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The Cytoskeleton

Diverse Roles in a Plant's Life

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Preface

The cytoskeleton, microtubules, and actin filaments together have been found to play diverse roles in the growth and development of plants. The role of cytoskeleton is well established in providing shape and size to plants cells. Be it the jigsaw shape of pavement cells or the conical shape of petal epidermis or the grain shape of rice, it is all brought about by the coordination of the cytoskeleton. The plant cell uses microtubules for trafficking CESA to the cell wall. The orientation of microfibrils corresponds to the microtubular orientation, thereby giving a functional role to the microtubules in context to cell wall structure. The cytoskeleton not only provides for the geometric dimensions but is also very important in physiological processes. Actin filaments are known to play roles in dynamics of stomata and chloroplast, both of which have physiological consequences which are related to adaptations pertaining to abiotic stresses. Recent studies show the interactions between microtubules and actin filaments. Also, it is evident from hormonal cross talks that the cytoskeleton in plants is needed for proper distribution of hormones. Advances in imaging techniques have made the functional studies of cytoskeleton more fascinating in plants. In this book, the authors would like to bring out the role of plant cytoskeleton in context to its interactions and functional affinity to other cellular organelles.

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Chapter 1
Cortical Region of Diffusively Growing Cells as a Site of Actin–Microtubule Cooperation in Cell Wall Synthesis

Kateřina Schwarzerová and Judith García-González

Abstract The cortical cytoskeleton, consisting of microtubules and actin filaments, plays a crucial role in the shaping and synthesis of the cell wall. Microtubules and actin filaments engage in cross-talk in plant cells, and this interplay is mediated by a number of molecular interactors and signaling pathways. This work is focused on the interconnected role of actin and microtubules during cell wall formation. Proteins possibly involved in the cross-talk between microtubules and actin filaments for cell wall assembly control are described, and pathways connecting their function in specialized cells with complex shapes are discussed. These include *Arabidopsis* trichomes, interdigitating epidermal pavement cells, and xylem vessel cells. Mutual interactions between microtubules and actin filaments in these cells are based on restrictive cooperation, often controlled by overlapping regulatory pathways, rather than direct cross-link between both cytoskeletons. A specialized formation of the cytoskeletal structure, the preprophase band, is further discussed as an example of direct microtubules and actin filaments cross-linking.

1.1 Cortical Cytoplasm

The cortical cytoskeleton, which consists of microtubules (MTs) and actin filaments (AFs), plays a crucial role in the synthesis and shaping of the cell wall (Szymanski and Cosgrove 2009). The role of MTs in cell wall deposition is under intense investigation, which has uncovered many molecular details of the process. On the other hand, although the importance of AFs in cell wall deposition is unquestionable, the molecular mechanisms underlying its role are less clear. Cortical MTs and AFs clearly interact with each other (for review see Takeuchi et al. 2017). MTs and AFs

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cross-talk is mediated by a number of molecular interactors (reviewed in Petrášek and Schwarzerová 2009; Krtková et al. 2016; Takeuchi et al. 2017). This work is focused on MTs–AFs cross-talk mechanisms involved in cell wall assembly. Firstly, from the list of proteins that cross-link MTs and AFs, candidate proteins possibly involved in cell wall assembly control are selected. Secondly, we discuss signaling pathways connecting MT and AF cytoskeletons in specialized cells with polar expansion. These include *Arabidopsis* trichomes cells, interdigitating epidermal pavement cells, and xylem vessel cells depositing the secondary cell wall in unique patterns. Current data suggest that in these specialized cells, MTs and AFs delimit different cortical zones on the plasma membrane for localized deposition of the cell wall with distinct mechanical properties. Mutual interactions between MTs and AFs are based on restrictive cooperation, often controlled by one upstream regulator rather than direct cross-link between both types of polymers. A specialized formation of cytoskeletal structure, the preprophase band, is further discussed as an example of direct MTs and AFs cross-linking.

1.2 Plant Cell Wall and Its Deposition

Plant cell walls are semirigid, composite structures. The main component is polysaccharides, which strengthen the cell wall while maintaining enough plasticity for growth (Cosgrove 2005; Szymanski and Cosgrove 2009; Bashline et al. 2014). The polysaccharides that make up the cell wall are cellulose, hemicelluloses, and pectins.

1.2.1 Microtubules and Cellulose Synthesis

Cellulose is the main load-bearing polymer of the cell wall. The molecular mechanism of cellulose synthesis is based on a multimeric enzyme complex, localized in the plasma membrane. Enzymes that synthesize cellulose are called cellulose synthases (CESA). The CESA complexes move within the plane of plasma membrane during cellulose synthesis. Each protein complex consists of 18–36 single catalytic subunits of CESA; 18 subunits are favored by current models (Vandavasi et al. 2016). Cellulose is synthesized into the cell wall, where it crystallizes, and the crystallization process is believed to generate lateral movement of CESA complexes within the plasma membrane (Paredez et al. 2006).

Since the deposition of cellulose significantly influences cell wall properties, plant cells control CESA movement through a unique mechanism. The process involves cortical MTs in close association with the plasma membrane to define the direction of CESA movement (Paredez et al. 2006). Several proteins mediate the interaction between the MTs and the CESA complex. An MT-associated protein (MAP)

CELLULOSE SYNTHASE INTERACTING1 (CSI1) links cortical MTs to CESA complexes (Bringmann et al. 2012; Li et al. 2012). After the identification of CSI1, several other proteins involved in the control of CESA attachment to MTs were discovered. This suggests that cellulose synthesis is controlled by large protein machinery. COMPANION OF CELLULOSE Synthase (CC) proteins (Endler et al. 2016) are also a component of the CESA complex. Here, CC proteins function as a MAP independently of CSI1, and their function is based on the control of microtubular dynamics under stress conditions, ensuring that cellulose synthesis is sustained (Kesten et al. 2019). The physical interaction between MTs and CESA is controlled by another kind of MAP called cellulose synthase–microtubule uncouplings (CMU) (Liu et al. 2016). The cellulose-producing protein factory probably has more components regulating cellulose synthesis, such as KORRIGAN (Vain et al. 2014), CHITINASE-LIKE1 (Sanchez-Rodriguez et al. 2012), or COBRA (Liu et al. 2013). These proteins partake in cellulose synthesis and may be associated with CESA complexes as well.

1.2.2 Actin and Cellulose Synthesis

MTs, CESA protein complexes, and associated regulatory proteins are crucial for cellulose synthesis and plants impaired in this mechanism usually display strong morphogenic defects. However, what is the role of the actin cytoskeleton in cellulose deposition, and is microtubular and actin cytoskeletons cross-talk important for this process? The cortical actin cytoskeleton is spatially and structurally associated with MTs (Sampathkumar et al. 2011). The actin cytoskeleton is involved in the process of cellulose deposition as well. For example, 5-day-old etiolated seedlings of *act2act7* mutants contain less cellulose (Sampathkumar et al. 2013). CESA complexes are assembled in Golgi apparatus (GA) and are delivered to the PM in vesicles (Crowell et al. 2009; Gutierrez et al. 2009). The delivery of CESA-containing vesicles was impaired and the lifetime of CESA complexes in the PM in double mutants *act2 act7* was longer (Sampathkumar et al. 2013).

Interestingly, there is a bidirectional relationship between cellulose synthesis and actin cytoskeleton; a decrease in AFs dynamics was observed in plants treated with isoxaben, a cellulose synthesis inhibitor (Tolmie et al. 2017). These data clearly suggest that the actin cytoskeleton plays a role in cellulose deposition. However, molecular players linking the actin cytoskeleton to components of the cellulose synthesis machinery are missing. Recently, Zhang et al. (2019) demonstrated the role of myosins XI in the tethering and fusion of vesicles containing CESA complexes to the plasma membrane. Triple myosin mutant *xik xi1 xi2* displays decreased cellulose content, and a detailed analysis shows that AFs depolymerization and myosin inhibition lead to the fusion failure of CESA-containing vesicles with the plasma membrane. Interestingly, inhibition of myosin, but not the actin cytoskeleton,

leads to decreased CESA density and motility in the PM, which suggests an actin-independent role of myosin in this process (Zhang et al. 2019). The report of Zhang et al. (2019) thus identified the myosin as the first molecule linking the actin cytoskeleton to CESA function.

1.2.3 Deposition of Noncellulose Polymers

The deposition of noncellulosic polysaccharides is less understood. Pectin and hemicellulose are synthesized in the GA and delivered to the plasma membrane through vesicular transport (Kim and Brandizzi 2014). Vesicles are delivered to the plasma membrane in a cytoskeleton-dependent manner. FRA1 is a kinesin-4 family member, which is believed to transport vesicles originating in the GA to the plasma membrane. FRA1 moves along MTs in a CESA-independent manner, and *fra1* mutants show cell wall defects (Zhu et al. 2015). AFs are also necessary for the delivery of vesicles containing cell wall components to the plasma membrane. Plants with disrupted actin-dependent transport show defects in cell wall deposition and expansion, which has been demonstrated in plants treated with latrunculin B (Baluska et al. 2001), in plants with knocked-out myosins (Peremyslov et al. 2010) and in plants lacking vegetative actin isoforms (Kandasamy et al. 2009; Sampathkumar et al. 2013). However, the molecular mechanism, chiefly an actin-associated protein involved in actin-vesicle interaction, PM targeting, and fusion, remains unknown.

Fine AFs may participate in vesicles fusion with the plasma membrane in a manner similar to that described for tip-growth (Ketelaar 2013). The coordination of cellulose and noncellulosic polymer deposition is also not well understood. Considering the crucial roles of both MTs and AFs in cell wall deposition, it is tempting to speculate that a mechanism linking these two cytoskeletal arrays may be involved in this regulation. Therefore, here we discuss a number of candidate proteins, which are known to cross-link MTs and AFs, and whose function is related to cell wall assembly during diffuse growth.

1.3 Proteins Interacting with Actin and Microtubules with Potential Function in Cell Wall Assembly

1.3.1 Kinesins

Kinesins are molecular motors that convert chemical energy into directional movement along MTs. The family of plant kinesins is exceptionally large, which is considered a consequence of the absence of dynein motors in land plants (Wickstead and Gull 2007). The plant-specific kinesin-14 family contains kinesins with a unique capability to translocate toward the minus end of MTs (Gicking et al. 2018). A

subgroup of the kinesin-14 family is comprised of kinesins with a calponin homology (CH) domain, which have been shown to interact with MTs and AFs (KCH subgroup) in species such as cotton (Preuss et al. 2004), rice (Frey et al. 2009; Maruta et al. 2010), *Arabidopsis* (Buschmann et al. 2011), and tobacco (Klotz and Nick 2012). If some of these kinesins aid in cell wall synthesis through MTs and AFs cross-linking, a co-localization with both cytoskeletal systems should be observed in the plant cortex. Indeed, Preuss et al. (2004) demonstrated co-localization of cotton KCH with transverse AFs and Frey et al. (2009) showed OsKCH co-localization with crossover between MTs and AFs in the cell cortex. However, cell wall changes have not been reported for respective mutants. A phenotype associated with altered cell expansion has been reported for mutants lacking tobacco NtKCH (Klotz and Nick 2012), but the NtKCH association with cortical MTs was actin-independent. Therefore, the question of possible participation of these cross-linking kinesins in cell wall assembly remains open.

1.3.2 DREPP/MDP25

DREPP (Developmentally-Regulated Plasma Membrane Polypeptide) proteins represent plant-specific proteins that interact peripherally with the plasma membrane (Gantet et al. 1996; Vosolsobě et al. 2017). The *Arabidopsis* member of the DREPP family, called MDP25, has been shown to bind and destabilize cortical MTs in *Arabidopsis hypocotyls* (Li et al. 2011) and to bind and sever AFs in pollen tubes (Qin et al. 2014) in a Ca^{2+}-dependent manner. Since *mdp25* mutants had longer hypocotyls, and overexpression of MDP25 resulted in shorter hypocotyls, the microtubule-destabilization activity of MDP25 seems to play a role in cell elongation, probably through the control of cortical MTs stability in elongating cells (Li et al. 2011). It has not been demonstrated whether MDP25 function in the cortical region of diffusively expanding cells involves regulation of AFs as well. However, its interaction with cortical MTs, its function in cell elongation, as well as its dual function in the binding of MTs and AFs, make MDP25 another candidate for an actin- and microtubule-interacting protein during cell wall expansion.

1.3.3 Formins

Formins are evolutionary conserved multidomain proteins, which contain a typical FH (formin homology) domain in their structure. One of formin's roles is to promote AFs polymerization. Likewise, some family members also interact with MTs in animal (Bartolini and Gundersen 2010) and plant (Deeks et al. 2010; Wang et al. 2013; Rosero et al. 2013; Sun et al. 2017) cells. Plant formins comprise a large protein family with three clades: Class I, Class II, and Class III (Deeks et al. 2002; Grunt et al. 2008), where Class I often contains transmembrane members, and Class II members

peripherally interact with membranes (Cvrčková 2013). Membrane association and the ability to bind MTs and AFs thus make formins a good candidate for the molecular link between both cytoskeletons regarding cell wall assembly control. Moreover, formin mutants often exhibit growth defects. For example, the loss of a Class I AtFH1 protein results in changes in the MT and AF cytoskeletons and altered cell expansion (Rosero et al. 2013, 2016). Rice Class II formin FH5 binds AFs and MTs, and functional analysis of respective mutants proved the role of FH5 in cell expansion (Zhang et al. 2011a). Sun et al. (2017) demonstrated that rice formin OsFH5 is involved in actin assembly, and the binding and cross-linking of AFs and MTs. The overexpression of OsFH5 results in the formation of larger grains due to altered cell expansion. Here the dense actin network formed in overexpressing plants probably promoted cell expansion, presumably through stabilization of the plasma membrane-associated cytoskeleton and speeded cell wall assembly (Sun et al. 2017). The role in cross-linking of AFs and MTs has been demonstrated also for formin AtFH4 (Deeks et al. 2010) and Class II formin AthFH16 (Wang et al. 2013). Since no cell wall defects have been reported for plants lacking these formins, it is not known whether they participate in cell wall assembly. During cell division, a specific role is played by the class II formin AFH14. AFH14 was shown to interact with both AFs and MTs. The T-DNA insertion mutant *afh14* of *Arabidopsis* shows defects in meiosis and tetrad formation, suggesting a specific role during cell division (Li et al. 2010). In summary, at least AtFH1, rice FH5, and OsFH15 formins clearly link the control of AFs and MTs regulation with cell wall assembly. Although the mechanism needs to be elucidated, the control of cortical cytoskeleton seems to be tightly connected with formin function. Importantly, AtFH1 was shown to connect the cell wall with the plasma membrane and the cortical cytoskeleton, and its localization in the plasma membrane to specific domains was restricted by MTs (Martinière et al. 2011), which may be the mechanism of communication within the cytoskeleton–plasma membrane–cell wall continuum.

1.3.4 Arp2/3

Arp2/3 is an evolutionarily conserved protein complex, whose main function is the nucleation of AFs (Welch et al. 1998). The complex consists of 7 subunits (Welch et al. 1997), which are also encoded in plant genomes. *Arabidopsis* plants lacking a functional Arp2/3 complex display a broad spectrum of phenotypes, which suggests a specific role of Arp2/3-controlled actin nucleation in cell wall building. Defects in cell–cell adhesion have been reported for cotyledons and etiolated hypocotyls of mutants lacking Arp2/3 complex subunits (Le et al. 2003; Mathur et al. 2003a, b; El-Assal et al. 2004; Kotchoni et al. 2009; Zhang et al. 2013; Pratap Sahi et al. 2017). The hallmark phenotype of Arp2/3 mutants is distorted trichomes (Szymanski et al. 1999; Mathur et al. 1999; Schwab et al. 2003; Le et al. 2003; Li et al. 2003; El-Assal et al. 2004; Basu et al. 2004, 2005), which fail to form narrow and pointed trichome branches in the absence of an active Arp2/3 complex. As demonstrated by

Yanagisawa et al. (2015), the distorted phenotype is based on aberrant cell wall deposition within the growing apex. In the work of Sahi et al. (2017), mutants lacking several Arp2/3 subunits were shown to deposit cell walls of altered composition in inflorescence stems; these cell walls contained less cellulose and more pectin. Interestingly, several reports noted microtubular changes in Arp2/3 mutants, which indicated that the Arp2/3 complex may also be involved in the regulation of MTs (Saedler et al. 2004a; Zhang et al. 2005). A direct interaction of Arp2/3 with MTs was suggested by Havelková et al. (2015), who showed that the Arp2/3 complex subunit ARPC2 binds to MTs. Since the Arp2/3-nucleated actin cytoskeleton is clearly involved in the control of cell wall deposition, despite the mechanism not being clear, its interaction with MTs makes the Arp2/3 complex another candidate in MT-AF cross-talk during cell wall assembly.

1.4 Patterning for Polar Cell Wall Deposition in Diffusively Growing Cells: Cooperation by Restriction?

Cellulose synthesis and deposition of noncellulosic polymers must be coordinated in isotropically expanding plant cells. Our knowledge of cellulose synthesis and its cytoskeletal control has increased considerably in recent years due to several important findings, but the mechanism and control of noncellulosic polymer deposition during cell wall expansion is less understood. In many cases, diffusively growing plant cells deposit the cell wall in a polarized manner, which allows them to achieve complex shapes. Several cell types that undergo complex morphogenesis became models for research in cytoskeletal control of cell deposition.

Current data suggest that during polarized cell wall deposition and expansion, specific mechanisms are often activated based on the cooperative restriction of both cytoskeletons, rather than direct cross-linking. Several examples indicate that AFs and MTs may delineate cortical regions to enable polarized cell expansion by differential deposition of cell wall. This leads to the formation of cell wall domains, with distinct mechanical properties that respond differentially to turgor pressure, thus enabling various cell shapes. This concept is supported by recently uncovered molecular mechanisms that control the differential deposition of the cell wall in plant cells with complex shapes, as reviewed later in this chapter. Perhaps understanding the specific roles of AFs and MTs in cell wall deposition in these specialized cases will help to uncover the general mechanism coordinating cellulose and noncellulose polysaccharide deposition in diffusively expanding cells.

1.4.1 *Developing Trichomes and Cytoskeletal Patterning of Cell Wall Deposition*

The unicellular trichomes of Arabidopsis leaves are a classical model of plant morphogenesis (Hulskamp et al. 1998). Trichome development is under the control of both the microtubular and actin cytoskeleton (Szymanski 2009). MTs contribute to trichome branch initiation and trichome growth during anisotropic cell expansion of the main stalk (Mathur and Chua 2000; Folkers et al. 2002). To direct cell expansion, cortical MTs are oriented perpendicularly to the direction of growth, forming a collar (Sambade et al. 2014). Close inspection suggests that MTs are involved in branch marking, but they are not needed for bulge formation (Sambade et al. 2014). Actin has been shown present at the tip of newly emerging trichomes and trichome branches. Data indicate that, in the context of trichome branching, actin would be crucial to determining future sites of MTs localization by affecting microtubule dynamics (Schwab et al. 2003; Saedler et al. 2004a; Sambade et al. 2014). During branch elongation, the Arp2/3 complex plays a specific role, as distorted trichomes are typical phenotypes of mutants lacking functional Arp2/3 (see abovementioned roles of Arp2/3 and references there). The role of the Arp2/3 complex in trichome branch growth was analyzed in detail by Yanagisawa et al. (2015). The authors demonstrated that during trichome branch elongation, cell wall of gradually increasing thickness is deposited at the growing branch tip, which endows the trichome cell wall with specific biomechanical properties. The Arp2/3-nucleated actin cytoskeleton at the branch tip aids in polar cell wall deposition, because mutants defective in activated Arp2/3 failed to form pointed trichome structure (Yanagisawa et al. 2015). A detailed mechanism of the Arp2/3-nucleated AFs in branch elongation was shown by Yanagisawa et al. (2018). SPIKE1 is a protein that functions as a GEF, which also controls Arp2/3 complex activation through the Arp2/3-activating complex WAVE/SCAR (Basu et al. 2008). SPIKE1 localizes to the trichome branch tip, where it is concentrated and stabilized during the course of trichome development. SPIKE1 restriction to the branch tip is controlled by microtubular bundles found on the flank, but absent in the tips of growing trichomes (Yanagisawa et al. 2018). MTs in the growing trichome branches thus develop a microtubule-depleted zone (MDZ) in the tip (Yanagisawa et al. 2015). SPIKE1 patches are destabilized in the vicinity of MTs and have a shorter lifetime than SPIKE1 in MDZ, suggesting its stabilization in the tip of the branch. The stabilized population of SPIKE1 then marks the place of WAVE/SCAR complex activation, Arp2/3 activation, and AFs assembly (Yanagisawa et al. 2018). The authors presumed that the polymerized actin mediates the flow of cytoplasm and the delivery of vesicles containing cell wall material into the tip of growing trichome branches. Here, a specialized cell wall domain with a characteristic thickness gradient is believed to enable proper trichome development. The developing trichome branch thus represents an example of MTs restricting AFs polymerization for differential cell wall deposition, as is necessary for polarized growth.

1.4.2 *Leaf Epidermal Cells*

Leaf epidermal cells are an outstanding example of plant cell shape control. Through a multistep morphogenic process, they develop from polygon-shaped cells into large cells with a complex interlocking pattern of lobes and necks, similar to that of a puzzle (Vőfély et al. 2019).

Careful time-lapse investigations following cell morphogenesis indicate that pavement cells go through a phase of lobe initiation followed by a phase of cell expansion (Armour et al. 2015; Zhang et al. 2011b). This set of subcellular responses is thought to be regulated by tissue-wide molecular and mechanical stimuli. Both MTs and AFs are involved in the control of pavement cell shape, and the current hypothesis is based on their mutual interaction during cell expansion and cell wall building. Preliminary observations hinted at microtubule organization and cellulosic cell wall deposition as main players in PC morphogenesis. Briefly, cellulose microfibrils were visualized at the periclinal–anticlinal wall junctions of neck regions, which continued all the way down the anticlinal wall (Panteris et al. 1993a, b, 1994) mirroring microtubule orientation (Panteris et al. 1993a, b, 1994; Kirik et al. 2007; Fu et al. 2002, 2005; Zhang et al. 2011b). To date, visualization of MTs along anticlinal and periclinal cell surfaces during the initiation of lobe formation has yielded contradictory conclusions. While Armour et al. (2015) observed that a higher occupancy of MTs in anticlinal and periclinal walls was a predictor of lobe formation, Belteton et al. (2018) could not correlate the presence of MTs with the emergence or existence of lobes. Since MTs are highly dynamic structures, higher spatiotemporal resolution will be of utmost importance to elucidate their role during cell morphogenesis. AFs were introduced into the model, indicating the need for them in lobe elongation. Mutants lacking a functional Arp2/3 complex (Basu et al. 2004, 2005, 2008; Brembu et al. 2004; Frank et al. 2003; Frank and Smith 2002; Le et al. 2003, 2006; Li et al. 2003; Mathur et al. 2003a, b; Qiu et al. 2002; Saedler et al. 2004b) were shown to develop reduced lobes in pavement cells. Actin has been detected in lobes (Fu et al. 2002, 2005; Armour et al. 2015), and the involvement of the cytoskeleton in the process was further confirmed in pharmacological studies, where depolymerization of MTs (Akita et al. 2015) and AFs (Rosero et al. 2016) impacts pavement cell morphogenesis. A molecular model was established involving Rho GTPase of plants (ROP) signaling cascade controlling lobes formation through the control of cortical cytoskeleton. In this model, activated ROP2/ROP4 triggers the activity of ROP-interactive CRIB motif-containing protein 4 (RIC4), promoting the formation of dynamic AFs in the lobe outgrowth (Fu et al. 2005). In this context, AFs were suggested to participate in the delivery of vesicles containing new cell wall material or cell wall loosening enzymes to the elongating lobes (Smith 2003). Furthermore, activation of ROP6 localized at the neck regions would induce the rearrangement of MTs at the sites of growth restriction via the action of its RIC1 effector (Fu et al. 2009), which activates KATANIN protein with microtubule-severing activity (Lin et al. 2013). Spatial restriction of actin and microtubular cytoskeletons at the cell cortex would be mediated through the ROP2 molecule,