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Invited Expert Review

Targeting and Regulation of Cell Wall Synthesis During Tip Growth in Plants[F]

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Abstract

Root hairs and pollen tubes are formed through tip growth, a process requiring synthesis of new cell wall material and the precise targeting and integration of these components to a selected apical plasma membrane domain in the growing tips of these cells. Presence of a tip-focused calcium gradient, control of actin cytoskeleton dynamics, and formation and targeting of secretory vesicles are essential to tip growth. Similar to cells undergoing diffuse growth, cellulose, hemicelluloses, and pectins are also deposited in the growing apices of tip-growing cells. However, differences in the manner in which these cell wall components are targeted and inserted in the expanding portion of tip-growing cells is reflected by the identification of elements of the plant cell wall synthesis machinery which have been shown to play unique roles in tip-growing cells. In this review, we summarize our current understanding of the tip growth process, with a particular focus on the subcellular targeting of newly synthesized cell wall components, and their roles in this form of plant cell expansion.

Keywords: Cellulose; membrane trafficking; plant cell wall; pollen tube; root hair; tip growth.

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Introduction

Plant cells are surrounded by a rigid extracellular matrix, the plant cell wall, a unique structure that not only defines cell shape but also provides sufficient tensile strength to support plant tissues and organs. During cell growth, cellular expansion is determined by the targeted deposition of newly synthesized cell wall components, which is thought to occur in one of two distinct, generalized mechanisms. During diffuse growth, new cell wall components are uniformly deposited along one or more entire cell surfaces (Nielsen 2009). However, in tip growth, new cell wall component secretion occurs in a strictly controlled fashion, and deposition of these components occurs in a limited region of the cell surface (Carol and Dolan 2002; Cosgrove 2005; Cheung and Wu 2008; Lee and Yang 2008; Nielsen 2009).

Root hairs and pollen tubes are two well-characterized tip growth models in *Arabidopsis*. Root hairs are long tubular structures that extend from the basal ends of epidermal cells and can achieve lengths of 1 mm before growth stops (Carol and Dolan 2006). The growth and elongation of the tubular hair element in these cells occurs primarily by tip-restricted expansion (Tominaga-Wada et al. 2011). Root hairs function in water and nutrient uptake and participate in nodule organogenesis in legumes (Patriarca et al. 2004; Tominaga-Wada et al. 2011). However, in laboratory conditions, root hairs appear to be dispensable for *Arabidopsis* growth and reproduction. This has resulted in the identification of a large set of *Arabidopsis* root hair mutants in which normal tip growth has been abolished or altered (Schiefelbein and Somerville 1990; Parker et al. 2000). Pollen tubes germinate from pollen grains and in response to directional cues, traverse floral tissues to fertilize the female gametophyte

(Cheung and Wu 2008). Both root hairs and pollen tubes display rapid, highly polarized growth that can be easily established in liquid culture medium conditions and measured using time-lapse light microscopy, making root hairs and pollen tubes ideal tools for studying tip growth and the associated polarized membrane trafficking events that support this mode of plant cell expansion. In this review, we focus on some recent advances in our understanding of how this polarized cell expansion process is organized, and the mechanisms by which primary cell wall deposition occurs during tip growth.

Subcellular Organization of Tip Growth in Plant Cells

In *Arabidopsis*, root epidermal cells differentiate into two distinct cell types, root hair forming cells called trichoblasts, or non-hair forming cells called atrichoblasts (Tominaga-Wada et al. 2011; Grebe 2012). Trichoblast cell fate is determined by its relative position to the underlying cortex cells, and our current understanding of this process was recently reviewed elsewhere (Grebe 2012; Ryu et al. 2013). In both pollen and root hairs, initiation of tip growth is accompanied by accumulation of ROP GTPases in a plasma membrane domain that will become the future apex of the growing pollen tube or root hair (Fu et al. 2001; Molendijk et al. 2001; Jones et al. 2002). In the case of root hairs, ROP2 (At1g20090. Full list of the names and AGI numbers of the genes introduced in this review can be found in **Table 1**) triggers the formation of reactive oxygen species (ROS) in a process requiring the presence of RHD2 (At5g51060), which encodes an nicotinamide adenine dinucleotide phosphate oxidase (Foreman et al. 2003; Jones et al. 2007). ROS accumulation in the tips of growing root hairs ultimately results in activation of calcium channels in these cells, and in turn the influx of Ca^{2+} further activates RHD2 which produces more ROS (Takeda et al. 2008). A tip-focused calcium gradient is then thought to be formed through this positive feedback and the cytosolic Ca^{2+} concentration reaches approximately 1 μM at the tip and 100–200 nM in the rest of the cell (Wymer et al. 1997; Foreman et al. 2003; Carol and Dolan 2006; Lee and Yang 2008). This tip-focused Ca^{2+} gradient is maintained as long as tip growth occurs in these cells, and disappears when these cells stop growing (Wymer et al. 1997).

In addition to tip-focused Ca^{2+} gradients, cytoskeleton dynamics play a particularly prominent role in the organization of the subapical cytoplasmic region in tip-growing cells (**Figure 1**), and intact F-actin networks are essential for the maintenance of tip growth in both pollen tubes and root hairs (Carol and Dolan 2002; Lee and Yang 2008). While microtubules are essential for determining the polarity of root hairs, vesicle delivery to the apical region was not blocked by depolymerization of microtubules, indicating they are not required for tip growth (Van Bruaene et al. 2004; Preuss et al. 2004). F-actin accumulates in the tips of newly initiated root hair bulges, but treatment with latrunculin B, which interferes with F-actin polymerization, did not block this process, indicating selection of sites for future root hair tip growth occurs in an actin independent process (Baluska et al. 2000). Once tip-restricted elongation of the root hair is initiated, secretory vesicles containing newly synthesized cell wall components are transported to the sub-apical region along actin bundles (Pei et al. 2012). Disruption of actin polymerization by treatment with latrunculin B blocks root hair and pollen tube growth (Gibbon et al. 1999; Baluska et al. 2000). At the tip region, the high calcium concentration increases the actin turnover rate and faciliates fusion of these vesicles with the the apical plasma membrane and the subsequent delivery of cell wall cargo to the expanding root hair cell wall (**Figure 1**; Pei et al. 2012). In pollen tubes, initiation of F-actin polymerization requires the

Table 1. *Arabidopsis thaliana* Gene Index (AGI) number for the genes mentioned in this review

Gene	AGI no.	Gene	AGI no.	Gene	AGI no.	Gene	AGI no.
AFH1	At3g25500	*DRP1C*	At1g14830	*P4H2*	At3g06300	*RHD4*	At3g51460
AFH3	At4g15200	*EXPA7*	At1g12560	*P4H5*	At2g17720	*RHS8/XUT1*	At1g63450
AFH8	At1g70140	*EXPA18*	At1g62980	*P4H13*	At2g23096	*RIC3*	At1g04450
AFH13	At5g58160	*FER*	At3g51550	*PI4Kβ1*	At5g64070	*RIC4*	At5g16490
AFH19	At5g07780	*GAE6*	At3g23820	*PIP5K3*	At2g26420	*ROP GEF1*	At4g38430
ANX1	At3g04690	*GAUT1*	At3g61130	*PIP5K4*	At3g56960	*ROP1*	At3g51300
CESA3	At5g05170	*GH9C1*	At1g48930	*PPME1*	At1g69940	*ROP2*	At1g20090
CESA6	At5g64740	*HvEXPB1*	AY351785	*RAB A4B*	At4g39990	*THE1*	At5g54380
CSLD1	At2g33100	*KOR1*	At5g49720	*RAB A4D*	At3g12160	*UER1*	At1g63000
CSLD2	At5g16910	*KOR2*	At1g65610	*RAB B1C*	At4g17170	*XXT1*	At3g62720
CSLD3	At3g03050	*KOR3*	At4g24260	*RGXT1*	At4g01770	*XXT2*	At4g02500
CSLD4	At4g38190	*LRX3*	At4g13340	*RGXT2*	At4g01750	*XXT5*	At1g74380
CSLD5	At1g02730	*OsEXPB5*	Os04g46650	*RHD2*	At5g51060		

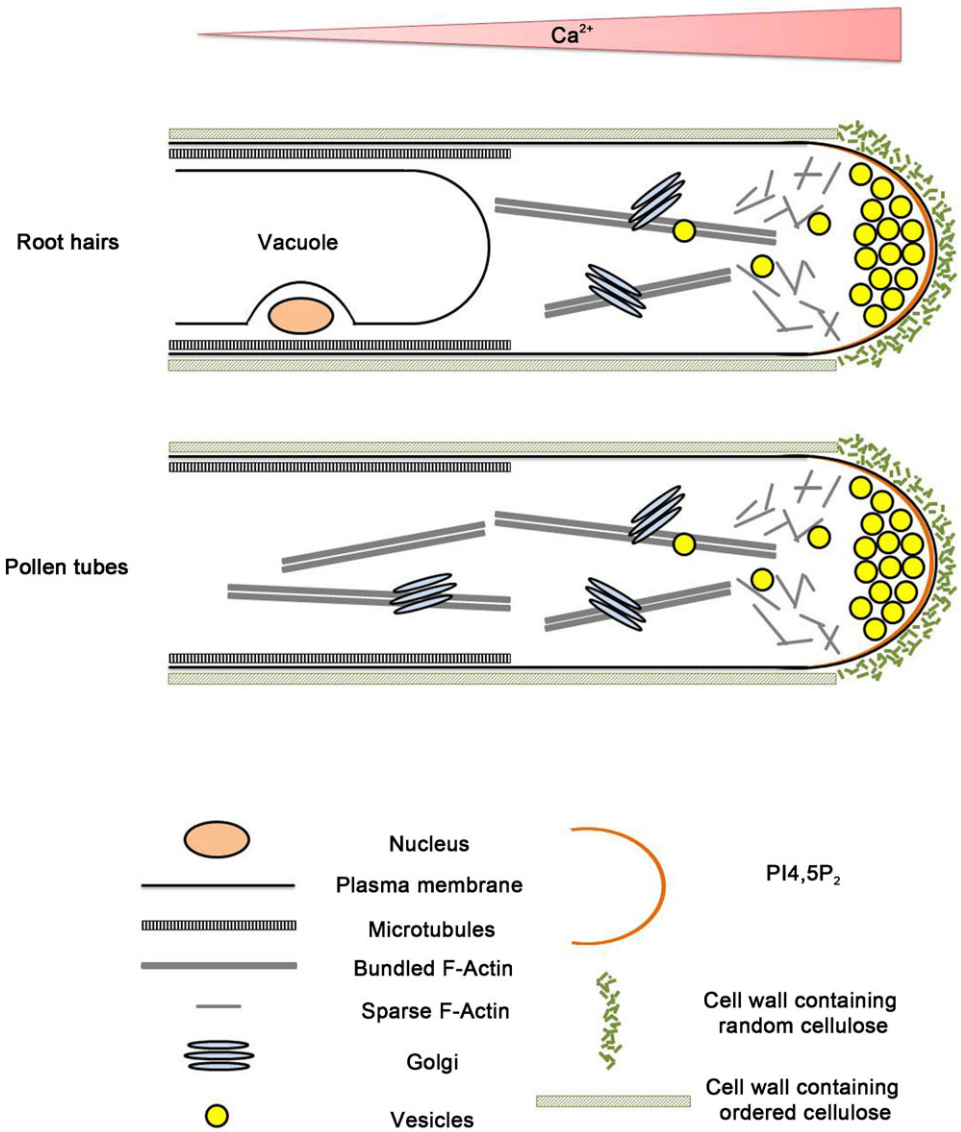

Figure 1. A schematic model of root hairs and pollen tubes.

The concentration of Ca^{2+} is higher in the tip and $PI4,5P_2$ is enriched at the apical plasma membrane. Vesicles are transported from the trans-Golgi network and the trafficking requires the involvement of F-actins. These vesicles accumulate in the apical region, which is also designated the vesicle rich zone. Cell walls deposited in the tips of root hairs typically contain short, randomly oriented cellulose microfibrils. A second layer of cell wall is deposited behind the actively growing tip, and the orientation of the cellulose is helical or longitudinal along the length of root hairs and pollen tubes, respectively.

involvement of several nucleating factors, including formins (Cheung and Wu 2004; Ye et al. 2009; Cheung and Niroomand 2010). There are 21 formins found in *Arabidopsis* and 11 of these are classified as class I formins due to the presence of membrane insertion signals at the N-terminus (Cvrcková et al. 2004). Overexpression of *Arabidopsis* class I formins AFH1 (At3g25500), AFH3 (At4g15200), or AFH8 (At1g70140) stimulates the formation of F-actin and causes pollen tubes or root hairs to swell (Cheung and Wu 2004; Yi et al. 2005; Ye et al. 2009). Much less is known about the function of class II formins in tip-growing cells, although one class II formin, AFH13 (At5g58160), appears to be expressed selectively in pollen (Pina et al. 2005). Additionally, the class II formin AFH19 (At5g07780), appears to act in opposition to that of AFH1 (Zheng et al. 2012), although whether this antagonism plays important roles in tip-growing cells remains unclear. F-actin

dynamics are also controlled by a pollen tube tip-localized ROP GTPase, ROP1 (At3g51300), and its two target proteins, RIC3 (At1g04450) and RIC4 (At5g16490) (Gu et al. 2005). These two RIC proteins are thought to play antagonistic roles, with RIC3 disassembling F-actin cables, and RIC4 stablizing them (Gu et al. 2005). The counteracting effects of these two proteins are thought to be responsible for the oscillatory growth patterns observed during pollen tube elongation (Gu et al. 2005; Cole and Fowler 2006; Lee et al. 2008). Similarly in root hairs, disruption of F-actin in pollen tubes affects the organization of the subapical cytoplasm, and interferes with efficient vesicle delivery in pollen (Lee et al. 2008). These results showed that a precise regulation of tip growth can be accomplished through the dynamics of F-actin polymerization and turnover, and that an imbalance in these dynamics causes defects in this process.

RAB GTPases are key regulators of membrane trafficking and different RAB proteins target vesicles to different membranes (Nielsen 2006; Nielsen et al. 2008). In tip growth, newly synthesized cell wall components are packaged in secretory vesicles and delivered to the apical plasma membrane region of elongating root hairs and pollen tubes, and the formation and delivery of these secretory vesicles is regulated by the specific recruitment of members of the RAB A family of plant RAB GTPases to these membrane compartments (Preuss et al. 2004; Szumlanski and Nielsen 2009; Ovecka et al. 2010). RAB-A4B (At4g39990) recruits the lipid kinase, PI-4Kβ1 (At5g64070), and regulation of PI-4P levels by this lipid kinase, and a PI-4P phosphatase, RHD4 (At3g51460), play important roles in regulation and delivery of secretory cargo to the tips of growing root hairs and pollen tubes (Preuss et al. 2004, 2006; Thole et al. 2008; Szumlanski and Nielsen 2009). Likewise, enzymes that regulate the level of PI-4,5P_2 are also essential for tip growth (Helling et al. 2006; Kusano et al. 2008; Sousa et al. 2008). Disruption of two genes encoding key kinases that produce PI-4,5P_2, PIP5K3 (At2g26420), and PIP5K4 (At3g56960), caused various defects in root hairs and pollen tubes, respectively (Helling et al. 2006; Kusano et al. 2008; Sousa et al. 2008). Both PIP5K4 and a dynamin-related protein (DRP1C, At1g14830) were localized at the flanks of the apical zone in pollen tubes where endocytosis was inhibited in a *pip5k4* mutant, indicating that PI-4,5P_2 is essential for vesicle trafficking during tip growth (Konopka et al. 2008; Sousa et al. 2008). Members of the RAB B family, which show high sequence similarity to ER-localized Rab2 in animal and yeast cells, are also thought to regulate endoplasmic reticulum (ER)-to-Golgi trafficking in plants. *Arabidopsis* RAB-B1C (At4g17170, previously designated RAB2) is highly expressed in pollen and a dominant negative mutant of a tobacco RAB B homolog blocking pollen tube growth, highlighting the importance of secretory trafficking during polarized cell expansion observed in tip-growing cells (Moore et al. 1997; Cheung and Chen 2002).

Cellulose

Cellulose microfibrils are thought to be the primary element of plant cell walls that provide their mechanical strength (Cosgrove 2005). Cellulose differs from most other plant cell wall polysaccharides in that its site of synthesis occurs in plasma membrane-localized multiprotein complexes, termed rosette terminal complexes (Mueller and Brown 1980; Brown et al. 1996). According to current models, cell walls contain multiple layers of load-bearing cellulose microfibrils that are deposited sequentially during cell growth and differentiation (Emons 1994; Emons and Mulder 2000; Somerville 2006). The catalytic subunits of the cellulose synthase complex, called CESA proteins, have been identified using a combination of genetic screens, and sequence similarity to bacterial cellulose synthases (Cosgrove 2005; Somerville 2006; Guerriero et al. 2010; Endler and Persson 2011; Zhang and Zhou 2011). Each of the numerous CESA proteins that are thought to be present in a rosette terminal complex are thought to synthesize and extrude individual β-1,4-glucan polymers through the plasma membrane, and these polymers then assemble into paracrystalline cellulose microfibrils, which are then incorporated into the innermost layer of the plant cell wall (Emons and Mulder 2000; Cosgrove 2005; Guerriero et al. 2010). In cells undergoing diffuse growth, the innermost layer of cellulose microfibrils is deposited transversely to the expansion axis of the cell, and this orientation is thought to provide the asymmetric expansion characteristics observed during this form of polarized cell growth (Green 1962; Baskin 2005). Co-localization experiments showed the movement of functional fluorescently tagged CESA proteins in discrete particles within the plasma membrane, and these are co-localized with underlying cortical microtubules in the cytoplasm (Paredez et al. 2006). Although the existence of cellulose in root hairs has been postulated for decades (Newcomb and Bonnett 1965), until recently, the presence and possible role of cellulose during tip growth remained relatively unstudied.

Early ultrastructural studies indicated that root hair cell walls were comprised of two distinct layers that appeared to be organized based on when and where they were deposited during root hair growth (**Figure 1**). Initial root hair cell walls are deposited during tip growth at the extreme apical region of the root hair cell, typically restricted to the apical 30–50 μm of the root hair (Newcomb and Bonnett 1965; Galway et al. 1997). Fibrillar cell wall elements were observed in these primary root hair walls, but unlike in cells undergoing diffuse expansion, these appeared to be somewhat shorter in length, and were randomly oriented (Newcomb and Bonnett 1965; Emons and Mulder 2000). An additional inner cell wall layer containing parallel arrays of cellulose microfibrils appeared to be deposited later during root hair growth and differentiation, and these cellulose microfibril arrays were often organized in a helical orientation

along the length of the root hair (Emons and Wolters-Arts 1983; Emons 1994). Interestingly, the orientation of the cell wall fibrils and CESA6 movement in pollen tube plasma membranes were recently shown to occur in a longitudinal orientation to the pollen tube growth axis, a similar orientation to that observed earlier in root hairs (Chebli et al. 2012). Additionally, several *cesa* mutants display significant defects in pollen germination and pollen tube growth suggesting important roles for cellulose in these tip-growing cells (Persson et al. 2007). More recent examination of the role of cellulose in root hair cells have determined that cellulose synthesis is also required for root hair tip growth (Galway et al. 2011; Park et al. 2011). Cellulose is enriched in the tip-growing region, and treatment of growing root hairs with exogenous cellulase interferes with the integrity of these cell walls and induces rupture of growing root hair cells (Galway et al. 2011; Park et al. 2011). Examination of microarray-based spatiotemporal root gene expression maps has indicated that only CESA genes implicated in primary cellulose synthesis are expressed in these cells (Brady et al. 2007). Although the mutants of major primary cellulose synthases display root hair developmental defects to varying degrees, tip growth in these mutants was not abolished (Desnos et al. 1996; Caño-Delgado et al. 2000) (**Figure 2**). Additionally, eYFP-CESA3 (At5g05170) and eGFP-CESA6 (At5g64740), two of the major primary cellulose synthases tagged with fluorescent proteins, did not display significant localization to the apical plasma membrane region of growing root hairs where new cellulose synthesis would occur (Park et al. 2011). These results indicate that synthesis of cellulose, or cellulose-like polysaccharides, in the tips of growing root hairs likely involves proteins other than those CESA subunits identified to be involved in cellulose synthesis in diffusely expanding cells.

Interestingly, CSLD3 (At3g03050), a member of the cellulose synthase-like (CSL) super family, is required for root hair growth (Wang et al. 2001; Bernal et al. 2008; Galway et al. 2011; Yin et al. 2011). Both CSLD and CESA gene families display a higher degree of sequence similarity to each other than to the other CSL gene families (Richmond and Somerville 2000). CSLD5 (At1g02730) and a combination of CSLD2 (At5g16910) and CSLD3 displayed moderately increased mannan synthase activity when transiently overexpressed in tobacco leaves (Yin et al. 2011). However, in a separate study a functional, N-terminal tagged CSLD3 was enriched at the tips of growing root hairs (Park et al. 2011). Further analysis showed that CSLD3 is a plasma membrane protein, and a CSLD3 chimera containing a CESA6 catalytic domain was able to rescue the root hair growth defects of a *csld3* null mutant (Park et al. 2011). These findings are consistent with a model in which CSLD3 provides the biosynthetic activity for cellulose or cellulose-like β-1,4-glucan polysaccharide synthesis in tip-growing root hairs. Further investigation will be required to unravel the precise biochemical activity of these CSLD proteins. Supporting a role

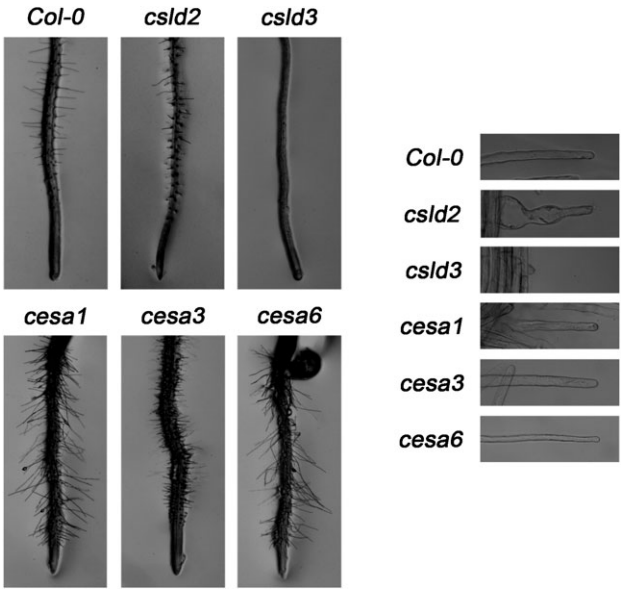

Figure 2. Phenotypes of different *cesa* and *csld* mutants.

In *csld3* mutants, root hairs fail to form, and *csld2* mutant root hairs are significantly shorter than wild-type and display abnormal bulges. In the *cesa1*, *cesa3*, and *cesa6* mutants diffuse growth is significantly impacted, leading to reduced root elongation and radial swelling. However, root hairs undergoing tip-restricted expansion can be observed in all three *cesa* mutants. While *cesa3* mutants display somewhat shortened root hairs, root hairs achieve wild-type lengths in both *cesa1* and *cesa6* mutant plants. Representative root hairs are shown on the right. Scale bar: 1 mm (left) and 0.1 mm (right).

for CSLD function during tip growth, mutations in four of the six *Arabidopsis* CSLD genes have been shown to affect tip growth in either root hairs or pollen (**Figure 2**). Both CSLD2 and CSLD3 play essential roles during root hair tip growth, with *csld3* mutants displaying no root hairs and *csld2* mutants displaying significantly shorter root hairs that often form abnormal bulges compared to wild type (Bernal et al. 2008; Yoo et al. 2012). The growth defects observed in *csld2* root hairs are likely due to repeated cycles of cell rupture followed by re-initiation of tip growth (Bernal et al. 2008). Both *csld1* (At2g33100) and *csld4* (At4g38190) mutants show defects in pollen germination (Bernal et al. 2008; Wang et al. 2011), consistent with important roles for these pollen-specific CSLDs in pollen tube tip growth. CSLD2 expressed under control of the constitutive 35S promoter was able to rescue the mutant phenotype of *csld3* and vice versa (Yin et al. 2011), but more recent analysis of the specific functions of these two CSLD genes revealed some level of divergence according to the difference of the phenotype when a single copy of CSLD2 or CSLD3 was interrupted (Yoo et al. 2012).

In addition to CESA proteins, which are thought to synthesize cellulose, a number of additional "accessory" genes have been identified whose products alter cellulose deposition by the plasma membrane-localized rosette terminal complexes (Nicol et al. 1998; Schindelman et al. 2001; Pagant et al. 2002; Gu et al. 2010). Mutation of the KORRIGAN1 (At5g49720), β-1,4-endo-glucanase, results in defects in both primary and secondary cell wall deposition (Nicol et al. 1998; Szyjanowicz et al. 2004). KORRIGAN1 (KOR1) is unique among a larger family of predicted β-1,4-endo-glucan hydrolases, termed the GH9A family, in that this protein is predicted to contain an amino terminal transmembrane domain (Zuo et al. 2000). It has been suggested that KOR1 (also known as GH9A1) co-localizes in the plasma membrane with CESA-containing cellulose synthase complexes (Crowell et al. 2010). Two other β-1,4-endo-glucanases with predicted transmembrane domains, called KORRIGAN2 (KOR2, At1g65610) and KORRIGAN3 (KOR3, At4g24260), have been described in the *Arabidopsis* genome (Mølhøj et al. 2001), and similar transmembrane GH9A1 sequences appear to be conserved in a number of sequenced genomes from both monocot and dicot plants (F. Gu, E. Nielsen, unpubl. data, 2011). Expression pattern analysis has shown that KOR1 is broadly expressed throughout the plant, while KOR2 displays a more restricted root hair-specific expression pattern, raising the possibility that KOR2 may function during root hair tip growth (Mølhøj et al. 2001). Other members of the GH9 family of β-1,4-endo-glucanases also have been implicated in the regulation of cell wall deposition in root hairs, and mutation of a member of the class C GH9 glucan hydrolases, ATGH9C1 (At1g48930), displays severe root hair phenotype and only bulges are formed after root hair initiation (Del Campillo et al. 2012). It has been hypothesized that endo-glucanases like KORRIGAN1 may initiate cellulose synthesis or remove the synthesized cellulose from the synthase complex (Cosgrove 2005; Crowell et al. 2010). However, the precise roles of these endo-glucanases during cellulose synthesis and deposition remains a mystery.

Xyloglucan

Xyloglucan is the main form of hemicellulose found in primary cell walls of dicots, and these polysaccharides are thought to link and tether neighboring cellulose microfibrils (Cavalier et al. 2008; Liepman et al. 2010). This cross-linking activity has been thought to provide overall integrity to the load-bearing polysaccharide network of the plant cell wall (Somerville et al. 2004; Cosgrove 2005). However, recent characterization of several xyloglucan xylosyltransferase mutants and examination of the effects of xyloglucan-specific endoglucanases on isolated hypocotyl cell walls have revealed few apparent defects in overall cell wall integrity, leading to a re-examination of the extent and roles of xyloglucan–cellulose interactions in plant cell walls (Cavalier et al. 2008; Park and Cosgrove 2012; Zabotina et al. 2012).

The polysaccharide backbones of both cellulose and xyloglucan are comprised of β-1,4-linked glucan polymers. However, while the unmodified β-1,4-glucan polymers of cellulose assemble into higher order cellulose microfibrils, the glucan polymer backbone of xyloglucan is extensively modified with side-chains containing xylose, galactose, and fucose sugars (Liepman et al. 2010). Xylosyl residues are attached to the xyloglucan glucan backbone through the action of a series of xylosyltranferases, or XXTs. Surprisingly, *Arabidopsis xxt1/xxt2* (At3g62720, At4g02500) double mutants with undetectable xyloglucan levels grew normally, and significant morphological defects were mainly observed in root hairs, which were significantly shorter and often displayed bulges at root hair bases (Cavalier et al. 2008). Mutation of a third AtXXT, AtXXT5 (At1g74380), also displayed increased numbers of short, irregular, and tip-bulging root hairs compared to wild-type plants (Zabotina et al. 2008). These effects appear to be additive, as *xxt1/xx2/xxt5* triple mutants have even shorter root hairs and more extensive bulging defects than double or single *xxt* mutants (Zabotina et al. 2012). Importantly, while overall length of these root hairs was affected, this did not appear to be reflected in decreased cell wall integrity as few of these shorter root hairs displayed evidence of cellular rupture (Cavalier et al. 2008; Zabotina et al. 2012). These results indicate that although xyloglucan is essential to root hair growth, tip growth is not abolished by the absence of xyloglucan in root hairs.

Further highlighting root hair-specific roles for xyloglucan, immunohistochemical studies using several monoclonal antibodies raised against distinct xyloglucan epitopes differentially labeled xyloglucan in root hair cell walls and cell walls of other root tissues (Zabotina et al. 2012). An intriguing possibility for these differences was provided by the recent identification of a novel root hair-specific acidic xyloglucan which incorporates galacturonic acid residues (Peña et al. 2012). Disruption of ROOT HAIR-SPECIFIC 8 or RHS8 (At1g63450) eliminates the accumulation of this acidic xyloglucan, and the *RHS8* gene has been renamed to XYLOGLUCAN-SPECIFIC GALACTURONOSYLTRANSFERASE 1 or XUT1 to reflect this novel root hair-specific xyloglucan enzyme activity (Won et al. 2009; Peña et al. 2012).

Pectin

Pectic polysaccharides comprise approximately 35–40% of *Arabidopsis* primary cell walls (Mohnen 2008; Liepman et al. 2010). Homogalacturonan (HG), rhamnogalacturonan I (RG-1), and rhamnogalacturonan II (RG-II) are three major forms of pectin and they differ in their backbones and side-chain residues

(Mohnen 2008). Although a single α-1,4-galacturonosyltransferase (GAUT1, At3g61130) and two α-1,3-xylosyltransferases (RGXT1, At4g01770 and RGXT2, At4g01750) have been characterized as responsible for the synthesis of the HG and RG-II backbone polymers, respectively, whether mutation of these enzymes affects root hair tip growth was not described (Egelund et al. 2006; Sterling et al. 2006). Nonetheless, disruption of UER1 (At1g63000) or GAE6 (At3g23820), two enzymes required for synthesis of pectin precursors, affected root hair length, suggesting that presence of these pectic polymers may play important roles during tip growth in root hairs (Pang et al. 2010).

Further evidence for roles of pectins in tip-growing root hairs comes from the recent use of FucA1, a sugar analog fucose alkyne, to examine RG-I synthesis and deposition in *Arabidopsis* (Anderson et al. 2012). FucA1-labeled RG-1 accumulated in newly initiated root hair bulges, suggesting a prominent role for these polysaccharides during root hair initiation steps (Anderson et al. 2012). However, in a separate study that used propidium iodide to visualize pectin distribution, pectin was primarily localized to more distal portions of the root hair (Rounds et al. 2011). Alternatively, pectin polysaccharide incorporation into newly deposited cell walls has been more clearly established in the tips of growing pollen tubes, and the morphology of pollen tubes during elongation requires the involvement of pectin methylesterases (PMEs; Tian et al. 2006; Rounds et al. 2011). Knockout of a pollen-specific PME, PPME1 (At1g69940), caused a stunted pollen tube morphology (Tian et al. 2006). Further, treatment of growing pollen tubes with exogenously added pectinases eliminated the apical accumulation of polysaccharides that could be labeled with cellulose-specific stains, raising the possibility that cellulose networks are not stable without pectins in these tip-growing cells (Chebli et al. 2012). Whether pectin is also responsible for stabilizing cellulose in root hair cell walls is an intriguing area for future investigation.

Cell Wall Proteins

In addition to the major polysaccharide classes, numerous proteins are secreted and incorporated into growing plant cell walls. As discussed above, a number of these secreted proteins are extracellular enzymes that are responsible for various modifications of the cell wall that occur during cell wall deposition, cell expansion, and differentiation. However, diverse classes of structural cell wall proteins are also secreted and integrated into the plant cell wall (Cosgrove 2005; Cheung and Wu 2011). One class of these structural cell wall proteins, the "expansin" protein family, has been proposed to play key roles in wall-loosening, and disruption of several expansin genes results in reduction of leaf size or plant height (Cho and Cosgrove 2000; Choi et al. 2003). Expansins can be divided into two subgroups according to their specificity to distinct polysaccharide substrates, and four members of the expansin family (EXPA7, At1g12560; EXPA18, At1g62980; OsEXPB5, Os04g46650; HvEXPB1, AY351785) have been shown to be specifically expressed in root hairs (Cho and Cosgrove 2002; Won et al. 2010). Treatment with exogenous expansin resulted in cucumber root hair swelling or rupture in a dose-dependent manner (Cosgrove et al. 2002). Disruption of Expansin A7 by RNAi also affected root hair elongation (Lin et al. 2011), and reduced levels of EXPA7 transcription in these RNAi lines correlated with significantly shorter root hair lengths (Lin et al. 2011). Therefore, an *in vivo* role for expansin proteins in tip growth is supported.

Many hydroxyproline-rich glycoproteins are found in cell walls, including "extensins" and proline-rich proteins (Mohnen and Tierney 2011; Velasquez et al. 2011). In *Arabidopsis*, the proline residues in these proteins are post-translationally hydroxylated to form hydroxyproline, in a process catalyzed by membrane-bound prolyl 4-hydroxylases (P4Hs; Gorres and Raines 2010; Mohnen and Tierney 2011; Velasquez et al. 2011). Elimination of the root-expressed P4H genes, P4H2 (At3g06300), P4H5 (At2g17720), or P4H13 (At2g23096), resulted in shortened root hairs, and yeast two-hybrid analysis showed that P4H5 binds LRX3 (At4g13340), a root hair-specific extensin (Velasquez et al. 2011). Although the functions of extensins are not well characterized, it was hypothesized that extensins in root hairs are modified by P4Hs prior to secretion into plant cell walls, and that incorporation of this class of cell wall proteins assists in the appropriate assembly of different polysaccharides into cell walls (Velasquez et al. 2011).

Receptor-Like Kinases and Sensing of Cell Wall Integrity

Recent experiments have highlighted important roles for members of the *Catharanthus roseus* RLK1-like (CrRLK1L) family of lectin-containing receptor-like kinases in regulating aspects of cell wall integrity in tip-growing cells (Hématy and Höfte 2008; Cheung and Wu 2011; Boisson-Dernier et al. 2011). THESEUS1 (At5g54380), the first characterized member of the CrRLK1L family, functions as a suppressor of *prc1*, a *cesa6* mutant (Hématy et al. 2007). The *the1* mutant rescues the short hypocotyl phenotype of *prc1* when grown in dark and therefore it was hypothesized that THE1 represses cell expansion when it detects the wall integrity is impaired (Hématy et al. 2007; Hématy and Höfte 2008). Another member of the CrRLK1L family, FERONIA (At3g51550), was initially identified based on fertilization defects associated with inappropriate pollen tube-synergid cell interactions observed in *fer* mutants (Huck 2003). Root hair defects were later observed in other *fer* mutant

backgrounds in which the majority of root hairs were significantly shorter and often root hairs burst (Duan et al. 2010). FER was subsequently shown to interact with an ROPGEF1 (At4g38430) and current models for the function of FER receptors involve the recruitment and activation of ROP GTPase-based signaling pathways upon perception of upstream cell wall-associated signals by the FER receptor (Duan et al. 2010; Cheung and Wu 2011). While the downstream pathway of FER is elucidated to some extent (Duan et al. 2010), the upstream signal that activates FER still remains unknown. However, the extracellular domains of FER as well as THE1 and ANX1 (At3g04690), another member of the CrRLK1L family, contain two malectin-like domains, providing a hint in their potential substrates (Boisson-Dernier et al. 2011; Cheung and Wu 2011). In animals, malectin is an ER membrane protein that shows specificity for Glc_2-*N*-glycan moieties, and binds α-linked disaccharides, such as maltose (glucose-α-1,4-glucose) and nigerose (glucose-α-1,3-glucose) (Schallus et al. 2008). This raises the intriguing possibility that the two malectin domains present in the extracellular domains of CrRLK1L family members may recognize similar oligosaccharides or polysaccharides in the plant cell wall.

Conclusion and Perspectives

Examination of tip-growing cells such as root hairs and pollen tubes using electron microscopy has provided important information regarding the ultrastructural organization of cell wall components during polarized cell expansion in plants (Emons and Wolters-Arts 1983; Emons 1994). More recently, the dispensable nature of root hairs to overall plant growth, and the relative ease by which growth and development of tip-growing root hairs and pollen tubes can be observed and manipulated using live-cell imaging methods has resulted in the generation of detailed models for the subcellular organization that underpins the polarized secretion and integration of cell wall components in these cells. Given the central role the plant cell wall plays in growth and development, study of the mechanisms that govern overall plant growth has proven relatively recalcitrant to genetic approaches due either to the lethal nature of, or genetic redundancy observed for, the cellular components involved in generalized cell wall biosynthesis. In this respect, examination of root hair- or pollen-specific cell wall defects that are observed upon mutation of members of larger gene families that display cell type-specific expression has proven a useful tool for identifying possible roles of a number of gene families involved in synthesis or regulation of plant cell wall biogenesis.

While our understanding of how specific classes of cell wall polysaccharides are synthesized and deposited in expanding cell walls has greatly improved in recent years, key questions still remain. What are the identities of proteins responsible for synthesis of cellulose or cellulose-like polysaccharides in the tips of growing root hairs and pollen tubes, and how does the regulation of these enzyme complexes differ from cells undergoing diffuse expansion? What specifically are the roles of xyloglucan and pectin polysaccharides with regard to spatial organization and cross-linking of cellulose microfibrils in the cell wall, and how do these two physical characteristics contribute to the extensibility and integrity of cell walls overall? Finally, how similar, or distinct, is the overall organization of these three main polysaccharide classes between cells undergoing tip-restricted or diffuse expansion? Experiments aimed at providing insight into these questions should provide interesting challenges, but also novel insights into our overall knowledge of plant cell wall biosynthesis, and how the organization of this process compares between cells undergoing either tip-restricted or diffuse cell expansion.

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