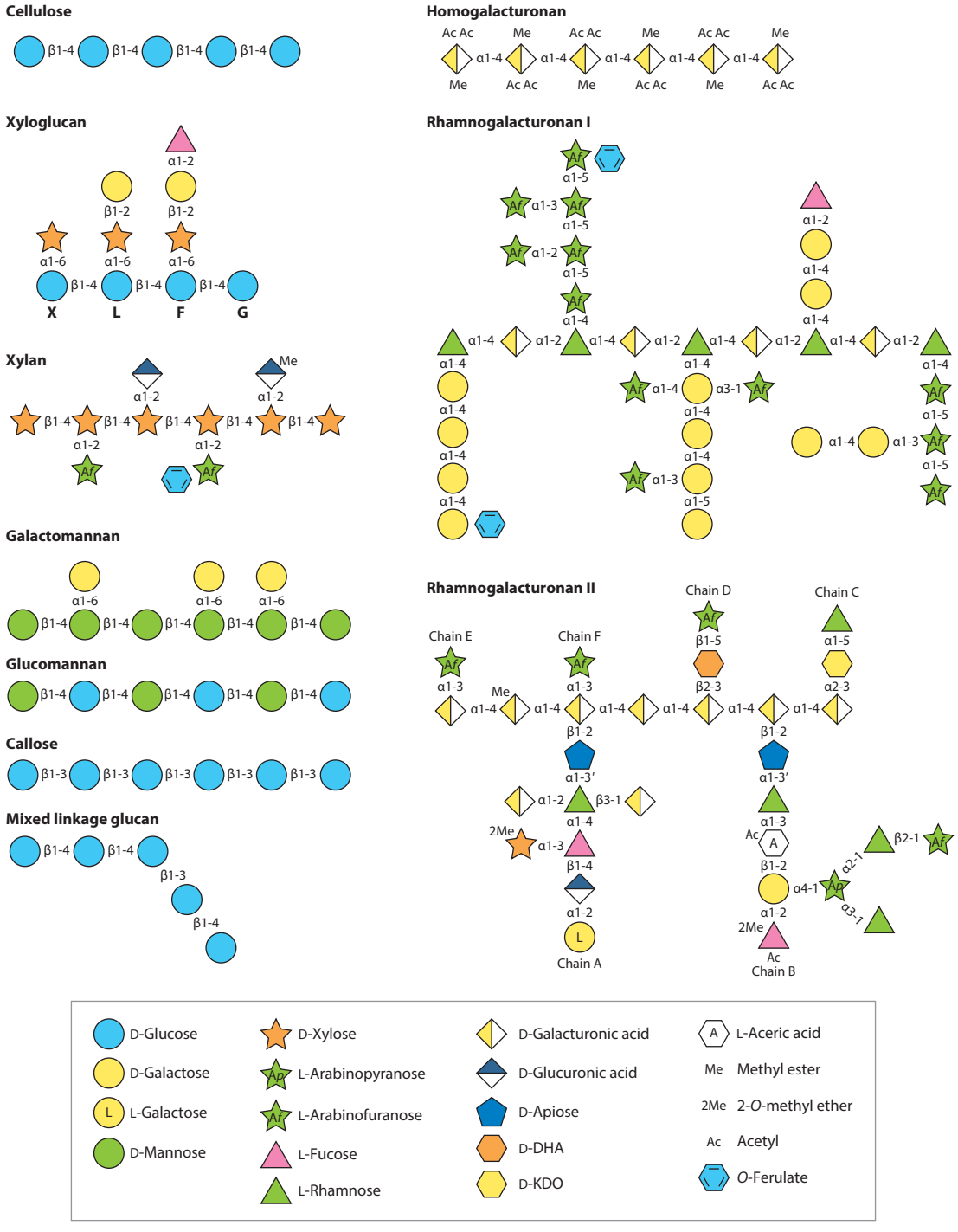

Figure 1

Schematic examples of the structures of cell wall polysaccharides. Sugar symbols follow the Consortium for Functional Glycomics notation (152). Xyloglucan residues are abbreviated according to Reference 67. Structures of polysaccharides, especially of side chains, are not comprehensive; the structure of rhamnogalacturonan-II is based on Reference 130.

It is synthesized in specific developmental and environmental contexts and at particular subcellular regions, including cell plates during cytokinesis, plasmodesmata, and sieve plates. Callose is often degraded soon after its synthesis, making it one of the most dynamic cell wall polymers (58).

Pectins: acidic polysaccharides that are abundant in the primary cell walls of some plant taxa

Pectins are acidic polysaccharides that contain galacturonic acid residues (1, 2, 7). Homogalacturonan, the most abundant pectin, is composed of continuous α-1,4-linked chains of galacturonic acid that are sometimes decorated with xylose or apiose side chains. Homogalacturonan can be methylesterified at the C6 carboxyl groups of its galacturonic acid residues, in addition to being acetylated at its O2 and O3 positions. The backbone of rhamnogalacturonan I, another pectin domain, consists of alternating galacturonic acid and rhamnose residues, and anchors arabinan, galactan, and arabinogalactan side chains. Rhamnogalacturonan II, a highly conserved and complex pectin, has a backbone of homogalacturonan decorated with side chains containing 13 different sugars and over 20 distinct glycosyl linkages. Collectively, hemicelluloses and pectins are called matrix polysaccharides.

Structural proteins are key components of plant cell walls, although their complex glycosylation patterns and large gene families have made them challenging to functionally characterize (18). These include extensins, which are hydroxyproline-rich glycoproteins, proline-rich proteins, and arabinogalactan proteins (178). Enzymes and nonenzymatic proteins are also present in plant cell walls, as are ions, small metabolites, and water in both bulk and surface-associated forms. However, some metabolites that are abundant in the cytoplasm of living cells, such as adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH), are scarce in the wall, making it an energetically distinct compartment.

Although groups of cell wall polysaccharides are often discussed as independent entities, accumulating evidence points to the existence of covalent linkages among the different classes. For instance, the ARABINOXYLAN PECTIN ARABINOGALACTAN PROTEIN (APAP1) glycoprotein (187) contains pectin and arabinoxylan domains that are covalently linked to a polypeptide core, and covalent linkages between pectins and xyloglucans have been detected in some cell walls (147, 148, 193). Likewise, researchers have recently proposed that pectin-cellulose (24) and pectin-xylan (23) complexes exist in some wall types. It is unclear whether these hybrid molecules arise as the immediate products of intracellular synthetic events, from the ligation of independently synthesized domains shortly after synthesis, or after transglycosylation reactions that occur in the wall (see the section titled Cell Wall Modification During Growth).

Lignin, which is abundant in secondary walls, is a hydrophobic, polyphenolic compound made of covalently linked monolignol subunits that undergo redox-mediated polymerization (199). Lignin can be covalently linked to the ferulate side chains of xylans (88).

Functional Interactions Between Wall Components

In isolation, no single wall polysaccharide is capable of recapitulating the mechanics and behaviors of in vivo–assembled plant cell walls. Thus, interactions between wall polymers that arise during the assembly of the cell wall are crucial for determining the mechanical characteristics and growth capacity of plant cell walls. For example, specialized biomechanical hot spots of cellulose-xyloglucan interfaces have been identified as the targets of expansins, proteins that noncatalytically loosen the wall (206). Pectins have been detected in close proximity to cellulose by solid-state nuclear magnetic resonance (NMR) (46), and mutants with altered homogalacturonan molecular weight show changes in homogalacturonan-cellulose interactions and the relative mobility of both polymers (144). Demethylesterified homogalacturonan can be cross-linked via calcium bridges to form mechanically robust networks, rhamnogalacturonan II monomers can be cross-linked by borate diesters, and the feruloylated side chains of rhamnogalacturonan-I can also be cross-linked

via ferulate diesters. These pectic interactions might be key determinants of cell–cell adhesion, which typically keeps plant cells locked in their relative locations during development but can be disrupted to drive cell separation events, for example, during leaf shedding or seed release (41).

Glycosyltransferase: an enzyme that transfers a sugar from an activated nucleotide sugar donor to an acceptor, forming a glycosidic linkage

The forces that drive interactions between wall polymers are sometimes obscure, with electrostatic, hydrogen bonding, van der Waals, and hydrophobic/hydrophilic forces all being implicated. The substitution of every other xylose in the xylan backbone with acetyl groups is thought to favor the adoption of a twofold helical screw conformation, allowing for binding to the hydrophilic surface of cellulose (77). However, despite in vitro evidence pointing to the neutral side chains of pectins being responsible for their interactions with cellulose (229), evidence of close apposition between homogalacturonan and cellulose (46) points to potential direct interactions between homogalacturonan backbones, which are negatively charged, and cellulose microfibrils. One classic model of the plant cell wall (102) envisions the wall as a single unified macromolecule joined by many covalent interactions; although this model is unlikely to be fully correct because many wall components, in particular pectins, can be extracted by treatments that do not break covalent bonds (66), the growing list of covalent linkages between different wall components (23, 24, 147, 148, 193) does lend partial credence to this view.

In addition to the direct physical interactions between components in the cell wall, evidence is emerging of signaling pathways by which changes in the status of one cell wall component can result in compensatory changes in the dynamics and status of other wall components. For example, mutations in xyloglucan synthesis genes that eliminate detectable xyloglucan in *Arabidopsis* (29, 140) result in reduced cellulose synthesis and alter the expression of wall-integrity-sensing genes (217); despite this, the mutant plants can grow and reproduce with only slightly abnormal morphology.

Interactions Between Cell Walls and the Plasma Membrane

Because of the close proximity between the plasma membrane and the cell wall and due to the fact that at least some wall components, e.g., cellulose, are synthesized at the cell surface, connections between the wall and the plasma membrane, as well as those extending across the plasma membrane into the cytoplasm, have been hypothesized to be important routes of communication between the outside and inside of plant cells (104). Direct evidence for these connections stems from plasmolysis experiments, in which placing plant cells in high concentrations of osmolytes results in the formation of Hechtian strands that bridge the cell wall to the shrinking protoplast (110). Likewise, membrane-embedded receptor proteins that bind to wall components (44, 63) are points of direct contact between the wall and the plasma membrane, although whether these interactions are transient or permanent in vivo has not been firmly established.

POLYMERIZATION AND ASSEMBLY OF CELL WALL COMPONENTS

Golgi Body–Localized Synthesis of Matrix Polysaccharides

Pectins and hemicelluloses are synthesized in the lumen of the Golgi body using activated nucleotide sugars as substrates (173) and then transported to the cell surface where they can be further modified in the wall (**Figure 2**). The enzymes that catalyze the assembly of these polymers include glycosyltransferases, methyltransferases, and acetyltransferases, whereas those that modify them postsynthesis include glycosyl hydrolases, transglycosylases, polysaccharide lyases, and carbohydrate esterases (28). The most prominent group of these enzymes includes the Golgi body–localized glycosyltransferases, with individual enzymes forming specific types of glycosidic linkages (168). Glycosyltransferases encompass large families of enzymes that can be broadly

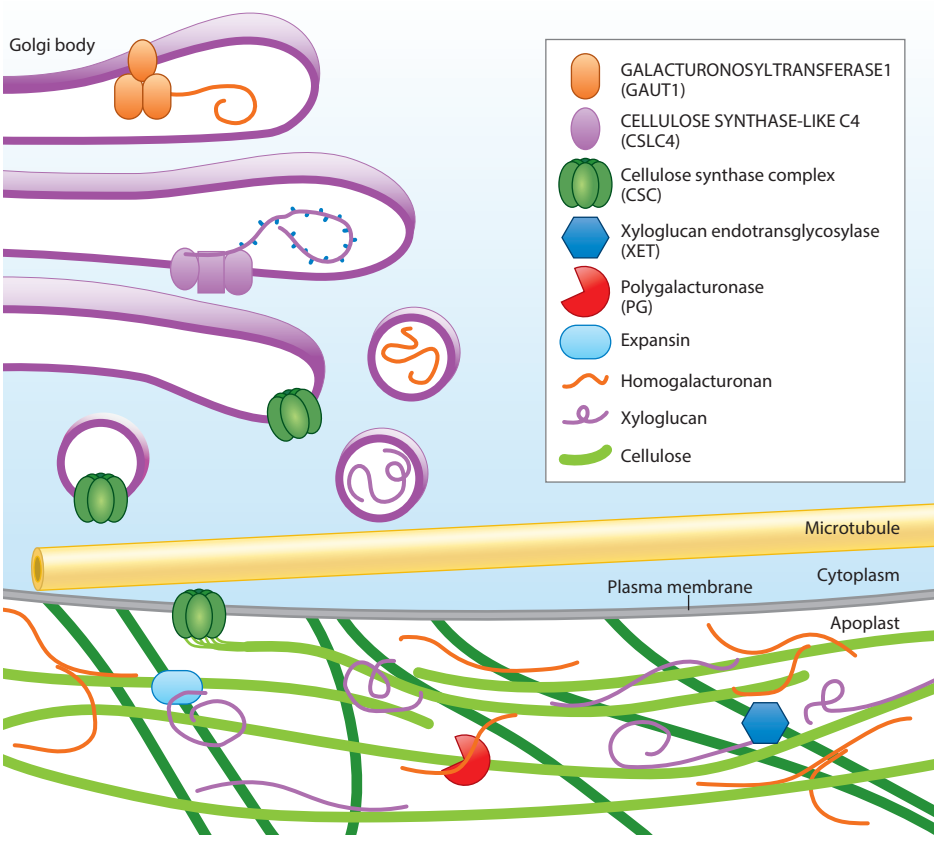

Figure 2

Cell wall assembly and structure in plants. Matrix polysaccharides, including pectic homogalacturonan (*orange*), xyloglucan (*purple*), and xylan (not shown), are synthesized in the Golgi body by glycosyltransferases, including GALACTURONOSYLTRANSFERASE1 (GAUT1) and CELLULOSE SYNTHASE-LIKE C4 (CSLC4), which can also decorate the polysaccharide backbones with side chains. These synthetic enzymes often act in complexes that are integral or tethered to the Golgi membrane. Post-Golgi trafficking of matrix polysaccharides can involve the formation of vesicles directly from Golgi cisternae or from the trans-Golgi network (not shown), and might involve trafficking along microtubules (*yellow*) at the cell cortex. Cellulose synthase complexes (CSCs, *green*) are also trafficked from the Golgi body to the cell cortex, where they can interact with microtubules during delivery to the plasma membrane, after which they synthesize cellulose and move through the membrane, driven by the polymerization force of cellulose (*green*). In the apoplast, matrix polysaccharides and cellulose interact, and proteins, including polygalacturonases (PGs, *red*), xyloglucan endotransglycosylases (XETs, *dark blue*), and expansins (*light blue*), can modify the structure of the wall to enable growth and developmental events.

classified into two types: Type I glycosyltransferases synthesize the backbones of pectins and hemicelluloses in the Golgi body, processively adding sugar moieties to backbone chains, whereas type II glycosyltransferases decorate the backbones by adding one sugar moiety at a time to form side chains (168). Type I glycosyltransferases are integral membrane proteins with their catalytic domains oriented in either the cytosol or the lumen of the Golgi body; Type II glycosyltransferases have a single-pass transmembrane domain with their catalytic domain generally facing into the lumen. The biochemical activities of only a small subset of all glycosyltransferases have been elucidated; for example, researchers have suggested that pectin biosynthesis requires more than 65

distinct enzyme activities, including those of glycosyltransferases, methyltransferases, and acetyltransferases (127), but only a few of these have been genetically and biochemically characterized.

Trans-Golgi network (TGN): a membrane-delimited compartment, often associated with the trans cisterna of the Golgi body, that functions as a sorting hub for cargo trafficking to the plasma membrane and/or vacuole

Cellulose synthase (CESA): a widely conserved enzyme that polymerizes the glucan chains of cellulose

Cellulose synthase complex (CSC): a multimeric complex responsible for polymerizing the glucan chains of cellulose microfibrils

Precisely where in the Golgi body the various enzymatic steps involved in the synthesis of matrix polysaccharides occur is not fully understood. The Golgi body is composed of a series of flattened membrane-enclosed disks known as cisternae, which occur as distinct compartments that include the cis-face, which interacts with the endoplasmic reticulum (ER), the medial Golgi, the trans-Golgi cisternae, and finally the trans-Golgi network (TGN) where secretory vesicles form (182, 183). The TGN can either be associated with the Golgi body or move independently in the cell. In the case of xyloglucan, quantitative immunolabeling experiments using antibodies targeting either the backbone or the fucosylated side chains revealed that the xyloglucan backbone occurs in the trans cisternae, but that the fucosylated side chains occur in both the trans cisternae and the TGN (52, 224). Likewise, the enzymes that sequentially elaborate xyloglucan side chains are localized in different Golgi body subcompartments (32). A similar model of step-like biosynthesis was developed for pectin biosynthesis using antibodies specific for the low-methylesterified pectin backbone or highly methylesterified pectin (51, 136, 224). Thus, these glycans are synthesized in an assembly-line-like manner as they move through the Golgi body (220).

More recent analysis of protein–protein interactions among various enzymes involved in xyloglucan biosynthesis paints a different picture. Chou et al. (33) examined interactions among various enzymes involved in the biosynthesis of xyloglucan in *Arabidopsis*, including enzymes catalyzing the synthesis of the glycan backbone [CELLULOSE SYNTHASE-LIKE C4 (CSLC4) and XYLOGLUCAN XYLOSYLTRANSFERASE1–XYLOGLUCAN XYLOSYLTRANSFERASE5 (XXT1–XXT5)], as well as enzymes that decorate the side chains (e.g., the FUT1 fucosyltransferase and the MUR3 and XLT2 galactosyltransferases). From coimmunoprecipitation and bimolecular fluorescence complementation experiments, these authors found interactions between the CSLC4/XXT complexes and CSLC4, MUR3, and XLT2. This suggests that the enzymes that synthesize both the backbone and side chains of xyloglucan are present as a large, multisubunit complex, presumably present in the same Golgi domain (**Figure 2**). Consistent with these results, Lund et al. (116) used a reversible *Renilla* luciferase protein complementation assay to demonstrate interactions between XXTs, MUR3, and FUT1 in transiently transformed *Nicotiana benthamiana* cells.

The presence of a large protein complex acting in the synthesis of both the backbone and side chains of xyloglucan is at odds with a model predicting the spatial separation of these biosynthetic steps drawn from the localization of different xyloglucan epitopes and green fluorescent protein (GFP)-localized biosynthetic enzymes in different subcompartments of the Golgi body (32, 52, 224). One contributing factor to this paradox could be the masking of polysaccharide epitopes (93) that might undermine the conclusion that different xyloglucan species are present in distinct locations within the Golgi body. A second issue is that overexpressed fusion proteins might be mislocalized in transiently transformed cells. Alternatively, multiple protein complexes with differing compositions involved in xyloglucan synthesis might be present in distinct regions of the Golgi body. Further studies are required to understand the spatial distribution of glycan synthesis in the Golgi body, and ideally, these studies will analyze the steps of glycan synthesis in vivo with high spatiotemporal resolution.

Synthesis and Assembly of Cellulose Synthase Complexes

Cellulose is synthesized by cellulose synthases (CESAs), enzymes that are active at the plasma membrane as a protein complex called the cellulose synthase complex (CSC) (129, 180). Each CSC subunit is thought to catalyze the polymerization of a single glucan chain using cytosolic

uridine diphosphate-glucose (UDP-glucose) as a substrate. CESAs are integral membrane proteins that are often encoded by multigene families. At least three different CESA isoforms are likely required to form a functional CSC (45, 189, 190). Given that cellulose microfibrils are likely composed of 18–24 glucan chains (65, 109, 132, 191, 192, 225), a model in which 18 CESA proteins form a functional CSC in the membrane as a hexamer of trimers has been proposed based on stoichiometric, electron microscopy, and modeling evidence (74, 94, 135).

In *Arabidopsis*, CESA1, CESA3, and CESA6 interact to form CSCs that function in primary wall biosynthesis (5, 62, 143, 169). CESA2, CESA5, and CESA9 also function in primary wall synthesis and are partially redundant with CESA6 (45, 143). The differences in function among these partially redundant CESAs do not simply reflect distinct patterns of expression, as neither *CESA2* nor *CESA5* driven by a *CESA6* promoter fully complements a *cesa6* mutant (45, 143). CESA4, CESA7, and CESA8 form distinct rosettes that function in secondary wall biosynthesis (6, 69, 189). Within the CSCs, each of the three CESA isoforms occurs in equimolar amounts as determined by coimmunoprecipitation, mass spectrometry, and quantitative immunoblot studies (74, 94).

CESA proteins are synthesized in the ER and transported to the cis Golgi body via coat protein complex II (COPII)-coated vesicles (21). Freeze-fracture studies of vascular plant cells detected CSCs in the Golgi body, but not the ER, suggesting that CSCs assemble in the Golgi body before being transported to the plasma membrane (39, 80, 82). However, CSCs have been observed in the ER in moss (159), suggesting either that CSCs assemble in the ER only in nonvascular plants or that some assembly occurs in the ER in most plants and this simply has not yet been observed in angiosperms. CSC assembly involves the STELLO1 and STELLO2 proteins, which localize to the Golgi body where they interact with multiple CESA proteins and are required for the normal multimerization, cellular trafficking, and plasma membrane insertion of CESAs (226). STELLO1 and STELLO2 contain a domain with homology to glycosyltransferases that is localized to the Golgi lumen, suggesting that they might glycosylate CESAs, but their precise molecular function is currently unclear.

Trafficking of Cellulose Synthase Complexes

Trafficking of CSCs plays a critical role in regulating the level of cellulose synthesis (11, 39, 40, 146, 208) (**Table 1**). After assembly in the Golgi body, CSCs are likely packaged into vesicles in the TGN for initial delivery to the plasma membrane. Consistent with this, proteomic analysis of immunoisolated SYNTAXIN OF PLANTS61-containing vesicles revealed that CESA1, 2, 3, and 6 are present in these vesicles (50). Further, the *det3* mutation, a weak allele of *VHA-C* that specifically affects the pH of the TGN and early endosomes, impairs secretion and recycling of CSCs, suggesting a role for TGN and early endosomes in CSC trafficking (118). A small Golgi-localized GTPase, RabH1B, has also been linked to CESA exocytosis: *rabh1b* mutations result in decreased secretion of CESA from the Golgi body, reduced cellulose levels, and an accumulation of vesicles around the Golgi body (89). However, CESA endocytosis is also decreased, and the density of CESA particles at the plasma membrane is elevated in this mutant (89), likely reflecting complex feedback regulation involved in CESA trafficking and function. The concurrence of increased CESA particle density at the plasma membrane with decreased cellulose levels in the mutant suggests that CESA trafficking is important for removing nonfunctional CSCs from the cell surface.

The exocyst complex has also been implicated in the delivery of CSCs to the plasma membrane (219). The exocyst is a multisubunit assembly that functions in a variety of developmental events (214, 222). CESA6, CELLULOSE SYNTHASE INTERACTIVE PROTEIN1 (CSI1), and

Table 1 Genes identified that play a role in the trafficking of CSCs

Gene	ID	Role	Mutant phenotype	Reference(s)
DET3	AT1G12840	Impairs trafficking of the CSC by affecting the pH of the TGN and early endosomes	Reduced cellulose levels as a result of reduced delivery of CSCs to the PM	118
RabH1B	AT2G44610	Decreased exo- and endocytosis of CSCs	Reduced cellulose levels, but elevated CSC density at the PM	89
Exo84B	AT5G49830	Components of exocyst complex; interact with PTL1 and CSI1 to regulate CSC trafficking	Reduced cellulose levels as a result of reduced delivery of CSCs to the PM	228
Exo70B1	AT5G58430			
Exo70A1	AT5G03540			
Sec5B	AT1G21170			
Sec6	AT1G71820			
PTL1	AT5G06970	Plays a role in trafficking proteins, including CESA, to the PM	Reduced cellulose levels as a result of reduced delivery of CSCs to the PM	228
POM2/CSI1	AT2G22125	Tethers CSCs to microtubules; serves as landmark for CSC insertion sites at the PM	Reduced levels of cellulose, but the level of CSCs at the PM is normal	22, 115, 228
SHOU4	AT1G78880	Directly interact with CESA and negatively regulate CSC exocytosis	Increased cellulose levels as a result of increased CSC levels at the PM	145
SHOU4L	AT1G16860			
CC1	AT1G45688	Required for efficient recycling of CSCs in conditions of abiotic stress	Hypersensitive to elevated saline	60, 111
CC2	AT5G42860			
AP2M/μ2	AT5G46630	Required for clathrin-mediated endocytosis	Increased CSCs at the PM	10
TML	AT5G57460	Required for endocytosis as part of the TPLATE complex	Reduced cellulose levels, elevated CSC density at the PM	12, 166
TPLATE	AT3G01780			
TWD40-2	AT5G24710			
KORRIGAN	AT5G49720	Plays a role in intracellular relocalization of CSCs	Decreased cellulose synthesis (required for optimal cellulose synthesis)	196

Abbreviations: AP2M/μ2, AP-2 complex subunit mu; CC, COMPANION OF CELLULOSE SYNTHASE; CESA, cellulose synthase; CSC, cellulose synthase complex; CSI, CELLULOSE SYNTHASE INTERACTIVE PROTEIN; DET, DE-ETIOLATED; PM, plasma membrane; PTL, PATROL; TGN, trans-Golgi network; SHOU4L, SHOU4-LIKE; TML, TPLATE COMPLEX MUNISCIN-LIKE.

PATROL1 all interact with multiple exocyst subunits, suggesting a role for this complex in CESA trafficking (228). PATROL1 functions in the trafficking of proteins to the plasma membrane (87), and disruption of *PATROL1* results in slower CESA delivery rates to the plasma membrane and lower cellulose levels (228). Based on detailed analysis of the cellular dynamics of these proteins, Zhu et al. (228) proposed an elegant model in which *POM2/CSI1* serves as a landmark for the insertion of CESA-containing exocytotic vesicles along cortical microtubules, with PATROL1 priming the fusion of these vesicles.

The paralogous *SHOU4* and *SHOU4L* genes also play an important role in regulating exocytosis of CESAs (145). *SHOU4* was identified in a suppressor screen of the cellulose-deficient *fei1 fei2* mutant. Disruption of both *SHOU4* and *SHOU4L* results in elevated CESA density at the plasma membrane due to enhanced exocytosis, which in turn leads to increased amorphous cellulose. Because SHOU4 and SHOU4L interact directly with primary wall CESAs and the levels

of SHOU4 and SHOU4L appear to be critical to their functions, SHOU4/SHOU4L might act as a counter of CESA levels at the plasma membrane, with the complex generating a signal to responsively inhibit CESA exocytosis and maintain optimal levels of cellulose biosynthesis.

The residence time of CESA proteins in the plasma membrane has been estimated to be as short as 7–8 min, but the half-lives of the proteins are much longer (95, 162), suggesting that CESA proteins are likely recycled between intracellular locations and the plasma membrane, dynamically controlling the level of cellulose synthesis in cells. CESA proteins have been detected in microtubule-associated vesicles, called microtubule-associated cellulose synthase compartments (MASCs) or small CESA compartments (SmaCCs), which might arise from endocytosis of CSCs from the plasma membrane and could serve as intracellular storage depots of CSCs (40, 60, 80). Treatment with the cellulose inhibitor isoxaben or with hyperosmotic conditions induces the formation of SmaCCs/MASCs. The CSI and COMPANION OF CELLULOSE SYNTHASE1 (CC1) and CC2 proteins are required for efficient recycling of CSCs in the presence of abiotic stress, at least in part through the regulation of SmaCC/MASC formation and by controlling microtubule dynamics (60, 111).

One mechanism underlying CSC recycling is clathrin-mediated endocytosis (83, 138). The μ-adaptin subunit of the AP2 complex, *AP2M-1*, which docks to the plasma membrane and recruits the clathrin-mediated endocytosis machinery, interacts with CESA6 (10, 125). *ap2m-1* mutants show reduced endocytosis and increased CESA density at the plasma membrane (10). Likewise, a component of the large adaptor TPLATE complex, TWD40, is required for general endocytosis and for maintaining normal CESA density at the plasma membrane (12). The higher CESA density in these mutants correlates with reduced levels of cellulose, similar to the situation with the *rabh1b* mutant (89). In addition to TWD40, other members of the TPLATE complex, TML and TPLATE, also interact with CESAs to mediate their endocytosis (166). Phosphoinositides (PIs) also modulate CESA trafficking, with PI3K and PI4K playing distinct roles: PI3K regulates clathrin-mediated endocytosis of CESAs, whereas PI3K functions in the exit of CESAs from the Golgi body (68).

Cellulose Synthesis at the Cell Surface

Several inputs regulate CESA function at the plasma membrane (146). The β-1,4 glucanase KORRIGAN interacts with primary and secondary wall CESAs and influences both CSC trafficking and cellulose synthesis (120, 196). Another potential regulator of cellulose synthesis is *COBRA*, which encodes a putative glycosylphosphatidylinositol (GPI)-anchored extracellular protein (157, 171). Cells in the elongation zone of *cobra* mutant roots display radial expansion that is correlated to a reduction in the level of crystalline cellulose. Posttranslational modifications also regulate CESA function (146). Phosphorylation of CESAs occurs at multiple residues within the hypervariable regions in the N terminus and in the central cytosolic loop (181) and likely regulates multiple aspects of CESA function, including degradation by the 26S proteasome (188), CSC movements in the plasma membrane (31), and response to hormones (165) and environmental factors (15). CESA proteins are also regulated by *S*-acylation; CESA7 is *S*-acylated at four cysteines in variable region 2 and the C-terminal domain, and mutation of these sites results in a reduction of crystalline cellulose, likely as a consequence of aberrant CESA trafficking (108).

The CESA-interacting protein CSI1/POM2 likely links CSCs to microtubules to pattern cellulose deposition (22, 115). Disruption of CSI1, and its paralogs CSI2 and CSI3, leads to reduced movement of CESA particles, lower cellulose content, and defective cell elongation (22, 79, 115). The CC1 and CC2 proteins also tether the CSCs to microtubules and are necessary to traffic CSCs back to the plasma membrane following their internalization after exposure to salt stress

(60). CC1 affects microtubule bundling and dynamics via an intrinsically disordered region in the N-terminal domain (103).

Trafficking of Polysaccharides from the Golgi Body to the Cell Wall

While it is clear that pectins and hemicelluloses are synthesized in the Golgi body and then delivered to the cell surface, the mechanisms underlying this trafficking are not well understood. The timely and spatially accurate delivery of cell wall components is essential for proper functioning of the wall and must be dynamic, adjusting with development and in response to environmental stimuli. There are two general modes of secretion to the apoplastic space, called the conventional and unconventional protein secretion pathways (34, 207). The unconventional pathway has been reviewed recently and is not discussed in depth here (42, 133, 151, 155). It is thought that pectins and hemicelluloses primarily traffic through the conventional pathway (179, 197). In the conventional pathway, cargos move from the trans face of the Golgi to the TGN, where they are trafficked to different cellular destinations (201). Correct targeting of TGN-derived vesicles that contain glycans to distinct regions of the plasma membrane involves transport along a cytoskeletal element and recognition and fusion to the plasma membrane, likely mediated in part by motor and SNARE proteins (227).

The TGN and vesicles emerging from it have been shown to contain matrix polysaccharide epitopes by immunocytochemistry (101, 164, 194) and glycome profiling (209), suggesting that pectin and hemicellulose cargos are sorted and packaged in the TGN. The function of the TGN is regulated by diverse inputs, including RAB GTPases, SNARE proteins, and accessory proteins. Indeed, mutations in several of these elements disrupt the proper targeting of Golgi body–derived glycans. Mutations in genes encoding YPT/RAB GTPase Interacting Protein 4a (YIP4a), YIP4b, or their interacting protein ECHIDNA disrupt secretion of matrix polysaccharides (71). Further studies revealed that disruption of *ECHIDNA* results in accumulation of pectins in post-Golgi vesicles and ER-derived bodies and mistargeting to the central lytic vacuole (121). Individual null mutants in *RABA1*, *RABA2*, and *RABA4* affect the levels of pectin, cellulose, and hemicellulose, respectively, in the cell walls of *Arabidopsis* stems, although these mutants do not display obvious morphological or growth phenotypes (117). Pectin has been colocalized with SECRETORY CARRIER MEMBRANE PROTEIN2 (SCAMP2) vesicles in tobacco BY-2 cells (194), suggesting a role for this conserved component of secretory vesicles in the trafficking of pectins and/or other glycans. In contrast, in border cells derived from alfalfa root tips, xylogalacturonan is packaged into large vesicles that derive directly from the margins of trans-Golgi compartments (205), suggesting that the sorting of Golgi-derived wall polysaccharides into exocytic vesicles might occur as early as the trans Golgi.

Are Golgi body–derived polysaccharides and proteins packaged together or into discrete vesicles? Several lines of evidence suggest that these macromolecules traffic through distinct routes from the Golgi to the plasma membrane. For example, mutations in the FRAGILE FIBER 1 kinesin reduce the delivery of pectins to the cell wall, but do not appreciably affect CESA3 trafficking (227), suggesting that they may be trafficked in distinct vesicles. Consistent with this, whereas *echidna* mutations result in a mislocalization of wall polysaccharides to the vacuole as noted above, proteins are not mistargeted to the vacuole in the mutant (121), suggesting that distinct post-TGN trafficking components might mediate downstream events in their secretion from the TGN. However, counter to this idea, both CESA proteins and matrix polysaccharides have been detected in isolated SYP61-containing vesicles (50, 209), suggesting at least some overlap between the pathways.

Different Golgi body–derived wall polysaccharides may be targeted to distinct secretory vesicles. The proton ionophore monensin, which inhibits the proton gradient of the secretory

pathway, causes intracellular accumulation of hemicelluloses but does not affect pectin secretion (223). Another study used double labeling with antibodies specific for either xylogalacturonan or xyloglucan to demonstrate that each of these glycans occupies distinct vesicles (205). In contrast, double labeling of vesicles moving from the TGN to the plasma membrane in *Vicia* root hair cells determined that polygalacturonic acid and xyloglucan epitopes are present together in most vesicles (176). A model consistent with these results includes multiple classes of vesicles derived from the TGN (or in some cases directly from the Golgi body) that contain distinct sets of multiple glycans.

Apoplast: the space outside the plasma membrane of a plant cell, usually occupied by the cell wall

Golgi stacks move around the cell via actin-myosin-driven cytoplasmic streaming in a stop-and-go fashion (16, 99, 131), and cargos are likely unloaded during Golgi pauses (39, 131). Several studies suggest that the delivery of glycans to the apoplast occurs nonuniformly across the cell surface. In growing pollen tubes, pectin subtypes are distributed heterogeneously from the tip to the shank of the tube (30, 158). In *Arabidopsis* root cells, polysaccharides that are metabolically labeled to allow for fluorescent tagging (4) first localize in the cell wall as distinct puncta that likely mark the sites of vesicle-mediated delivery of new polysaccharides. Consistent with the dynamic nature of glycan delivery, this pattern of incorporation changes significantly along the developmental gradient of the root (4).

Initiation of Interactions Between Wall Components in the Apoplast

Insights into the initial interactions between wall components can be gained from the analysis of cytokinesis in plant cells, during which cell wall components are sequentially delivered to the growing and developing cell plate that separates daughter nuclei (163). This de novo wall formation begins with the deposition of positively charged extensins and negatively charged pectins that potentially interact electrostatically (26), followed by the deposition of callose, and finally the synthesis of cellulose (126). However, whether these ordered delivery and synthesis events persist during the interphase deposition of cell walls is unclear, and the question of how exactly cellulose-matrix interactions arise in vivo is an open one. Given the potential separability of the secretory pathways for CSCs and matrix polysaccharides mentioned in the section titled Trafficking of Polysaccharides from the Golgi Body to the Cell Wall, it should be possible to functionally dissect how these initial interactions take place and how altering their kinetics and patterning affects wall structure immediately after its initial assembly.

WALL MECHANOPERCEPTION AND INTEGRITY SENSING

Physical Deformation of the Cell Wall During Growth

Plant cells can expand tremendously in volume over their lifetimes, swelling to thousands of times their initial volumes. This growth, which is driven by the turgor pressure imposed by water influx into the cell, is accompanied by increases in cell surface area, which can arise from the expansion and thinning of existing wall materials or from the spatially controlled deposition of additional wall components. Both mechanisms appear to exist in plants and their relatives: For example, dark-grown *Arabidopsis* seedlings deposit thick outer epidermal walls during a slow elongation phase that then become thinner during a subsequent rapid elongation phase (142, 153), whereas expanding cells of the charophyte green alga *Penium margaritaceum* lay down new wall material at an isthmus that displaces older wall regions toward the cell tips (49), and tip-growing cells such as pollen tubes and root hairs grow via polarized wall deposition (9). In certain cell types, such as the protoxylem cells of *Arabidopsis* roots that form circular and spiral thickenings of secondary wall material (172), cell growth can persist even after the deposition of secondary wall layers, but once

secondary walls fully encase plant cells, their growth ceases and they often undergo programmed cell death.

Cell wall integrity: the molecular composition, polymer configuration, and physical status of the wall of a plant cell that enables its proper function

CSCs move in the plasma membrane along linear tracks determined by underlying cortical microtubules (139), driven by the force generated by cellulose polymerization (43, 48, 92, 128). Because cellulose is the main load-bearing element of the wall, its orientation often dictates the extent and direction of cell expansion. In elongating cells, such as root or hypocotyl cells, cellulose is deposited with a net orientation perpendicular to the axis of expansion, thus constricting radial expansion (13, 78, 186). However, cellulose fibers change in orientation in elongating cells (3), suggesting that after transverse synthesis, cellulose might passively reorient in growing cells to generate differentially oriented wall layers. Additionally, recent evidence (19, 141) suggests that polarized pectin modification might underlie the initiation of anisotropic growth in some elongating cells, with the deposition pattern of cellulose responding to and reinforcing this initial growth asymmetry.

Mechanoperception of Environmental Stimuli Mediated by the Cell Wall

Although gravity is thought to be sensed primarily by intracellular amyloplasts, it is also possible that deformation of the cell wall itself by gravity results in changes in wall status that are sensed by the plant. Weakening of the cell wall due to abnormalities in wall synthesis or remodeling can result in mechanical failure of plant stems near the ground level under the forces imposed by gravity and/or wind, which is called stem lodging (97, 215), causing major reductions in crop yields. Plants also respond to wind-induced bending and touch. The *arad1 arad2* double mutant, which is deficient in arabinan synthesis, produces stems that are deficient in their responses to touch (200). Additionally, the hydration status of the wall is likely to alter both wall-membrane contacts and the interactions of wall polymers themselves, resulting in changes in wall integrity signaling (64).

Genetic and Developmental Evidence for Wall Integrity Sensing

The dynamic nature of growing and developing plant cell walls, combined with the effects of biotic and abiotic stresses, necessitates an ability of plant cells to monitor the integrity of their walls and modulate wall synthesis and properties in order to allow for appropriate expansion and/or to resist bursting in different conditions (96, 98, 145, 184, 204, 210–212). Cell wall integrity sensing is best understood in the budding yeast *Saccharomyces cerevisiae*, which employs a family of five cell-surface sensors that contain an extracellular domain that is highly *O*-mannosylated and acts as a nanospring embedded in the wall (57, 112). These sensors control the activity of the guanine nucleotide exchange factor (GEF) Rom2, which in turn regulates the activity of a small GTPase called Rho1. Rho1 activates a mitogen-activated protein kinase (MAPK) cascade that ultimately phosphorylates transcription factors, which modulate the expression of genes involved in cell wall synthesis and function.

In contrast to our understanding of wall integrity sensing in yeast, our understanding of wall integrity sensing in plants is still in its infancy. An early clue suggesting the existence of a wall integrity sensing system in plants was that tomato cell cultures that had been adapted to the presence of the cellulose synthesis inhibitor 2,6-dichlorobenzonitrile could grow, despite the fact that they synthesize virtually no cellulose (175). A number of reports indicated compensatory changes in plants in response to chemical or genetic inhibition of cellulose synthesis, including ectopic lignification, enhanced callose deposition, increased pectin synthesis and demethylesterification, elevated jasmonic acid levels, increased production of reactive oxygen species, and changes in

Table 2 Putative RLK cell wall integrity sensors in plants

Genes	Cell wall components bound	Other signaling components	Cell-wall-related phenotype	Reference(s)
Wall-associated kinases				
WAK1–WAK5	Pectin (Ca^{2+}-dependent) and pectin-derived oligogalacturonides	MAP kinase cascade	Defects in cell elongation and the response to pathogens	25, 44, 47, 106
CrRLK1l family				
THE1	Pectin; cell wall fragments?	GEF4, partially functionally redundant with HERK1/2	*the1* suppresses growth defects, ectopic lignification, and gene expression in response to cellulose deficiency; double mutants with *herk1* display reduced stature	90, 150
FER	Pectin; FER also acts as a RALF peptide sensor	ABI2 (phosphatase), RIPK (kinase), LRE (GPI-anchored protein), Ca^{2+}, GEF, Rac/ROP GTPases, ROS, β-subunit of G proteins, MARIS (kinase)	*fer* mutants are pleiotropic; phenotypes include pollen tube overgrowth, shorter hypocotyls, reduced stature, hypersensitivity to salt stress, defective root hairs, larger seeds, and cotyledons	53, 56, 63, 70, 113, 114
ANX1/2	Unknown	MARIS (kinase)	Plays a role in pollen tube growth; mutants show reduced fertility	134
Leucine-rich repeat genes				
FEI1 FEI2	Unknown	SOS5, 1-aminocyclopropane-1-carboxylic acid (ACC)	Conditional reduction of cellulose synthesis	218
MIK2	Unknown	Interacts with THE1 in response to biotic and abiotic stress	Root skewing and salt stress sensitivity; links cell wall integrity to biotic and abiotic stress	198

Abbreviations: ABI, ABA-insensitive; ANX, ANXUR; FER, FERONIA; GEF, guanine nucleotide exchange factor; GPI, glycosylphosphatidylinositol; LRE, LORELEI; MIK2, MDIS1-interacting receptor-like kinase 2; RALF, rapid alkalinization factor; ROS, reactive oxygen species; THE, THESEUS; WAK, wall-associated kinase

carbohydrate metabolism (reviewed in 84). These studies imply the existence of a cell wall integrity monitoring system that perceives perturbation of the wall and signals to the cell to execute compensatory changes.

Wall Integrity Sensing by Membrane-Localized Receptors

Genetic studies have implicated a number of receptor-like kinases (RLKs) from several distinct clades in cell wall sensing (reviewed in 146, 184, 210, 212) (**Table 2**). The best characterized of these are in the *Catharanthus roseus* protein-kinase-1-like (CrRLK1L) family. These proteins are composed of an extracellular N-terminal domain with two tandem malectin-like domains, a single transmembrane domain, and a cytosolic serine/threonine kinase domain. In animals, malectin binds dimeric and oligomeric glucose chains (167), suggesting that by analogy, the CrRLK1L family members might bind to wall components. One member of the CrRLK1L family, *THESEUS1*, was identified as a suppressor of the growth defects of the *cesa6*^{prc1} mutant. Loss-of-function mutants in *THESEUS1* suppress growth defects, ectopic lignification, and many of the gene-expression changes that occur in response to cellulose deficiency, but do not restore

RLK: receptor-like kinase

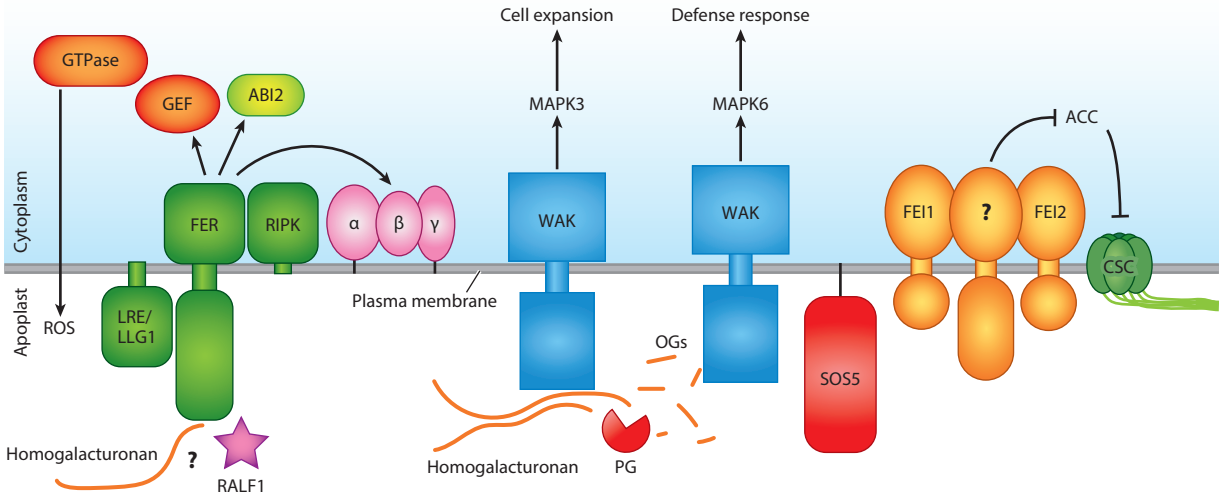

Figure 3

Examples of wall integrity sensing and responsive wall modification. The RLK FER (*green*) binds to pectic homogalacturonan and the RALF1 peptide, interacts with a GEF, and results in GTPase activation, driving alterations in ROS in the apoplast. FER also interacts with LRE and LLG1, as well as the ABI2 phosphatase, RIPK, and the β-subunit of heterotrimeric G proteins. WAK family members (*blue*) can bind to demethylesterified homogalacturonan and activate the MAPK3 kinase signaling pathway, but when homogalacturonan is degraded to OGs, WAK binds these pectin fragments with higher affinity and activates defense responses via MAPK6 signaling (106). FEI1 and FEI2 (*orange*) act on the same pathway as the arabinogalactan protein, SOS5, and potentially another RLK to facilitate cellulose synthesis by blocking ACC, which downregulates cellulose synthesis. Question marks indicate unknown connections or interactions. Abbreviations: ACC, 1-aminocyclopropane-1-carboxylic acid; CSC, cellulose synthase complex; FER, FERONIA; GEF, guanine nucleotide exchange factor; LLGI, LORELEI-LIKE-GPI-ANCHORED PROTEIN 1; LRE, LORELEI; OG, oligogalacturonide; PG, polygalacturonase; RALF1, RAPID ALKALINIZATION FACTOR1; RIPK, RPM1-induced protein kinase; RLK, receptor-like kinase; ROS, reactive oxygen species; WAK, WALL-ASSOCIATED KINASE.

wild-type cellulose levels (90). This suggests that the response to cellulose deficiency, including reduced growth, is an active process, and that this response requires THESEUS1. More recently, a new hypermorphic allele of *THESEUS1* was identified that expresses a truncated protein lacking the kinase domain (124), suggesting that kinase activity is not required for signal activation, but rather that it attenuates THESEUS1 signaling.

FERONIA, the best-studied member of the CrRLK1L family, has pleiotropic roles in growth and development, including pollen tube reception in the female gametophyte, root hair development, brassinosteroid and ethylene signaling, and wall integrity perception (reviewed in 113) (**Figure 3**). FERONIA is required to maintain cell wall integrity during salt stress via pectin binding and by inducing Ca^{2+} transients that are necessary for cell acclimation to salt stress (63). FERONIA, together with a set of extracellular leucine-rich repeat extensins, is also required to coordinate changes in cell wall acidification and loosening with an increase in vacuole size in expanding atrichoblast cells in *Arabidopsis* roots (56). FERONIA appears to signal through a GEF to regulate the activity of a GTPase (Rac/ROP or a RHO), which in turn regulates NADPH oxidase-derived reactive oxygen species levels during root hair growth and in the female gametophyte postpollination (54, 55). The cytoplasmic kinase domain of FERONIA likely also acts through other signaling elements, including the phosphatase, ABI2, RPM1-induced protein kinase, and the β-subunit of heterotrimeric G protein (53). These diverse signaling outputs likely mediate

the pleiotropic effects of FERONIA in different cell types. Further, two GPI-anchored proteins, LORELEI and LORELEI-LIKE-GPI-ANCHORED PROTEIN 1, directly interact with FERONIA and likely act as chaperones and coreceptors (114). Related CrRLK1L family members, such as ANXUR1 and 2, have also been linked to cell wall synthesis, though they may primarily affect pectin synthesis (134). Interestingly, THESEUS1, FERONIA, ANX1/2, and BUPS1/2 also act as receptors for rapid alkalinization factor (RALF) peptides (70, 75, 86). How the binding of RALF peptides and a glycan in the extracellular domains of these proteins relate to each other is not yet clear.

Another pair of RLKs implicated in regulated cell wall synthesis is FEI1/FEI2 (218) (**Figure 3**). Double *fei1 fei2* mutants display reduced growth anisotropy that is correlated to reduced cellulose biosynthesis. The FEI extracellular domain has relatively few leucine-rich repeat (LRR) proteins, suggesting that it might act in concert with other RLKs to accomplish wall sensing or signaling (**Figure 3**). Genetic analyses indicate that the FEIs act in a linear pathway with SOS5, a GPI-anchored protein that has been linked to the regulation of cellulose biosynthesis (14, 218). The SOS5-FEI2 pathway is required to produce cellulosic rays that anchor the pectic components of mucilage to the seed surface (85, 123). Radial swelling in *fei1 fei2* roots is suppressed by inhibitors of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) but not by inhibitors of ethylene signaling or by genetic disruption of ethylene perception, implicating ACC but not ethylene in cell wall sensing. Consistent with this, inhibition of cell expansion in response to isoxaben in root epidermal cells is dependent on ACC but not on ethylene (195). Similar results were found when these cells were treated with the peptide elicitor flg22, perhaps linking ACC to defense activation in response to wall perturbation. The *fei1 fei2* phenotype is also reverted by mutations in auxin biosynthesis genes, which can also partially suppress other cellulose-deficient mutants such as $cesa6^{prc1-1}$, *cobra*, and *sos5*, consistent with a role for auxin in cell wall integrity signaling (185).

Wall Integrity Signaling and Plant Defense

Plants possess surveillance systems to detect potential pathogen infection, including the perception of so-called damage associated molecular patterns (DAMPs), which are host-derived molecules that activate plant defense responses. DAMPs include wall fragments released in response to pathogen infection that are perceived by the wall integrity sensing system (8, 17, 119). Consistent with this, mutations affecting cellulose biosynthesis can affect pathogen interactions. For example, the *cev1* mutant, a hypomorphic allele of *CESA3*, causes constitutive expression of defense-related genes and resistance to powdery mildew pathogens (27, 59). Likewise, mutations in secondary wall *CESA* genes induce defense genes and reduce susceptibility to broad-host necrotrophic and vascular pathogens (91). Chemical inhibition of cellulose biosynthesis also leads to the induction of defense-response genes (27, 61, 198), and the treatment of *Arabidopsis* seedlings with three different inhibitors was used to identify a set of robust immune markers that are also induced by cell wall damage (198). The induction of these genes by the inhibition of cellulose biosynthesis required another LRR-RLK, MIK2.

The cellulose biosynthesis inhibitor isoxaben and *Basidiomycetes*-derived wall-degrading enzymes induce similar wall damage responses (61, 73). Phenotypic clustering reveals that the activation of these responses depends on a set of RLKs, with THESEUS1, MIK2, and FEI2 playing the most prominent roles, and that pattern-triggered immunity repressed the responses to cell wall damage (73). Gigli-Bisceglia et al. (73) proposed that wall damage induces a negative feedback loop, in which the wall integrity response triggers an immunity-related signal that in turn represses cell wall integrity signaling. Intriguingly, cellulose perturbation also induces

genes encoding enzymes that degrade cytokinin (*CKX2* and *CKX3*), which in turn leads to the downregulation of cell cycle genes (73), suggesting a link between cell wall integrity sensing, hormone signaling, and the cell cycle.

WAK: wall-associated kinase

Pectins have been linked to disease resistance in multiple reports (8). This is likely due to pectins acting as a physical barrier to ingress by pathogens and to their release of pectin-derived DAMP glycans upon their degradation that activate defense signaling, the latter of which is relevant for wall integrity sensing. Application of oligogalacturonides (OGs), which are oligomers of α-galacturonosyl residues derived from pectin, to plants can trigger activation of MAPKs, production of H_2O_2, induction of defense genes, and callose deposition. One protein family that likely functions in the perception of these OGs are wall-associated kinases (WAKs) (**Figure 3**), which are a family of RLKs, some of whose extracellular domains bind to pectin in planta (105). In vitro, the WAK extracellular domain binds to pectin oligosaccharides in a Ca^{2+}-dependent manner (44, 106). A domain-swap experiment revealed that WAK1 could be activated by OGs (25), and WAK2 was found to be required for the induction of MAPK activity in response to pectin (106). WAKs have been linked to resistance to a number of pathogens, including *Fusarium*, *Botrytis*, and *Verticillium longisporum* in *Arabidopsis* (25, 47, 81), indicating that the WAK gene family plays an important role in wall-mediated plant defense. Although in a few cases it is clear that the WAK ligands are pectin or pectin-derived OGs, for most WAKs no clear ligand has been identified.

THESEUS1 has also been linked to plant defense. Loss-of-function *the1* mutants are more susceptible to *Botrytis cinerea*, and *THESEUS1* overexpression results in reduced disease severity for this pathogen (150). Several genes that are differentially expressed in response to *B. cinerea* infection are altered in a *THESEUS1* overexpressing line and likely represent the downstream transcriptional targets of this signaling pathway during pathogen response. THESEUS1 interacts directly with the RHO OF PLANTS (ROP) GEF4, and altered GEF4 function affects susceptibility to *B. cinerea*. These results suggest that THESEUS1 might be involved in the perception of cell wall fragments released during *Botrytis* infection and, similar to FERONIA, likely signals through a GEF to activate defense responses.

A recent study identified oligosaccharides derived from xyloglucans called Xh that might also act as DAMPs (35). Xh oligosaccharides trigger a signaling cascade similar to that observed in response to OGs. Purified Xh activates defense gene expression, H_2O_2 elevation, and MAPK activity in *Arabidopsis* and grape (*Vitis vinifera*). Exogenous application of purified Xh resulted in resistance to *B. cinerea* (grape and *Arabidopsis*) and *Hyaloperonospora arabidopsidis* (*Arabidopsis*), acting through the jasmonate, salicylate, and ethylene pathways.

RESPONSIVE CELL WALL REMODELING

Cell Wall Modification During Growth

In response to growth stimuli, plant cell walls must loosen to allow for turgor-driven expansion (37). This can be facilitated by the binding activity of nonenzymatic proteins called expansins, which are hypothesized to disrupt the interactions between cellulose and xyloglucan that mechanically constrain wall expansion (36). However, enzymatic modification and cleavage of wall polymers can also occur during growth and likely contribute to at least part of the increase in wall surface area. Homogalacturonan crosslinking by calcium is enabled by demethylesterification of galacturonic acid residues by pectin methylesterases (PMEs) in the wall, and a cycle of calcium binding and unbinding might allow for remodeling of the pectin network in expanding, and even contracting, cells (20, 160). For example, a subset of polygalacturonases, enzymes that cleave homogalacturonan backbones, is required for normal cell expansion in *Arabidopsis* plants (160, 216). Homogalacturonan can also be cleaved by pectate lyases, although these are less well-characterized

at the molecular and genetic levels. Xyloglucan can also be remodeled in the wall by xyloglucan endotransglycosylases/hydrolases, which cleave and can religate xyloglucan molecules. This large class of enzymes has also recently been shown to possess the capability of performing additional transglycosylation reactions between cellulose (177) and xylan (100), opening up the possibility that hybrid molecules with covalent cellulose-matrix links might be generated in the wall. Thus far, however, direct evidence for the function of xyloglucan endotransglycosylase/hydrolase enzymes in cellular growth is sparse, although their functions might be masked by genetic redundancy and/or the dominant biomechanical roles played by other polysaccharide modifications.

Given that enzymes that modify matrix polysaccharides are secreted to the apoplast, likely along post-Golgi trafficking routes that are similar to or overlap with those of their polysaccharide targets, it is not surprising that many of these enzymes are inactive intracellularly and are activated upon secretion or in response to changes in wall conditions, such as pH. A common regulatory mechanism for wall-modifying enzymes is the presence of an auto-inhibitory domain, or an inhibitory binding partner, that is removed or inactivated to initiate the activity of the enzyme. For example, many PMEs are synthesized as proPMEs that contain inhibitory sequences, which must be cleaved (161). One PME, AtPME17, has been hypothesized to be cleaved from a proPME into an active form by the subtilisin-like protease SBT3.5 (174). PMEs also interact with PME-inhibitory PMEI proteins that are likely to dynamically bind and release PMEs, allowing them to demethylesterify homogalacturonan in response to signaling cues (213).

Wall Remodeling via Synthesis and Assembly

Remodeling of plant cell walls can be achieved by the selective degradation of specific wall components, but it can also be accomplished by the biased production and integration of new wall components. One recent study reported that the Golgi compartments of grasses and eudicots contain similar proportions of matrix polysaccharides and glycoproteins (137), which, given the compositional differences between these two types of cell walls, implies that selective trafficking or degradation of these initially similar contingents of components might account for the differences in wall composition between the two taxa. Likewise, suites of glycosyltransferase enzymes might be differentially expressed, post-translationally modified, and degraded to tune the composition of new layers of cell walls. Investigating the differential composition of wall layers before and after experimentally applied, transient stimuli might shed new light on how walls are modified to adapt to changing conditions and would go beyond the constitutive changes induced by gene mutations.

Regardless of the remodeling mechanism, clear connections have yet to be made between wall integrity sensing and its downstream signaling pathways (**Figure 3**) and the synthesis of new wall components or the proteins that deliver and remodel them. By controlling the spatiotemporal activation of integrity-signaling pathways and monitoring wall assembly and modification in real time, it should be possible to begin to understand the cellular mechanisms by which plant cells can dynamically tune their extracellular environment to optimize growth, protection, and morphogenesis.

CONCLUSIONS

Although they exist outside the boundary of the plasma membrane, plant cell walls are dynamic extracellular structures that undergo assembly, metabolism, modification, and controlled degradation and thus mirror many of the characteristics found in the living protoplast. Genetic identification of new players in wall synthesis, sensing, and modification has provided a rapidly expanding cast of characters that carry out myriad activities in the cell wall, but determining exactly how all

of these players function alone and in concert will require new, molecular-level information in living plants.

SUMMARY POINTS

1. Cell walls are composed of interacting networks of polysaccharides, proteins, and polyphenolic compounds whose complexity arises from the large number of genes responsible for their synthesis, the complex biochemical pathways by which they are assembled, and the variety of ways in which they are delivered, assembled, and modified in the wall.
2. Plant cell walls are synthesized and decorated sequentially by the cell and assembled in the apoplast, and their assembly can thus be thought of as modular, with certain macromolecules potentially being linked after synthesis or even after delivery to the wall.
3. Increasing knowledge concerning the trafficking and regulation of cellulose synthase proteins provides one paradigm for understanding how the localization and activity of wall-synthesizing enzymes relate to the processes of wall assembly, but the Golgi-localized synthesis of many matrix polysaccharides distinguishes them from cellulose, which is synthesized at the plasma membrane and extruded directly into the apoplast.
4. Cell wall components and wall-modifying proteins are delivered to the wall along multiple poorly resolved trafficking pathways, and the activity of the wall-modifying proteins appears in some cases to be regulated to prevent premature modification or degradation of wall polysaccharides that are delivered along the same pathways as their substrates.
5. The components of the cell wall do not act only as structural elements, but can bind to and stimulate wall integrity sensors that activate integrity-responsive signaling pathways, potentially resulting in changes in wall synthesis or modification that allow plants to adapt to changing environments.
6. The cell wall is not static in living tissues, and molecular and mechanical perturbation of the wall during growth, environmental variation, and/or attack by pathogens and predators results in the activation of responsive intracellular machinery that can remodel the wall itself and increase the fitness of the plant.

FUTURE ISSUES

1. What are the molecular architectures of different types of plant cell walls, and how do these change during wall deposition, growth, remodeling, and degradation over the lifetime of a plant?
2. How are the synthesis and trafficking of matrix polysaccharides coordinated with the trafficking and activity of cellulose synthase complexes to facilitate the correct assembly of the cell wall?
3. Which components of the cell wall act as signals of changes in cell wall integrity, and how do the sensors for these components coordinate to enable responsive and appropriate responses to these signals?
4. To what extent are plant cell walls self-healing, in that they are able to autonomously remodel themselves and relink broken polymers in response to stimuli or damage, and

how much cell wall remodeling requires intracellular signaling, gene expression, protein production and modification, and synthesis of new wall components?

5. Does environmental perturbation of plant cell walls release pectin-bound calcium that can enter the cell to activate signaling pathways, and what are the downstream components of these pathways?

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Anderson CT. 2016. We be jammin': an update on pectin biosynthesis, trafficking and dynamics. *J. Exp. Bot.* 67:495–502
2. Anderson CT. 2019. Pectic polysaccharides in plants: structure, biosynthesis, functions, and applications. In *Extracellular Sugar-Based Biopolymers Matrices*, ed. E Cohen, H Merzendorfer, pp. 487–514. Cham, Switz.: Springer
3. Anderson CT, Carroll A, Akhmetova L, Somerville C. 2010. Real-time imaging of cellulose reorientation during cell wall expansion in Arabidopsis roots. *Plant Physiol.* 152:787–96
4. Anderson CT, Wallace IS, Somerville CR. 2012. Metabolic click-labeling with a fucose analog reveals pectin delivery, architecture, and dynamics in *Arabidopsis* cell walls. *PNAS* 109:1329–34
5. Arioli T, Peng L, Betzner A, Burn J, Wittke W, et al. 1998. Molecular analysis of cellulose biosynthesis in *Arabidopsis*. *Science* 279:717–20
6. Atanassov II, Pittman JK, Turner SR. 2009. Elucidating the mechanisms of assembly and subunit interaction of the cellulose synthase complex of *Arabidopsis* secondary cell walls. *J. Biol. Chem.* 284:3833–41
7. Atmodjo MA, Hao Z, Mohnen D. 2013. Evolving views of pectin biosynthesis. *Annu. Rev. Plant Biol.* 64:747–79
8. Bacete L, Mélida H, Miedes E, Molina A. 2018. Plant cell wall-mediated immunity: Cell wall changes trigger disease resistance responses. *Plant J.* 93:614–36
9. Bascom CS Jr., Hepler PK, Bezanilla M. 2018. Interplay between ions, the cytoskeleton, and cell wall properties during tip growth. *Plant Physiol.* 176:28–40
10. Bashline L, Li S, Anderson CT, Lei L, Gu Y. 2013. The endocytosis of cellulose synthase in Arabidopsis is dependent on μ2, a clathrin-mediated endocytosis adaptin. *Plant Physiol.* 163:150–60
11. Bashline L, Li S, Gu Y. 2014. The trafficking of the cellulose synthase complex in higher plants. *Ann. Bot.* 114:1059–67
12. Bashline L, Li S, Zhu X, Gu Y. 2015. The TWD40-2 protein and the AP2 complex cooperate in the clathrin-mediated endocytosis of cellulose synthase to regulate cellulose biosynthesis. *PNAS* 112:12870–75
13. Baskin TI. 2005. Anisotropic expansion of the plant cell wall. *Annu. Rev. Cell Dev. Biol.* 21:203–22

14. Basu D, Tian L, Debrosse T, Poirier E, Emch K, et al. 2016. Glycosylation of a fasciclin-like arabinogalactan-protein (SOS5) mediates root growth and seed mucilage adherence via a cell wall receptor-like kinase (FEI1/FEI2) pathway in Arabidopsis. *PLOS ONE* 11:e0145092
15. Bischoff V, Desprez T, Mouille G, Vernhettes S, Gonneau M, Höfte H. 2011. Phytochrome regulation of cellulose synthesis in *Arabidopsis*. *Curr. Biol.* 21:1822–27
16. Boevink P, Oparka K, Cruz SS, Martin B, Betteridge A, Hawes C. 1998. Stacks on tracks: The plant Golgi apparatus traffics on an actin/ER network. *Plant J.* 15:441–47
17. Boller T, Felix G. 2009. A renaissance of elicitors: perception of microbe-associated molecular patterns and danger signals by pattern-recognition receptors. *Annu. Rev. Plant Biol.* 60:379–406
18. Borassi C, Sede AR, Mecchia MA, Salgado Salter JD, Marzol E, et al. 2016. An update on cell surface proteins containing extensin-motifs. *J. Exp. Bot.* 67:477–87
19. Bou Daher F, Chen Y, Bozorg B, Clough J, Jonsson H, Braybrook SA. 2018. Anisotropic growth is achieved through the additive mechanical effect of material anisotropy and elastic asymmetry. *eLife* 7:e38161
20. Boyer JS. 2016. Enzyme-less growth in *Chara* and terrestrial plants. *Front. Plant Sci.* 7:866
21. Brandizzi F. 2018. Transport from the endoplasmic reticulum to the Golgi in plants: Where are we now? *Semin. Cell Dev. Biol.* 80:94–105
22. Bringmann M, Li E, Sampathkumar A, Kocabek T, Hauser MT, Persson S. 2012. POM-POM2/ CELLULOSE SYNTHASE INTERACTING1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*. *Plant Cell* 24:163–77
23. Broxterman SE, Schols HA. 2018. Characterisation of pectin-xylan complexes in tomato primary plant cell walls. *Carbohydr. Polym.* 197:269–76
24. Broxterman SE, Schols HA. 2018. Interactions between pectin and cellulose in primary plant cell walls. *Carbohydr. Polym.* 192:263–72
25. Brutus A, Sicilia F, Macone A, Cervone F, De Lorenzo G. 2010. A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides. *PNAS* 107:9452–57
26. Cannon MC, Terneus K, Hall Q, Tan L, Wang Y, et al. 2008. Self-assembly of the plant cell wall requires an extensin scaffold. *PNAS* 105:2226–31
27. Caño-Delgado A, Penfield S, Smith C, Catley M, Bevan M. 2003. Reduced cellulose synthesis invokes lignification and defense responses in *Arabidopsis thaliana*. *Plant J.* 34:351–62
28. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The Carbohydrate-Active EnZymes database (CAZy): an expert resource for Glycogenomics. *Nucleic Acids Res.* 37:D233–38
29. Cavalier DM, Lerouxel O, Neumetzler L, Yamauchi K, Reinecke A, et al. 2008. Disrupting two *Arabidopsis thaliana* xylosyltransferase genes results in plants deficient in xyloglucan, a major primary cell wall component. *Plant Cell* 20:1519–37
30. Chebli Y, Kaneda M, Zerzour R, Geitmann A. 2012. The cell wall of the Arabidopsis pollen tube—spatial distribution, recycling, and network formation of polysaccharides. *Plant Physiol.* 160:1940–55
31. Chen S, Ehrhardt DW, Somerville CR. 2010. Mutations of cellulose synthase (CESA1) phosphorylation sites modulate anisotropic cell expansion and bidirectional mobility of cellulose synthase. *PNAS* 107:17188–93
32. Chevalier L, Bernard S, Ramdani Y, Lamour R, Bardor M, et al. 2010. Subcompartment localization of the side chain xyloglucan-synthesizing enzymes within Golgi stacks of tobacco suspension-cultured cells. *Plant J.* 64:977–89
33. Chou Y-H, Pogorelko G, Young ZT, Zabotina OA. 2014. Protein–protein interactions among xyloglucan-synthesizing enzymes and formation of Golgi-localized multiprotein complexes. *Plant Cell Physiol.* 56:255–67
34. Chung KP, Zeng Y. 2017. An overview of protein secretion in plant cells. *Methods Mol. Biol.* 2017:19–32
35. Claverie J, Balacey S, Lemaître-Guillier C, Brulé D, Chiltz A, et al. 2018. The cell wall-derived xyloglucan is a new DAMP triggering plant immunity in *Vitis vinifera* and *Arabidopsis thaliana*. *Front. Plant Sci.* 9:1725
36. Cosgrove DJ. 2015. Plant expansins: diversity and interactions with plant cell walls. *Curr. Opin. Plant Biol.* 25:162–72

37. Cosgrove DJ. 2018. Diffuse growth of plant cell walls. *Plant Physiol*. 176:16–27
38. Cosgrove DJ. 2018. Nanoscale structure, mechanics and growth of epidermal cell walls. *Curr. Opin. Plant Biol.* 46:77–86
39. Crowell EF, Bischoff V, Desprez T, Rolland A, Stierhof Y-D, et al. 2009. Pausing of Golgi bodies on microtubules regulates secretion of cellulose synthase complexes in *Arabidopsis*. *Plant Cell* 21:1141–54
40. Crowell EF, Gonneau M, Vernhettes S, Höfte H. 2010. Regulation of anisotropic cell expansion in higher plants. *C. R. Biol.* 333:320–24
41. Daher FB, Braybrook SA. 2015. How to let go: pectin and plant cell adhesion. *Front. Plant Sci.* 6:523
42. Davis DJ, Kang B-H, Heringer AS, Wilkop TE, Drakakaki G. 2016. Unconventional protein secretion in plants. *Methods Mol. Biol.* 2016:47–63
43. DeBolt S, Gutierrez R, Ehrhardt DW, Somerville C. 2007. Nonmotile cellulose synthase subunits repeatedly accumulate within localized regions at the plasma membrane in Arabidopsis hypocotyl cells following 2,6-dichlorobenzonitrile treatment. *Plant Physiol*. 145:334–38
44. Decreux A, Messiaen J. 2005. Wall-associated kinase WAK1 interacts with cell wall pectins in a calcium-induced conformation. *Plant Cell Physiol*. 46:268–78
45. Desprez T, Juraniec M, Crowell EF, Jouy H, Pochylova Z, et al. 2007. Organization of cellulose synthase complexes involved in primary cell wall synthesis in *Arabidopsis thaliana*. *PNAS* 104:15572–77
46. Dick-Perez M, Zhang Y, Hayes J, Salazar A, Zabotina OA, Hong M. 2011. Structure and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. *Biochemistry* 50:989–1000
47. Diener AC, Ausubel FM. 2005. *RESISTANCE TO FUSARIUM OXYSPORUM 1*, a dominant Arabidopsis disease-resistance gene, is not race specific. *Genetics* 171:305–21
48. Diotallevi F, Mulder B. 2007. The cellulose synthase complex: a polymerization driven supramolecular motor. *Biophys. J.* 92:2666–73
49. Domozych DS, Sorensen I, Popper ZA, Ochs J, Andreas A, et al. 2014. Pectin metabolism and assembly in the cell wall of the charophyte green alga *Penium margaritaceum*. *Plant Physiol*. 165:105–18
50. Drakakaki G, van de Ven W, Pan S, Miao Y, Wang J, et al. 2012. Isolation and proteomic analysis of the SYP61 compartment reveal its role in exocytic trafficking in *Arabidopsis*. *Cell Res*. 22:413–24
51. Driouich A, Follet-Gueye M-L, Bernard S, Kousar S, Chevalier L, et al. 2012. Golgi-mediated synthesis and secretion of matrix polysaccharides of the primary cell wall of higher plants. *Front. Plant Sci.* 3:79
52. Driouich A, Zhang GF, Staehelin LA. 1993. Effect of brefeldin A on the structure of the Golgi apparatus and on the synthesis and secretion of proteins and polysaccharides in sycamore maple (*Acer pseudoplatanus*) suspension-cultured cells. *Plant Physiol*. 101:1363–73
53. Duan Q, Cheung AY. 2018. Context-specific dependence on FERONIA kinase activity. *FEBS Lett*. 592:2392–94
54. Duan Q, Kita D, Johnson EA, Aggarwal M, Gates L, et al. 2014. Reactive oxygen species mediate pollen tube rupture to release sperm for fertilization in *Arabidopsis*. *Nat. Commun*. 5:3129
55. Duan Q, Kita D, Li C, Cheung AY, Wu HM. 2010. FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *PNAS* 107:17821–26
56. Dünser K, Gupta S, Herger A, Feraru MI, Ringli C, Kleine-Vehn J. 2019. Extracellular matrix sensing by FERONIA and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in *Arabidopsis thaliana*. *EMBO J.* 38:e100353
57. Dupres V, Alsteens D, Wilk S, Hansen B, Heinisch JJ, Dufrêne YF. 2009. The yeast Wsc1 cell surface sensor behaves like a nanospring *in vivo*. *Nat. Chem. Biol*. 5:857–62
58. Ellinger D, Voigt CA. 2014. Callose biosynthesis in Arabidopsis with a focus on pathogen response: what we have learned within the last decade. *Ann. Bot.* 114:1349–58
59. Ellis C, Turner JG. 2001. The Arabidopsis mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell* 13:1025–33
60. Endler A, Kesten C, Schneider R, Zhang Y, Ivakov A, et al. 2015. A mechanism for sustained cellulose synthesis during salt stress. *Cell* 162:1353–64

61. Engelsdorf T, Gigli-Bisceglia N, Veerabagu M, McKenna JF, Vaahtera L, et al. 2018. The plant cell wall integrity maintenance and immune signaling systems cooperate to control stress responses in *Arabidopsis thaliana*. *Sci. Signal*. 11:eaao3070
62. Fagard M, Desnos T, Desprez T, Goubet F, Refregier G, et al. 2000. *PROCUSTE1* encodes a cellulose synthase required for normal cell elongation specifically in roots and dark-grown hypocotyls of Arabidopsis. *Plant Cell* 12:2409–23
63. Feng W, Kita D, Peaucelle A, Cartwright HN, Doan V, et al. 2018. The FERONIA receptor kinase maintains cell-wall integrity during salt stress through Ca^{2+} signaling. *Curr. Biol.* 28:666–75.e5
64. Feng W, Lindner H, Robbins NE II, Dinneny JR. 2016. Growing out of stress: the role of cell- and organ-scale growth control in plant water-stress responses. *Plant Cell* 28:1769–82
65. Fernandes AN, Thomas LH, Altaner CM, Callow P, Forsyth VT, et al. 2011. Nanostructure of cellulose microfibrils in spruce wood. *PNAS* 108:E1195–203
66. Fry SC. 1988. *The Growing Plant Cell Wall: Chemical and Metabolic Analysis*. New York: Longman
67. Fry SC, York WS, Albersheim P, Darvill A, Hayashi T, et al. 1993. An unambiguous nomenclature for xyloglucan-derived oligosaccharides. *Physiol. Plant*. 89:1–3
68. Fujimoto M, Suda Y, Vernhettes S, Nakano A, Ueda T. 2015. Phosphatidylinositol 3-kinase and 4-kinase have distinct roles in intracellular trafficking of cellulose synthase complexes in *Arabidopsis thaliana*. *Plant Cell Physiol*. 56:287–98
69. Gardiner JC. 2003. Control of cellulose synthase complex localization in developing xylem. *Plant Cell* 15:1740–48
70. Ge Z, Bergonci T, Zhao Y, Zou Y, Du S, et al. 2017. *Arabidopsis* pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science* 358:1596–600
71. Gendre D, McFarlane HE, Johnson E, Mouille G, Sjödin A, et al. 2013. *Trans*-Golgi network localized ECHIDNA/Ypt interacting protein complex is required for the secretion of cell wall polysaccharides in *Arabidopsis*. *Plant Cell* 25:2633–46
72. Gigli-Bisceglia N, Engelsdorf T, Hamann T. 2019. Plant cell wall integrity maintenance in model plants and crop species-relevant cell wall components and underlying guiding principles. *Cell. Mol. Life Sci.* 2019:1–29
73. Gigli-Bisceglia N, Engelsdorf T, Strnad M, Vaahtera L, Khan GA, et al. 2018. Cell wall integrity modulates *Arabidopsis thaliana* cell cycle gene expression in a cytokinin- and nitrate reductase-dependent manner. *Development* 145:dev166678
74. Gonneau M, Desprez T, Guillot A, Vernhettes S, Höfte H. 2014. Catalytic subunit stoichiometry within the cellulose synthase complex. *Plant Physiol*. 166:1709–12
75. Gonneau M, Desprez T, Martin M, Doblas VG, Bacete L, et al. 2018. Receptor kinase THESEUS1 is a rapid alkalinization factor 34 receptor in *Arabidopsis*. *Curr. Biol*. 28:2452–58.E4
76. Gorshkova T, Chernova T, Mokshina N, Ageeva M, Mikshina P. 2018. Plant 'muscles': fibers with a tertiary cell wall. *New Phytol*. 218:66–72
77. Grantham NJ, Wurman-Rodrich J, Terrett OM, Lyczakowski JJ, Stott K, et al. 2017. An even pattern of xylan substitution is critical for interaction with cellulose in plant cell walls. *Nat. Plants* 3:859–65
78. Green PB. 1980. Organogenesis—a biophysical view. *Annu. Rev. Plant Physiol.* 31:51–82
79. Gu Y, Kaplinsky N, Bringmann M, Cobb A, Carroll A, et al. 2010. Identification of a cellulose synthase-associated protein required for cellulose biosynthesis. *PNAS* 107:12866–71
80. Gutierrez R, Lindeboom JJ, Paredez AR, Emons AM, Ehrhardt DW. 2009. *Arabidopsis* cortical microtubules position cellulose synthase delivery to the plasma membrane and interact with cellulose synthase trafficking compartments. *Nat. Cell Biol.* 11:797–806
81. Häffner E, Karlovsky P, Splivallo R, Traczewska A, Diederichsen E. 2014. ERECTA, salicylic acid, abscisic acid, and jasmonic acid modulate quantitative disease resistance of *Arabidopsis thaliana* to *Verticillium longisporum*. *BMC Plant Biol*. 14:85
82. Haigler CH, Brown RM. 1986. Transport of rosettes from the Golgi apparatus to the plasma membrane in isolated mesophyll cells of *Zinnia elegans* during differentiation to tracheary elements in suspension culture. *Protoplasma* 134:111–20

83. Halebian M, Morris K, Smith C. 2017. Structure and assembly of clathrin cages. *Subcell Biochem*. 83:551–67
84. Hamann T. 2015. The plant cell wall integrity maintenance mechanism—a case study of a cell wall plasma membrane signaling network. *Phytochemistry* 112:100–9
85. Harpaz-Saad S, McFarlane HE, Xu S, Divi UK, Forward B, et al. 2011. Cellulose synthesis via the FEI2 RLK/SOS5 pathway and CELLULOSE SYNTHASE 5 is required for the structure of seed coat mucilage in Arabidopsis. *Plant J*. 68:941–53
86. Haruta M, Sabat G, Stecker K, Minkoff BB, Sussman MR. 2014. A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science* 343:408–11
87. Hashimoto-Sugimoto M, Higaki T, Yaeno T, Nagami A, Irie M, et al. 2013. A Munc13-like protein in *Arabidopsis* mediates H^+-ATPase translocation that is essential for stomatal responses. *Nat. Commun.* 4:2215
88. Hatfield RD, Rancour DM, Marita JM. 2016. Grass cell walls: a story of cross-linking. *Front. Plant Sci.* 7:2056
89. He M, Lan M, Zhang B, Zhou Y, Wang Y, et al. 2018. Rab-H1b is essential for trafficking of cellulose synthase and for hypocotyl growth in *Arabidopsis thaliana*. *J. Integr. Plant Biol*. 60:1051–69
90. Hematy K, Sado PE, Van Tuinen A, Rochange S, Desnos T, et al. 2007. A receptor-like kinase mediates the response of *Arabidopsis* cells to the inhibition of cellulose synthesis. *Curr. Biol.* 17:922–31
91. Hernández-Blanco C, Feng DX, Hu J, Sánchez-Vallet A, Deslandes L, et al. 2007. Impairment of cellulose synthases required for *Arabidopsis* secondary cell wall formation enhances disease resistance. *Plant Cell* 19:890–903
92. Herth W. 1980. Calcofluor white and Congo red inhibit chitin microfibril assembly of Poterioochromonas: evidence for a gap between polymerization and microfibril formation. *J. Cell Biol*. 87:442–50
93. Hervé C, Marcus SE, Knox JP. 2011. Monoclonal antibodies, carbohydrate-binding modules, and the detection of polysaccharides in plant cell walls. *Methods Mol. Biol.* 2011:103–13
94. Hill JL Jr., Hammudi MB, Tien M. 2014. The Arabidopsis cellulose synthase complex: a proposed hexamer of CESA trimers in an equimolar stoichiometry. *Plant Cell* 26:4834–42
95. Hill JL Jr., Josephs C, Barnes WJ, Anderson CT, Tien M. 2018. Longevity in vivo of primary cell wall cellulose synthases. *Plant Mol. Biol.* 96:279–89
96. Höfte H. 2015. The yin and yang of cell wall integrity control: brassinosteroid and FERONIA signaling. *Plant Cell Physiol*. 56:224–31
97. Hongo S, Sato K, Yokoyama R, Nishitani K. 2012. Demethylesterification of the primary wall by PECTIN METHYLESTERASE35 provides mechanical support to the *Arabidopsis* stem. *Plant Cell* 24:2624–34
98. Humphrey TV, Bonetta DT, Goring DR. 2007. Sentinels at the wall: cell wall receptors and sensors. *New Phytol*. 176:7–21
99. Ito Y, Uemura T, Nakano A. 2014. Formation and maintenance of the Golgi body in plant cells. *Int. Rev. Cell Mol. Biol.* 310:221–87
100. Johnston SL, Prakash R, Chen NJ, Kumagai MH, Turano HM, et al. 2013. An enzyme activity capable of endotransglycosylation of heteroxylan polysaccharides is present in plant primary cell walls. *Planta* 237:173–87
101. Kang B-H, Nielsen E, Preuss ML, Mastronarde D, Staehelin LA. 2011. Electron tomography of RabA4b- and PI-4Kβ1-labeled *trans* Golgi network compartments in *Arabidopsis*. *Traffic* 12:313–29
102. Keegstra K, Talmadge KW, Bauer WD, Albersheim P. 1973. The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. *Plant Physiol*. 51:188–97
103. Kesten C, Wallmann A, Schneider R, McFarlane HE, Diehl A, et al. 2019. The companion of cellulose synthase 1 confers salt tolerance through a Tau-like mechanism in plants. *Nat. Commun.* 10:857
104. Kohorn BD. 2000. Plasma membrane-cell wall contacts. *Plant Physiol*. 124:31–38
105. Kohorn BD. 2016. Cell wall-associated kinases and pectin perception. *J. Exp. Bot.* 67:489–94

106. Kohorn BD, Johansen S, Shishido A, Todorova T, Martinez R, et al. 2009. Pectin activation of MAP kinase and gene expression is WAK2 dependent. *Plant J.* 60:974–82
107. Kubicki JD, Yang H, Sawada D, O'Neill H, Oehme D, Cosgrove D. 2018. The shape of native plant cellulose microfibrils. *Sci. Rep.* 8:13983
108. Kumar M, Wightman R, Atanassov I, Gupta A, Hurst CH, et al. 2016. S-Acylation of the cellulose synthase complex is essential for its plasma membrane localization. *Science* 353:166–69
109. Kuramae R, Saito T, Isogai A. 2014. TEMPO-oxidized cellulose nanofibrils prepared from various plant holocelluloses. *React. Funct. Polym.* 85:126–33
110. Lang I, Barton DA, Overall RL. 2004. Membrane-wall attachments in plasmolysed plant cells. *Protoplasma* 224:231–43
111. Lei L, Singh A, Bashline L, Li S, Yingling YG, Gu Y. 2015. CELLULOSE SYNTHASE INTERACTIVE1 is required for fast recycling of cellulose synthase complexes to the plasma membrane in Arabidopsis. *Plant Cell* 27:2926–40
112. Levin DE. 2011. Regulation of cell wall biogenesis in *Saccharomyces cerevisiae*: the cell wall integrity signaling pathway. *Genetics* 189:1145–75
113. Li C, Wu H-M, Cheung AY. 2016. FERONIA and her pals: functions and mechanisms. *Plant Physiol.* 171:2379–92
114. Li C, Yeh F-L, Cheung AY, Duan Q, Kita D, et al. 2015. Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. *eLife* 4:e06587
115. Li S, Lei L, Somerville CR, Gu Y. 2012. Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. *PNAS* 109:185–90
116. Lund CH, Bromley JR, Stenbæk A, Rasmussen RE, Scheller HV, Sakuragi Y. 2015. A reversible *Renilla* luciferase protein complementation assay for rapid identification of protein–protein interactions reveals the existence of an interaction network involved in xyloglucan biosynthesis in the plant Golgi apparatus. *J. Exp. Bot.* 66:85–97
117. Lunn D, Gaddipati SR, Tucker GA, Lycett GW. 2013. Null mutants of individual RABA genes impact the proportion of different cell wall components in stem tissue of *Arabidopsis thaliana*. *PLOS ONE* 8:e75724
118. Luo Y, Scholl S, Doering A, Zhang Y, Irani NG, et al. 2015. V-ATPase activity in the TGN/EE is required for exocytosis and recycling in *Arabidopsis*. *Nat. Plants* 1:15094
119. Malinovsky FG, Fangel JU, Willats WGT. 2014. The role of the cell wall in plant immunity. *Front. Plant Sci.* 5:178
120. Mansoori N, Timmers J, Desprez T, Alvim-Kamei CL, Dees DC, et al. 2014. KORRIGAN1 interacts specifically with integral components of the cellulose synthase machinery. *PLOS ONE* 9:e112387
121. McFarlane HE, Watanabe Y, Gendre D, Carruthers K, Levesque-Tremblay G, et al. 2013. Cell wall polysaccharides are mislocalized to the vacuole in *echidna* mutants. *Plant Cell Physiol.* 54:1867–80
122. Meents MJ, Watanabe Y, Samuels AL. 2018. The cell biology of secondary cell wall biosynthesis. *Ann. Bot.* 121:1107–25
123. Mendu V, Griffiths JS, Persson S, Stork J, Downie AB, et al. 2011. Subfunctionalization of cellulose synthases in seed coat epidermal cells mediates secondary radial wall synthesis and mucilage attachment. *Plant Physiol.* 157:441–53
124. Merz D, Richter J, Gonneau M, Sanchez-Rodriguez C, Eder T, et al. 2017. T-DNA alleles of the receptor kinase THESEUS1 with opposing effects on cell wall integrity signaling. *J. Exp. Bot.* 68:4583–93
125. Mettlen M, Chen P-H, Srinivasan S, Danuser G, Schmid SL. 2018. Regulation of clathrin-mediated endocytosis. *Annu. Rev. Biochem.* 87:871–96
126. Miart F, Desprez T, Biot E, Morin H, Belcram K, et al. 2014. Spatio-temporal analysis of cellulose synthesis during cell plate formation in Arabidopsis. *Plant J.* 77:71–84
127. Mohnen D. 2008. Pectin structure and biosynthesis. *Curr. Opin. Plant Biol.* 11:266–77
128. Morgan JLW, Strumillo J, Zimmer J. 2013. Crystallographic snapshot of cellulose synthesis and membrane translocation. *Nature* 493:181–86
129. Mutwil M, Debolt S, Persson S. 2008. Cellulose synthesis: a complex complex. *Curr. Opin. Plant Biol.* 11:252–57

130. Ndeh D, Rogowski A, Cartmell A, Luis AS, Baslé A, et al. 2017. Complex pectin metabolism by gut bacteria reveals novel catalytic functions. *Nature* 544:65–70
131. Nebenführ A, Gallagher LA, Dunahay TG, Frohlick JA, Mazurkiewicz AM, et al. 1999. Stop-and-go movements of plant Golgi stacks are mediated by the acto-myosin system. *Plant Physiol.* 121:1127–41
132. Newman RH, Hill SJ, Harris PJ. 2013. Wide-angle x-ray scattering and solid-state nuclear magnetic resonance data combined to test models for cellulose microfibrils in mung bean cell walls. *Plant Physiol.* 163:1558–67
133. Ng F, Tang BL. 2016. Unconventional protein secretion in animal cells. *Methods Mol. Biol.* 2016:31–46
134. Nissen KS, Willats WGT, Malinovsky FG. 2016. Understanding CrRLK1L function: cell walls and growth control. *Trends Plant Sci.* 21:516–27
135. Nixon BT, Mansouri K, Singh A, Du J, Davis JK, et al. 2016. Comparative structural and computational analysis supports eighteen cellulose synthases in the plant cellulose synthesis complex. *Sci. Rep.* 6:28696
136. Oikawa A, Lund CH, Sakuragi Y, Scheller HV. 2013. Golgi-localized enzyme complexes for plant cell wall biosynthesis. *Trends Plant Sci.* 18:49–58
137. Okekeogbu IO, Pattathil S, Gonzalez Fernandez-Nino SM, Aryal UK, Penning BW, et al. 2019. Glycome and proteome components of Golgi membranes are common between two angiosperms with distinct cell-wall structures. *Plant Cell* 31:1094–112
138. Paez Valencia J, Goodman K, Otegui MS. 2016. Endocytosis and endosomal trafficking in plants. *Annu. Rev. Plant Biol.* 67:309–35
139. Paredez AR, Somerville CR, Ehrhardt DW. 2006. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* 312:1491–95
140. Park YB, Cosgrove DJ. 2012. Changes in cell wall biomechanical properties in the xyloglucan-deficient *xxt1/xxt2* mutant of Arabidopsis. *Plant Physiol.* 158:465–75
141. Peaucelle A, Wightman R, Höfte H. 2015. The control of growth symmetry breaking in the *Arabidopsis* hypocotyl. *Curr. Biol.* 25:1746–52
142. Pelletier S, Van Orden J, Wolf S, Vissenberg K, Delacourt J, et al. 2010. A role for pectin de-methylesterification in a developmentally regulated growth acceleration in dark-grown Arabidopsis hypocotyls. *New Phytol.* 188:726–39
143. Persson S, Paredez A, Carroll A, Palsdottir H, Doblin M, et al. 2007. Genetic evidence for three unique components in primary cell-wall cellulose synthase complexes in *Arabidopsis. PNAS* 104:15566–71
144. Phyo P, Wang T, Xiao C, Anderson CT, Hong M. 2017. Effects of pectin molecular weight changes on the structure, dynamics, and polysaccharide interactions of primary cell walls of *Arabidopsis thaliana*: insights from solid-state NMR. *Biomacromolecules* 18:2937–50
145. Polko JK, Barnes WJ, Voiniciuc C, Doctor S, Steinwand B, et al. 2018. SHOU4 proteins regulate trafficking of cellulose synthase complexes to the plasma membrane. *Curr. Biol.* 28:3174–82
146. Polko JK, Kieber JJ. 2019. The regulation of cellulose biosynthesis in plants. *Plant Cell* 31:282–96
147. Popper ZA, Fry SC. 2005. Widespread occurrence of a covalent linkage between xyloglucan and acidic polysaccharides in suspension-cultured angiosperm cells. *Ann. Bot.* 96:91–99
148. Popper ZA, Fry SC. 2008. Xyloglucan-pectin linkages are formed intra-protoplasmically, contribute to wall-assembly, and remain stable in the cell wall. *Planta* 227:781–94
149. Popper ZA, Michel G, Herve C, Domozych DS, Willats WG, et al. 2011. Evolution and diversity of plant cell walls: from algae to flowering plants. *Annu. Rev. Plant Biol.* 62:567–90
150. Qu S, Zhang X, Song Y, Lin J, Shan X. 2017. THESEUS1 positively modulates plant defense responses against *Botrytis cinerea* through GUANINE EXCHANGE FACTOR4 signaling. *J. Integr. Plant Biol.* 59:797–804
151. Rabouille C. 2017. Pathways of unconventional protein secretion. *Trends Cell Biol.* 27:230–40
152. Raman R, Venkataraman M, Ramakrishnan S, Lang W, Raguram S, Sasisekharan R. 2006. Advancing glycomics: implementation strategies at the consortium for functional glycomics. *Glycobiology* 16:82R–90R
153. Refregier G, Pelletier S, Jaillard D, Höfte H. 2004. Interaction between wall deposition and cell elongation in dark-grown hypocotyl cells in Arabidopsis. *Plant Physiol.* 135:959–68
154. Rennie EA, Scheller HV. 2014. Xylan biosynthesis. *Curr. Opin. Biotechnol.* 26:100–7

155. Robinson DG, Ding Y, Jiang L. 2016. Unconventional protein secretion in plants: a critical assessment. *Protoplasma* 253:31–43
156. Rongpipi S, Ye D, Gomez ED, Gomez EW. 2018. Progress and opportunities in the characterization of cellulose—an important regulator of cell wall growth and mechanics. *Front. Plant Sci.* 9:1894
157. Roudier F, Schindelman G, DeSalle R, Benfey PN. 2002. The COBRA family of putative GPI-anchored proteins in Arabidopsis. A new fellowship in expansion. *Plant Physiol.* 130:538–48
158. Rounds CM, Lubeck E, Hepler PK, Winship LJ. 2011. Propidium iodide competes with Ca^{2+} to label pectin in pollen tubes and Arabidopsis root hairs. *Plant Physiol.* 157:175–87
159. Rudolph U. 1987. Occurrence of rosettes in the ER membrane of young *Funaria hygrometrica* protonemata. *Naturwissenschaften* 74:439
160. Rui Y, Xiao C, Yi H, Kandemir B, Wang JZ, et al. 2017. POLYGALACTURONASE INVOLVED IN EXPANSION3 functions in seedling development, rosette growth, and stomatal dynamics in *Arabidopsis thaliana*. *Plant Cell* 29:2413–32
161. Saez-Aguayo S, Ralet MC, Berger A, Botran L, Ropartz D, et al. 2013. PECTIN METHYLESTERASE INHIBITOR6 promotes *Arabidopsis* mucilage release by limiting methylesterification of homogalacturonan in seed coat epidermal cells. *Plant Cell* 25:308–23
162. Sampathkumar A, Gutierrez R, McFarlane HE, Bringmann M, Lindeboom J, et al. 2013. Patterning and lifetime of plasma membrane-localized cellulose synthase is dependent on actin organization in Arabidopsis interphase cells. *Plant Physiol.* 162:675–88
163. Samuels AL, Giddings TH Jr., Staehelin LA. 1995. Cytokinesis in tobacco BY-2 and root tip cells: a new model of cell plate formation in higher plants. *J. Cell Biol.* 130:1345–57
164. Samuels AL, Rensing K, Douglas C, Mansfield S, Dharmawardhana D, Ellis B. 2002. Cellular machinery of wood production: differentiation of secondary xylem in *Pinus contorta* var. latifolia. *Planta* 216:72–82
165. Sanchez-Rodriguez C, Ketelaar K, Schneider R, Villalobos JA, Somerville CR, et al. 2017. BRASSINOSTEROID INSENSITIVE2 negatively regulates cellulose synthesis in *Arabidopsis* by phosphorylating cellulose synthase 1. *PNAS* 114:3533–38
166. Sanchez-Rodriguez C, Shi Y, Kesten C, Zhang D, Sancho-Andres G, et al. 2018. The cellulose synthases are cargo of the TPLATE adaptor complex. *Mol. Plant* 11:346–49
167. Schallus T, Jaeckh C, Fehér K, Palma AS, Liu Y, et al. 2008. Malectin: a novel carbohydrate-binding protein of the endoplasmic reticulum and a candidate player in the early steps of protein *N*-glycosylation. *Mol. Biol. Cell* 19:3404–14
168. Scheible W-R, Pauly M. 2004. Glycosyltransferases and cell wall biosynthesis: novel players and insights. *Curr. Opin. Plant Biol.* 7:285–95
169. Scheible W-R, Eshed R, Richmond T, Delmer D, Somerville C. 2001. Modifications of cellulose synthase confer resistance to isoxaben and thiazolidinone herbicides in *Arabidopsis ixr1* mutants. *PNAS* 98:10079–84
170. Scheller HV, Ulvskov P. 2010. Hemicelluloses. *Annu. Rev. Plant Biol.* 61:263–89
171. Schindelman G, Morikami A, Jung J, Baskin TI, Carpita NC, et al. 2001. COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in *Arabidopsis*. *Genes Dev.* 15:1115–27
172. Schuetz M, Benske A, Smith RA, Watanabe Y, Tobimatsu Y, et al. 2014. Laccases direct lignification in the discrete secondary cell wall domains of protoxylem. *Plant Physiol.* 166:798–807
173. Seifert GJ. 1994. Nucleotide sugar interconversions and cell wall biosynthesis: how to bring the inside to the outside. *Curr. Opin. Plant Biol.* 7:277–84
174. Senechal F, Graff L, Surcouf O, Marcelo P, Rayon C, et al. 2014. Arabidopsis PECTIN METHYLESTERASE17 is co-expressed with and processed by SBT3.5, a subtilisin-like serine protease. *Ann. Bot.* 114:1161–75
175. Shedletzky E, Shmuel M, Delmer DP, Lamport DTA. 1990. Adaptation and growth of tomato cells on the herbicide 2,6-dichlorobenzonitrile leads to production of unique cell walls virtually lacking a cellulose-xyloglucan network. *Plant Physiol.* 94:980–87
176. Sherrier DJ, Vandenbosch KA. 1994. Secretion of cell wall polysaccharides in *Vicia* root hairs. *Plant J.* 5:185–95

177. Shinohara N, Sunagawa N, Tamura S, Yokoyama R, Ueda M, et al. 2017. The plant cell-wall enzyme AtXTH3 catalyses covalent cross-linking between cellulose and cello-oligosaccharide. *Sci. Rep.* 7:46099
178. Showalter AM, Basu D. 2016. Extensin and arabinogalactan-protein biosynthesis: glycosyltransferases, research challenges, and biosensors. *Front. Plant Sci.* 7:814
179. Sinclair R, Rosquete MR, Drakakaki G. 2018. Post-Golgi trafficking and transport of cell wall components. *Front. Plant Sci.* 9:1784
180. Somerville C. 2006. Cellulose synthesis in higher plants. *Annu. Rev. Cell Dev. Biol.* 22:53–78
181. Speicher TL, Li PZ, Wallace IS. 2018. Phosphoregulation of the plant cellulose synthase complex and cellulose synthase-like proteins. *Plants* 7:52
182. Staehelin LA, Giddings TH Jr., Kiss JZ, Sack FD. 1990. Macromolecular differentiation of Golgi stacks in root tips of *Arabidopsis* and *Nicotiana* seedlings as visualized in high pressure frozen and freeze-substituted samples. *Protoplasma* 157:75–91
183. Staehelin LA, Kang BH. 2008. Nanoscale architecture of endoplasmic reticulum export sites and of Golgi membranes as determined by electron tomography. *Plant Physiol.* 147:1454–68
184. Steinwand BJ, Kieber JJ. 2010. The role of receptor-like kinases in regulating cell wall function. *Plant Physiol.* 153:479–84
185. Steinwand BJ, Xu S, Polko JK, Doctor SM, Westafer M, Kieber JJ. 2014. Alterations in auxin homeostasis suppress defects in cell wall function. *PLOS ONE* 9:e98193
186. Taiz L. 1984. Plant cell expansion: regulation of cell wall mechanical properties. *Annu. Rev. Plant Physiol.* 35:585–657
187. Tan L, Eberhard S, Pattathil S, Warder C, Glushka J, et al. 2013. An *Arabidopsis* cell wall proteoglycan consists of pectin and arabinoxylan covalently linked to an arabinogalactan protein. *Plant Cell* 25:270–87
188. Taylor NG. 2007. Identification of cellulose synthase AtCesA7 (IRX3) in vivo phosphorylation sites—a potential role in regulating protein degradation. *Plant Mol. Biol.* 64:161–71
189. Taylor NG, Howells RM, Huttly AK, Vickers K, Turner SR. 2003. Interactions among three distinct CesA proteins essential for cellulose synthesis. *PNAS* 100:1450–55
190. Taylor NG, Laurie S, Turner SR. 2000. Multiple cellulose synthase catalytic subunits are required for cellulose synthesis in Arabidopsis. *Plant Cell* 12:2529–39
191. Thomas LH, Forsyth VT, Martel A, Grillo I, Altaner CM, Jarvis MC. 2015. Diffraction evidence for the structure of cellulose microfibrils in bamboo, a model for grass and cereal celluloses. *BMC Plant Biol.* 15:153
192. Thomas LH, Forsyth VT, Sturcova A, Kennedy CJ, May RP, et al. 2013. Structure of cellulose microfibrils in primary cell walls from collenchyma. *Plant Physiol.* 161:465–76
193. Thompson JE, Fry SC. 2000. Evidence for covalent linkage between xyloglucan and acidic pectins in suspension-cultured rose cells. *Planta* 211:275–86
194. Toyooka K, Goto Y, Asatsuma S, Koizumi M, Mitsui T, Matsuoka K. 2009. A mobile secretory vesicle cluster involved in mass transport from the Golgi to the plant cell exterior. *Plant Cell* 21:1212–29
195. Tsang DL, Edmond C, Harrington JL, Nühse TS. 2011. Cell wall integrity controls root elongation via a general 1-aminocyclopropane-1-carboxylic acid-dependent, ethylene-independent pathway. *Plant Physiol.* 156:596–604
196. Vain T, Crowell EF, Timpano H, Biot E, Desprez T, et al. 2014. The cellulase KORRIGAN is part of the cellulose synthase complex. *Plant Physiol.* 165:1521–32
197. van de Meene AML, Doblin MS, Bacic A. 2017. The plant secretory pathway seen through the lens of the cell wall. *Protoplasma* 254:75–94
198. Van der Does D, Boutrot F, Engelsdorf T, Rhodes J, McKenna JF, et al. 2017. The Arabidopsis leucine-rich repeat receptor kinase MIK2/LRR-KISS connects cell wall integrity sensing, root growth and response to abiotic and biotic stresses. *PLOS Genet.* 13:e1006832
199. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W. 2010. Lignin biosynthesis and structure. *Plant Physiol.* 153:895–905
200. Verhertbruggen Y, Marcus SE, Chen J, Knox JP. 2013. Cell wall pectic arabinans influence the mechanical properties of *Arabidopsis thaliana* inflorescence stems and their response to mechanical stress. *Plant Cell Physiol.* 54:1278–88

201. Viotti C. 2016. ER to Golgi-dependent protein secretion: the conventional pathway. *Methods Mol. Biol.* 2016:3–29
202. Vogel J. 2008. Unique aspects of the grass cell wall. *Curr. Opin. Plant Biol.* 11:301–7
203. Voiniciuc C, Pauly M, Usadel B. 2018. Monitoring polysaccharide dynamics in the plant cell wall. *Plant Physiol.* 176:2590–600
204. Voxeur A, Höfte H. 2016. Cell wall integrity signaling in plants: "To grow or not to grow that's the question." *Glycobiology* 26:950–60
205. Wang P, Chen X, Goldbeck C, Chung E, Kang B-H. 2017. A distinct class of vesicles derived from the trans-Golgi mediates secretion of xylogalacturonan in the root border cell. *Plant J.* 92:596–610
206. Wang T, Park YB, Caporini MA, Rosay M, Zhong L, et al. 2013. Sensitivity-enhanced solid-state NMR detection of expansin's target in plant cell walls. *PNAS* 110:16444–49
207. Wang X, Chung KP, Lin W, Jiang L. 2017. Protein secretion in plants: conventional and unconventional pathways and new techniques. *J. Exp. Bot.* 69:21–37
208. Wightman R, Turner S. 2010. Trafficking of the cellulose synthase complex in developing xylem vessels. *Biochem. Soc. Trans.* 38:755–60
209. Wilkop T, Pattathil S, Ren G, Davis DJ, Bao W, et al. 2019. A hybrid approach enabling large-scale glycomic analysis of post-Golgi vesicles reveals a transport route for polysaccharides. *Plant Cell* 31:627–44
210. Wolf S. 2017. Plant cell wall signalling and receptor-like kinases. *Biochem. J.* 474:471–92
211. Wolf S, Hematy K, Höfte H. 2012. Growth control and cell wall signaling in plants. *Annu. Rev. Plant Biol.* 63:381–407
212. Wolf S, Höfte H. 2014. Growth control: a saga of cell walls, ROS, and peptide receptors. *Plant Cell* 26:1848–56
213. Wormit A, Usadel B. 2018. The multifaceted role of pectin methylesterase inhibitors (PMEIs). *Int. J. Mol. Sci.* 19:2878
214. Wu B, Guo W. 2015. The exocyst at a glance. *J. Cell Sci.* 128:2957–64
215. Xiao C, Barnes WJ, Zamil MS, Yi H, Puri VM, Anderson CT. 2017. Activation tagging of *Arabidopsis* POLYGALACTURONASE INVOLVED IN EXPANSION2 promotes hypocotyl elongation, leaf expansion, stem lignification, mechanical stiffening, and lodging. *Plant J.* 89:1159–73
216. Xiao C, Somerville C, Anderson CT. 2014. POLYGALACTURONASE INVOLVED IN EXPANSION1 functions in cell elongation and flower development in *Arabidopsis*. *Plant Cell* 26:1018–35
217. Xiao C, Zhang T, Zheng Y, Cosgrove DJ, Anderson CT. 2016. Xyloglucan deficiency disrupts microtubule stability and cellulose biosynthesis in Arabidopsis, altering cell growth and morphogenesis. *Plant Physiol.* 170:234–49
218. Xu SL, Rahman A, Baskin TI, Kieber JJ. 2008. Two leucine-rich repeat receptor kinases mediate signaling, linking cell wall biosynthesis and ACC synthase in *Arabidopsis*. *Plant Cell* 20:3065–79
219. Xu Y, Sechet J, Wu Y, Fu Y, Zhu L, et al. 2018. Rice sucrose partitioning mediated by a putative pectin methyltransferase and homogalacturonan methylesterification. *Plant Physiol.* 174:1595–608
220. Young WW. 2004. Organization of Golgi glycosyltransferases in membranes: complexity via complexes. *J. Membr. Biol.* 198:1–13
221. Zabotina OA. 2012. Xyloglucan and its biosynthesis. *Front. Plant Sci.* 3:134
222. Zarsky V, Kulich I, Fendrych M, Pecenkova T. 2013. Exocyst complexes multiple functions in plant cells secretory pathways. *Curr. Opin. Plant Biol.* 16:726–33
223. Zhang GF, Driouich A, Staehelin LA. 1996. Monensin-induced redistribution of enzymes and products from Golgi stacks to swollen vesicles. *Eur. J. Cell Biol.* 71:332–40
224. Zhang GF, Staehelin LA. 1992. Functional compartmentation of the Golgi apparatus of plant cells: immunocytochemical analysis of high-pressure frozen- and freeze-substituted sycamore maple suspension culture cells. *Plant Physiol.* 99:1070–83
225. Zhang T, Zheng Y, Cosgrove DJ. 2016. Spatial organization of cellulose microfibrils and matrix polysaccharides in primary plant cell walls as imaged by multichannel atomic force microscopy. *Plant J.* 85:179–92

226. Zhang Y, Nikolovski N, Sorieul M, Vellosillo T, McFarlane HE, et al. 2016. Golgi-localized STELLO proteins regulate the assembly and trafficking of cellulose synthase complexes in *Arabidopsis*. *Nat. Commun.* 7:11656
227. Zhu C, Ganguly A, Baskin TI, McClosky DD, Anderson CT, et al. 2015. The Fragile Fiber1 kinesin contributes to cortical microtubule-mediated trafficking of cell wall components. *Plant Physiol.* 167:780–92
228. Zhu X, Li S, Pan S, Xin X, Gu Y. 2018. CSI1, PATROL1, and exocyst complex cooperate in delivery of cellulose synthase complexes to the plasma membrane. *PNAS* 115:E3578–87
229. Zykwinska AW, Ralet MC, Garnier CD, Thibault JF. 2005. Evidence for in vitro binding of pectin side chains to cellulose. *Plant Physiol.* 139:397–407