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The connection of cytoskeletal network with plasma membrane and the cell wall

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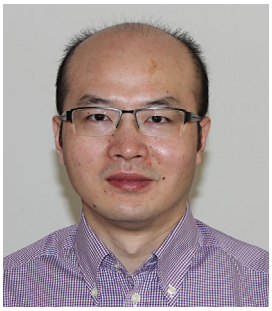
Invited Expert Review



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Abstract The cell wall provides external support of the plant cells, while the cytoskeletons including the microtubules and the actin filaments constitute an internal framework. The cytoskeletons contribute to the cell wall biosynthesis by spatially and temporarily regulating the transportation and deposition of cell wall components. This tight control is achieved by the dynamic behavior of the cytoskeletons, but also through the tethering of these structures to the plasma membrane. This tethering may also extend beyond the plasma membrane and impact on the cell wall, possibly in the form of a feedback loop. In this review, we discuss the linking components between the cytoskeletons and the plasma membrane, and/or the cell wall. We also discuss the prospective roles of these components in cell wall biosynthesis and modifications, and aim to provide a platform for further studies in this field.

Keywords: Cytoskeleton; plant cell wall; plasma membrane
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INTRODUCTION

Plant cells are surrounded by cell walls that typically are composed of polysaccharides, such as cellulose, hemicellulose and pectin, and proteins that may have structural or enzymatic functions (Somerville et al. 2004; Cosgrove 2005). The cytoskeleton, including microtubules and actin filaments, provides inner support for plant cells (Hussey et al. 2002; Wasteneys and Yang 2004; Hussey et al. 2006; Paradez et al. 2006). Plants treated with cytoskeleton inhibitors, or that have mutations in cytoskeleton components, display phenotypic similarities to plants with defects in cell wall synthesis, such as dwarfism, swollen epidermal cells and alterations in cell wall composition, suggesting an important role for the cytoskeleton in plant cell wall production (Sugimoto 2003; Ehrhardt and Shaw 2006; Buschmann and Lloyd 2008; Endler and Persson 2011). Indeed, the cytoskeleton can regulate cell wall deposition in several ways: first, the cytoskeleton is responsible for the trafficking of cell wall components that are synthesized in intracellular compartments, like the endoplasmic reticulum (ER) and the Golgi apparatus (Crowell et al. 2009; Sampathkumar et al. 2013; Bashline et al. 2014). The trafficking can also be polar, which induces polar cell wall growth, e.g. in pollen tubes and root hairs (Ketelaar 2013; Lazzaro et al. 2013; Rounds et al. 2014). Second, the cortical microtubules define insertion sites of cellulose synthase (CesA) complexes (CSC) and guide the direction of the CSCs during cellulose synthesis in growing plant cells (Paredez et al. 2006; Gutierrez et al. 2009). As cellulose is the main contributor to cell wall strength, the orientation of the microtubule array substantially contributes to the anisotropy of the cell walls. The cellulose, and thus microtubules, therefore allows cell growth perpendicular to their net orientation. Finally, the cytoskeletons also respond to cell wall signals, such as cell wall growth restrictions, mechanical stimuli and cell wall modifications (Takemoto and Hardham 2004; Uyttewaal et al. 2012; Jacques et al. 2013; Panteris et al. 2013; Sampathkumar et al. 2014a, 2014b). This response may alter the cytoskeletal organization, and thus cell wall deposition.

The close connection between the cytoskeleton and the cell wall imply a close association between on the one hand the cytoskeleton and the plasma membrane, and on the other between the plasma membrane and the cell walls. The plant cytoskeleton-plasma membrane-cell wall continuum has received considerable attention for quite some time (Baluška et al. 2003). However, proteins that link the three parts are still not well defined. In animals, integrins have been

shown to transduce the extracellular signals to the cytoskeleton (DeMali et al. 2003; Palazzo et al. 2004). The connection between the integrins and the extracellular matrix is based on an Arg-Gly-Asp (RGD) motif (Ruoslahti and Pierschbacher 1987). In plants, no obvious integrin homologs exist and the cytoskeleton and cell wall continuum is also less clear. However, plant cells can respond to RGD peptides (Schindler et al. 1989; Canut et al. 1998), suggesting that some forms of integrin-like signal transduction mechanism between the cytoskeleton and cell wall may exist in plants. Indeed, several distant integrin-like proteins have been identified in plants, such as NDR1 and Lec19. These proteins recognize the RGD peptide and lesions in the proteins cause cell wall-plasma membrane adhesion deficiencies during plasmolysis (Gouget et al. 2006; Knepper et al. 2011). Interestingly, mutations in another integrin-like protein, called At14A, resulted in aberrant organization of microtubules and actin filaments in *Arabidopsis* suspension cells (Lü et al. 2012). Nevertheless, the cell wall triggering components for these integrin-like proteins have not been identified, and it is not clear how integrin-like proteins regulate cytoskeleton functions in plants.

Considering that the cytoskeleton and cell wall biosynthesis and modification, e.g. trafficking and synthesis of cell wall components, polar cell wall deposition, cell wall and directional growth, and cell wall signaling responses have been intensely reviewed (Deinum and Mulder 2013; Ketelaar 2013; Lei et al. 2014; Thomas and Staiger 2014), we here focus on recent progress in understanding the interactions between the cytoskeleton, the plasma membrane and the cell wall in plant cells (Table 1).

PLANT MICROTUBULE-PLASMA MEMBRANE-CELL WALL CONNECTIONS

As alluded to above, cellulose is synthesized at the plasma membrane by CSCs that track along cortical microtubules (Paredez et al. 2006). The association between the CSC and the microtubules is mediated by the POM2/CSI1 protein (Bringmann et al. 2012; Li et al. 2012b) (Figure 1A). A lack of guidance, for example by impaired POM2/CSI function, leads to severe cell swelling and plant growth defects, caused by mis-aligned cellulose fibers (Bringmann et al. 2012; Li et al. 2012b; Landrein et al. 2013). These results indicate that the cortical microtubules have a crucial role in cell wall biosynthesis and plant morphology. Apart from the POM2/CSI1 interactions, the cortical microtubules may be tethered to the cytoplasmic side of the plasma membrane to maintain stable tracks for CSC movement. Indeed, contact sites between the cortical microtubules and the plasma membrane have been observed in different plant cells by electron microscopy (Murray 1983; Barton et al. 2008). These observations are supported by so-called plasma membrane ghost experiments, where plant protoplasts are attached to poly-L-lysine-coated glass slides followed by subsequent protoplast lysis. This results in release of cell material and only the membrane and membrane associated proteins remain on the slides (Marchant 1978). Microtubules are typically found together with the ghost membranes, suggesting a tight association of plant cortical microtubules and the plasma membrane (Marchant 1978; Akashi and Shibaoka 1991). The molecular mechanism for the association of cortical microtubules with plasma membrane is still largely unknown in plants, though both membrane-associated and -spanning proteins have been implicated in this process. The connection of the cellulose fiber-CSC-CSI1-microtubule forms a physical link between the cell wall, the plasma membrane and the microtubules. However, the lack of either CSI1, or of CSC subunits, did not result in detachment of microtubules from the plasma membrane, suggesting that this breach in connection is not severely affecting the microtubule tethering to the plasma membrane, and that other components therefore may contribute more substantially to this function.

CLASP

CLASP has been reported to link microtubule to plasma membrane in plants based on the observations that microtubule ends are frequently detached from plasma membrane in *clasp-1* mutant (Ambrose and Wasteneys 2008) (Figure 1A). CLASP is a conserved protein belonging to ORBIT/MAST/CLASP family of microtubule associated proteins (MAPs). In animal cells, CLASP also provides for the association between microtubules and plasma membrane (Lansbergen et al. 2006). It directly binds to microtubules and anchors the microtubule plus ends to the plasma membrane through the interaction with LL5β, which is a phosphatidylinositol-3,4,5-triphosphate (PIP3) binding protein (Lansbergen et al. 2006). However, plasma membrane binding partners of CLASP have not been identified in plants. Alternatively, CLASP was found to interact with retromer component sorting nexin 1, and to mediate the association of endosomes with microtubules (Ambrose et al. 2013). Therefore, it is possible that CLASP could form a transient association between the microtubules and the plasma membrane via retromer associated vesicles. Importantly, only short stretches of the microtubules displayed detachment from the plasma membrane in *clasp-1*, and the detached microtubules could also reattach to the cortex (Ambrose and Wasteneys 2008). This suggests that other components also partake in this process. It would be interesting to assess if the degree of microtubule detachment increases in a *pom2/csi1* and *clasp-1* double mutant, and also to investigate the behavior of the CSCs in *clasp-1*, in particular since *clasp-1* display a dwarf phenotype and altered cell shape (Ambrose et al. 2007; Ambrose and Wasteneys 2008).

Phospholipase D and phosphatidic acid

Phospholipase D (PLD) has been, and is, a hot candidate for the microtubule-plasma membrane connection; however, this connection has also been contested. The plant PLDs are subdivided into five subgroups, i.e. PLDα, PLDβ, PLDγ, PLDδ and PLDζ, based on their membrane association domains (Qin and Wang 2002). PLDs in *Arabidopsis* contain either PH/PX or C2 membrane association domains (Qin and Wang 2002), and transient expression of some PLDs does show plasma membrane localization (Andreeva et al. 2009; Zhang et al. 2012). Therefore, there is evidence for membrane association of the PLDs. A 90 kDa peptide (p90) in tobacco, sharing sequence similarity with *Arabidopsis* PLDδ, showed PLD activity and was associated with the plasma membrane and microtubules when transiently expressed in Bright Yellow2 (BY2) cells (Gardiner et al. 2001). Moreover, treatment with 1-

Table 1. Summary of proteins potentially involved in the connection of the cytoskeleton with the plasma membrane and the cell wall

Proteins	Subcellular localization	Connection with cytoskeleton, plasma membrane and cell wall	Reference
POM2/CSI1	Colocalization with CSC at the plasma membrane	Linker between CSC and cortical microtubules; contain a C2 domain that may bind to lipid surfaces.	Bringmann et al. 2012; Li et al. 2012b; Landrein et al. 2013
CLASP	Colocalization with microtubules	Forms a possible physical link between microtubules and the plasma membrane; microtubule-binding domain; mutants display microtubule-plasma membrane detachment phenotypes.	Ambrose et al. 2007; Ambrose and Wasteneys 2008; Ambrose et al. 2013
PLD	Plasma membrane and microtubules	Plausible microtubules-plasma membrane linking proteins; Contain PH/PX or C2 membrane association domains; PLDα generates PA that can affect MAP65-1 function.	Gardiner et al. 2001; Qin and Wang 2002; Ho et al. 2009; Zhang et al. 2012
		Possible mediators of the interaction between actin filaments and the plasma membrane; NtPLDβ directly binds to both F-actin and G-actin; its product PA could directly bind to the actin associated capping protein (CP).	Huang et al. 2006; Pleskot et al. 2010; Li et al. 2012a
ROPs and their interactors	Plasma membrane and microtubules	Signaling components that may link microtubules and plasma membranes; ROP2 and ROP6 associate with the plasma membrane via lipid-based posttranslational modifications; their interactor RIC1 directly binds to microtubules.	Fu et al. 2005; Fu et al. 2009; Lin et al. 2013
		ROP11/MIDD1/Kinesin13A complex forms a signaling bridge between the cortical microtubules and plasma membrane during secondary cell wall biosynthesis; both MIDD1 and Kinesin13A are microtubule associated proteins.	Oda et al. 2010; Oda and Fukuda 2013a, 2013b
CrRLKs	Plasma membrane	CrRLKs may form a link between the cortical microtubules and the cell wall; CrRLKs are plasma membrane integrated proteins with extracellular domains that could bind cell wall structures; The CrRLKs may transduce signals to microtubules via ROPs.	Hématy et al. 2007; Duan et al. 2010; Lindner et al. 2012
MDP25 (PCaP1)	Plasma membrane or cytosol depending on calcium concentration	Possible linkers between plasma membrane and microtubules; contains membrane association domains and microtubule binding domains; also binds to F-actin *in vitro*.	Nagasaki et al. 2008; Li et al. 2011; Qin et al. 2014
COBRA	Plasma membrane and cell wall	Binds to glucan chains via an extracellular cellulose binding module; connects to plasma membrane via a GPI anchor; microtubule localization pattern via immune-localization methods.	Roudier et al. 2005; Dai et al. 2011; Cao et al. 2012; Liu et al. 2013; Sorek et al. 2014
Class I Formin	Plasma membrane	Linkers between the plasma membrane, actin filaments, and possibly the cell wall; transmembrane domains; extracellular extension-like domains; microtubule and actin binding abilities.	Favery et al.2004; Cheung et al. 2010; Martinière et al. 2011; Yang et al. 2011; Zhang et al. 2011; Wang et al. 2012;

(Continued)

Table 1. (Continued)

Proteins	Subcellular localization	Connection with cytoskeleton, plasma membrane and cell wall	Reference
			Zheng et al. 2012; van Gisbergen and Bezanilla 2013; Wang et al. 2013
Class II Formin	Plasma membrane and microtubules	Linkers between the plasma membrane and actin filaments; membrane associated PTEN domains; microtubule and actin binding domains.	Li et al. 2010; van Gisbergen et al. 2012; Wang et al. 2013
NET1A	Plasma membrane and the plasmodesmata	Mediates actin and membrane interactions; directly binds to F-actin via NET actin-binding (NAB) domains.	Deeks et al. 2012
NET3C	ER-plasma membrane-associated puncta	Mediates the link between the plasma membrane and ER; forms a complex with VAP27, the actin and microtubule networks.	Wang et al. 2014

butanol, an agent affecting PLD activity, induced microtubule detachment from the plasma membrane in BY2 cells (Dhonukshe et al. 2003). Additionally, tubulin subunits were detected in pull-down assays, using PLDδ-GFP as bait, in transgenic *Arabidopsis* cell cultures (Ho et al. 2009). Based on these results, the PLDs were speculated to be linkers between microtubules and the plasma membrane. However, tubulin subunits are commonly detected in immunoprecipitation assays, and additional microtubule localizations of PLDs in plants have not been reported. Treatment with 1-butanol of *Arabidopsis* roots (Motes et al. 2005) and membrane ghosts (Hirase et al. 2006) displayed decreased microtubule signal, but the mechanisms behind this, either membrane detachment or effects on microtubules depolymerization, are unclear (Hirase et al. 2006). Hence, further investigations regarding the role of PLDs in microtubule-plasma membrane associations are necessary.

Apart from the plausible physical link of PLD between microtubules and plasma membrane, a function of PLDs via their product phosphatidic acid (PA) on microtubule activity is possible (Figure 1A). *In vivo*, PLDs hydrolyze plasma membrane phospholipids, such as phosphatidylcholine, to produce PA and free choline. When primary alcohols, such as 1-butanol, are exogenously applied, the phosphatidyl group is transferred to the alcohol and the PA production is inhibited. Therefore, the 1-butanol effects seen on microtubule detachment in BY2 cells could be due to decreased PA production (Dhonukshe et al. 2003; Gardiner et al. 2003; Motes et al. 2005). Interestingly, although PA cannot bind to tubulin directly it can interact with MAP65-1 (Zhang et al. 2012). This interaction promotes the association of MAP65-1 with the microtubules, and induces microtubule bundling (Smertenko et al. 2004). Therefore, decreased PA levels in a *pldα1* mutant resulted in microtubule depolymerization and salt sensitivity (Zhang et al. 2012). Based on these results, the authors proposed that the PLD generated PA forms a transient association between microtubules and plasma membrane via MAP65-1. Notably, cells lacking MAP65-1 only displayed microtubule depolymerization under salt stress without causing lateral movement of microtubules (Lucas et al. 2011).

Rho-GTPase in plants and their microtubule-related binding partners

Rho-GTPases in plants (ROPs) are Rho-like GTPases that act as signaling switches in plants (Zheng and Yang 2000; Craddock et al. 2012). Based on protein structures and on C-terminal hypervariable domains, ROPs are typically subdivided into two main types (Type I and Type II). ROPs are targeted to the plasma membrane by lipid-based posttranslational modifications, i.e. the addition of a lipid group to certain amino acids (Zheng and Yang 2000; Yang 2002). This addition promotes protein-membrane associations and protein-protein interactions, which influence plant growth and development (Running 2014). Three types of lipid modifications are known: prenylation, S-acylation and N-myristoylation. The first two modify cysteines that usually occur at the C-terminus of the proteins. Type I ROPs that contain a CaaX motif typically undergo prenylation (Sorek et al. 2007; Sorek et al. 2011). Type II ROPs that contain GC-CG boxes are S-acylated, and the polybasic region proximal to the box helps for a stable plasma membrane localization to occur (Lavy and Yalovsky 2006).

Among the ROPs, ROP2 and ROP6 together with their interactors, ROP-interactive CRIB motif-containing protein1 (RIC1), mediate microtubule-plasma membrane association in a dynamic manner (Wu et al. 2001) (Figure 1B). Although RIC1 only displayed weak microtubule affinity *in vitro*, it showed clear microtubule localization in pavement cells, indicating additional protein modifications or interactions of RIC1 *in vivo* (Fu et al. 2005). The association of RIC1 with microtubules causes microtubule bundling, which decreases cellulose crystallinity and therefore also affects cell wall characteristics (Fujita et al. 2011). Recent work from Lin et al. 2013 revealed that RIC1 also interacted with KTN1, the katanin p60 catalytic

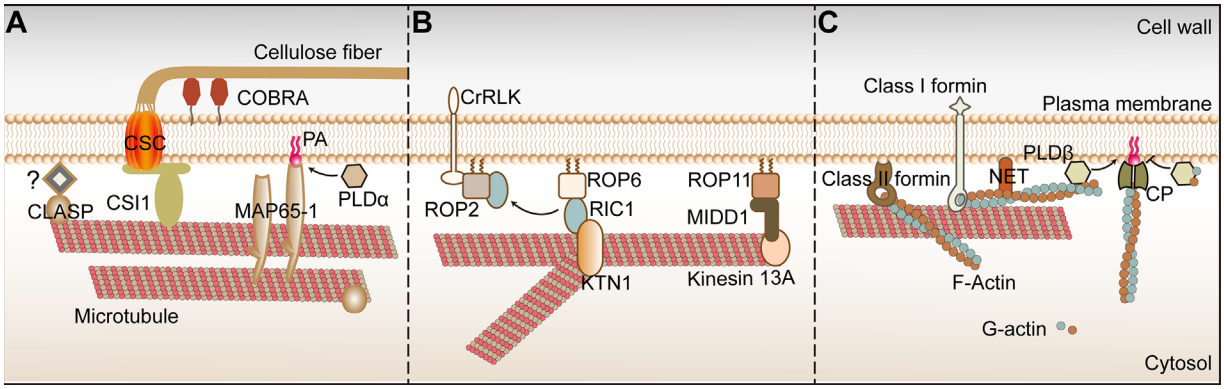

Figure 1. Components that potentially impact on the link between the cytoskeletons, plasma membrane and cell wall
(A) The cortical microtubules are tethered to the cytosolic side of the plasma membrane and form guiding tracks for the cellulose synthase complex (CSC); a process that is mediated by the cellulose synthase interactor 1 (CSI1). The active CSCs move along the cortical microtubule and synthesize cellulose chains that form para-crystalline microfibrils. The crystallization is potentially aided by the plasma membrane anchored protein COBRA. The microtubule plus end binding protein CLASP can anchor the microtubule end to the cell cortex, perhaps together with currently unknown interaction partners. The phospholipase Dα (PLDα) associates with the plasma membrane and hydrolyzes plasma membrane phospholipids, such as phosphatidylcholine, to produce phosphatidic acid (PA). The microtubule associating protein 65-1 (MAP65-1) binds to PA in the plasma membrane, and bundles antiparallel cortical microtubules, which make them less sensitive to salt stress. **(B)** The microtubule-plasma membrane-cell wall connection can be mediated by the Rho-GTPase in plants (ROPs) and their binding partner ROP-interactive CRIB motif-containing protein 1 (RIC1). ROP2 and ROP6 are prenylated to associate with the plasma membrane. The interaction of ROP6 with RIC1 can recruit the katanin subunit p60 (KTN1) to microtubule branching sites. The recruited KTN1 severs branched microtubules, and consequently form parallel cortical microtubules. ROP2 competes against ROP6 for the interaction with RIC1 and depletes RIC1 from the microtubules. This might be induced by the plasma membrane *Catharanthusroseus* receptor-like kinases (CrRLKs) which can interact with ROP2 and contains the predicted extracellular polysaccharide binding domain. During the secondary cell wall formation, the microtubule depletion domain 1 (MIDD1) is recruited by ROP11 and binds to microtubule ends. This plasma membrane-microtubule connection further recruits Kinesin13A, which depolymerizes microtubules, and determines the patterning of secondary cell wall pits. **(C)** Actin based interactions with the plasma membrane, cell wall and microtubules. The Class I formins contain a transmembrane domain and the extracellular part is predicted to bind to cell wall polysaccharides. The Class II formins associate with plasma membrane via their phosphatase and tensin (PTEN) domains. Formins could also bind to both actin filaments and microtubules. The networked (NET) superfamily of proteins can facilitate actin-membrane interactions. PLDs can also influence the actin filaments, for example PLDβ can directly bind to both actin filaments and monomeric G-actin. The G-actin interaction inhibits the PLDβ activity, while filamentous actin binding promotes the activity of PLDβ, which produces PA. PA then regulates the actin filament end dynamics by depleting the actin capping proteins (CPs). Please note that the relative sizes of the components are not drawn to scale.

subunit, in *Arabidopsis*, and thereby promoted microtubule-severing activity of KTN1 (Lin et al. 2013). The increased severing resulted in higher proportion of parallel microtubules and less lobes of *Arabidopsis* pavement cells (Lin et al. 2013). The binding of RIC1 to microtubules is antagonistically regulated by ROP2 and ROP6. Here, activated ROP2 binds to RIC1 and depletes RIC1 from microtubules, while active ROP6 promotes the association of RIC1 with cortical microtubules (Fu et al. 2005; Fu et al. 2009). By this dynamic interplay of activated ROPs, the pavement cell shape is tightly controlled to form the jigsaw-puzzle pavement cells. This highlights the important connections between ROPs, microtubules and the plasma membrane.

Another ROP, ROP11, was found to interact with the C-terminal of the microtubules depletion domain1 (MIDD1) and recruit MIDD1 to the plasma membrane domains where cortical microtubules are depolymerized by the microtubule end tracking proteins Kinesin13A (Oda et al. 2010; Oda and Fukuda 2013a) (Figure 1B). Simultaneously, the cortical microtubules also restrict the distribution of the ROP11/MIDD1/Kinesin13A complex along the plasma membrane, which results in shape and size variation of the pits in the xylem cells (Oda and Fukuda 2012). Both MIDD1 and Kinesin13A have microtubule binding activity *in vitro*, but *in vivo* Kinesin13A is recruited to microtubules by MIDD1 and then depolymerizes the cortical microtubules at sites where secondary cell wall pits are formed (Oda and Fukuda 2013b). Therefore, the ROP11/MIDD1/Kinesin13A complex forms a bridge between the cortical microtubules and the plasma membrane during secondary cell wall biosynthesis.

The kinesin13A was also found to genetically interact with THESEUS (THE1), one of 17 *Catharanthus roseus* receptor-like kinases (CrRLKs) in *Arabidopsis*, that is involved in the regulation of cell expansion (Hematy et al. 2007; Fujikura et al.

2014). THE1 may restrict cell expansion in primary wall cellulose deficient *Arabidopsis* seedlings. The report by Fujikura et al. 2014 therefore indicates that kinesin13A also influences primary cell wall formation. The CrRLK proteins contain an extracellular domain that shares sequence similarity with the carbohydrate binding malectin domain (Mal) from *Xenopus laevis* (Lindner et al. 2012). This suggests that the CrRLKs might recognize certain carbohydrates in the cell wall and perhaps sense the status of the cell wall in this way. Interestingly, another CrRLK protein FERONIA (FER) was co-immunoprecipitated with ROP_2 in a guanine nucleotide-regulated manner (Duan et al. 2010). The active ROPs can activate NAPDH-oxidases that produce reactive oxygen species (ROS), which controls root hair development and pollen tube growth by altering the cell wall architecture and intracellular signaling (Duan et al. 2010; Boisson-Dernier et al. 2013). Therefore, the CrRLKs may form an excellent linker between the cortical microtubules and the cell wall via ROPs and their putative interactors.

Other possible microtubule-plasma membrane linking components

Additionally, two *Arabidopsis* proteins, microtubule destabilizing protein25 (MDP25) and its homolog microtubule association protein18 (MAP18), which are also named as plasma membrane-associated cation binding protein 1 (PCaP1) and PCaP2, respectively, bind to the plasma membrane (Wang et al. 2007; Nagasaki et al. 2008; Kato et al. 2010; Li et al. 2011). Further, biochemical analysis revealed that both proteins could bind to microtubules and inhibit tubulin assembly *in vitro* (Wang et al. 2007; Li et al. 2011). Protein truncations and site directed mutagenesis revealed that the amino acids responsible for both membrane and microtubule interactions were located in the same region of MDP25 (Nagasaki et al. 2008; Li et al. 2011). These data were interpreted as that MDP25 could not bind to the plasma membrane and microtubule simultaneously. Therefore, the plasma membrane-microtubule connecting function of MDP25 is still obscure. One possibility is that the MDP25 plasma membrane localization is depleted when Ca^{2+} is released into the cytosol, and that it then can bind to and depolymerize microtubules.

The glycosylphosphatidylinositol (GPI) anchored protein COBRA might be another candidate connector for the cell wall-plasma membrane-microtubule continuum. Mutations in *COBRA* cause severe cell wall defects with swollen cells and decreased cellulose biosynthesis in different plant species (Roudier et al. 2005; Dai et al. 2011; Cao et al. 2012). Studies from both *Arabidopsis* and rice showed that COBRA can bind to cellulose via an extracellular cellulose binding module (Figure 1) (Liu et al. 2013; Sorek et al. 2014). This was proposed to aid the cellulose crystallization process, consistent with a decreased amount of crystalline cellulose in *cobra* mutants and in plants with reduced *COBRA* expression (Roudier et al. 2005; Liu et al. 2013; Sorek et al. 2014). Both live cell imaging and immune-labeling experiments showed that COBRA is located at the plasma membrane, possibly due to its GPI anchor (Liu et al. 2013; Sorek et al. 2014). COBRA was found to be secreted to the apoplast via a typical GPI-anchored protein path, and the secretion was disrupted by mutations in the putative GPI cleavage site or by truncation of the anchor region (Roudier et al. 2005; Liu et al. 2013). Interestingly, immune-localization analysis in *Arabidopsis* elongating root cells revealed a microtubule-like pattern of COBRA, which was disrupted by treatment of the microtubule disrupting agent oryzalin (Roudier et al. 2005). This indicates a connection of COBRA to the microtubules, probably in an indirect manner through the plasma membrane. However, the COBRA only partially overlapped with the microtubule signal (Roudier et al. 2005), and it is therefore unclear how the COBRA may connect to the microtubules.

Additionally, posttranslational modification of tubulin subunits may also influence the plasma membrane-microtubule interaction. Tubulin proteins contain palmitoylation (one type of S-acylation) sites and palmitoylated tubulin has been found in human and yeast (Wolff 2009). Palmitoylated α tubulin has also been reported in *Arabidopisis* (Hemsley et al. 2008). This could explain why microtubules are found in membrane fractions even after high salt treatment and after non-ionic treatment that normally remove membrane associated proteins, and abolish protein-protein interactions (Laporte et al. 1993; Sonesson et al. 1997). However, how, where and when plant tubulins are S-acylated and its effects on plant microtubules are unknown. An alternative way for the microtubules to attach to the plasma membrane would be via other membrane compartments, such as clathrin coated vesicles or the endoplasmic reticulum (Peña and Heinlein 2013). The insertion process of CSCs into the plasma membrane could generate one such possibility (Gutierrez et al. 2009).

PLANT ACTIN-PLASMA MEMBRANE-CELL WALL CONNECTIONS

The actin cytoskeleton plays a crucial role in the secretion of cell wall material in many cell types, including tip-growing cells, such as root hairs and pollen tubes (Mendrinna and Persson 2015). For example, in lily (*Lilium formosanum*) pollen tubes, disruption of filamentous actin by latrunculin B (LatB), an inhibitor that blocks actin polymerization by sequestering globular actin (Morton et al. 2000), resulted in aberrant pectin deposition (Rounds et al. 2014). The actin organization has also been shown to affect the distribution of CSCs at the plasma membrane in *Arabidopsis* interphase cells (Sampathkumar et al. 2013). Furthermore, mutations in subunits of the SCAR complex, a plasma membrane localized activator of the actin nucleator ARP2/3 complex, result in reduced cortical actin filaments and abnormal structure and composition of cell wall at intercellular junctions where SCAR complex are enriched in the adjacent plasma membrane (Dyachok et al. 2008). Taken together, membrane-actin contact sites are important for cell wall formation, and several proteins are proposed to mediate this connection.

Formin

A prominent candidate protein group for the associations of the plasma membrane and the actin cytoskeleton is the formins, an important actin nucleator protein family. Formins are defined by the formin homology 2 (FH2) domain, which

can bind to actin and generally are sufficient for actin nucleation (Cvrčková et al. 2004). As in other eukaryotes, *in vitro* biochemical analysis have shown that plant formins can regulate various aspects of actin dynamics, including actin nucleation/elongation, bundling, side binding and severing (Yang et al. 2011; Zhang et al. 2011; Wang et al. 2012; Zheng et al. 2012; Wang et al. 2013; van Gisbergen and Bezanilla 2013). Plant formins can be grouped into three clades (Class I–III). Class I and II members are found in angiosperms while members of the third clade (Class III) are only found in mosses and lycophytes (Cvrčková et al. 2004; Grunt et al. 2008; van Gisbergen and Bezanilla 2013).

Membrane anchoring properties have recently been proposed to be a common feature of plant formins, although the mechanisms for each clade appear different (Cvrčková 2013) (Figure 1C). Class I formins have a putative transmembrane domain, suggesting that they are integral membrane proteins (Cvrčková et al. 2004). Consistently, several Class I plant formins, including *Arabidopsis* FORMIN 1 (AtFH1), AtFH5 and AtFH6, are localized to the plasma membrane in *Arabidopsis* (Favery et al. 2004; Cheung et al. 2010; Martinière et al. 2011). Moreover, AtFH1 has an extracellular cell-wall extensin-like domain, which may link to the cell wall (Martinière et al. 2011). The AtFH1's ability to interact with both cell wall components and with the actin cytoskeleton is important for regulating the actin organization at the cell cortex, and consequently influences cytosolic trafficking (Martinière et al. 2011). Both formin inhibitors and mutations in AtFH1 induced actin bundling and hence a less dynamic actin cytoskeleton, which in turn caused reduced cell elongation (Rosero et al. 2013). A membrane association is also suggested for class II formins, albeit through a peripheral attachment. Class II formins often have an N-terminal domain with high sequence similarity to a phosphatase and tensin (PTEN) homolog thought to mediate lipid binding (Cvrčková et al. 2004; Grunt et al. 2008) (Figure 1C). For example, in moss, the PTEN domain of a Class II formin, For2A, can bind to $PI(3,5)P_2$, which is essential for formin function (van Gisbergen et al. 2012). Lastly, lower plant Class III and non-plant formins usually contain domains predicted to bind RHO GTPases that are membrane-associated (Cvrčková 2013).

Net protein family

A new family of actin-binding proteins, referred to as the networked (NET) superfamily, has been recently identified in plants (Deeks et al. 2012) (Figure 1C). In a screen of an *Arabidopsis* cDNA green fluorescent protein (GFP)-fusion expression library in tobacco leaves, the N terminus of NET1A, the founding member of this superfamily, was found to label actin filaments (Deeks et al. 2012). F-actin binding assays revealed that NET1A directly binds to F-actin *in vitro* via a novel actin-binding domain unique to plants, called the NET actin-binding (NAB) domain. Interestingly, the full length NET1A protein was associated with the plasma membrane and the plasmodesmata. A wider search, based on primary sequence homology, identified 13 NET proteins in *Arabidopsis* that could be grouped into four clades (Deeks et al. 2012). Proteins from each group decorated actin filaments in plant cells and labeled various membranes, such as the vacuole and nuclear membrane and pollen tube plasma membrane. Among them, NET3C localized to the ER plasma membrane-associated puncta and formed a complex with VAP27, actin and microtubule networks to link the plasma membrane and ER (Wang et al. 2014). These observations emphasize a potential role of NET superfamily members in mediating actin-membrane interactions. How the NET proteins may do so is, however, still obscure.

PLDs

Besides influencing microtubules as discussed above, PLDs and their product PA are also important regulators of the membrane-actin interface in plant cells (Figure 1C). Alterations in PLD activity and cellular PA levels can markedly change the actin organization in plant cells (Lee et al. 2003; Motes et al. 2005; Huang et al. 2006; Pleskot et al. 2010; Pleskot et al. 2012). Inhibition of PLD activity using 1-butanol led to disruption of actin filaments in multiple plant cells and organs (Motes et al. 2005; Pleskot et al. 2010), while elevation of PA levels, e.g. by exogenous application of PA, increased the density of cortical actin filaments (Huang et al. 2006; Pleskot et al. 2010; Li et al. 2012a). PLD and PA can regulate the actin cytoskeleton in at least two ways. On the one hand, recombinant plant PLDβ isoforms bind directly to both F-actin and G-actin, providing a potential link between the plasma membrane and the actin cytoskeleton (Pleskot et al. 2010). On the other hand, PA regulates actin organization via the capping protein (CP) (Huang et al. 2006; Li et al. 2012a). Here, PA can directly bind to CP and prevent its binding to the barbed end of actin filaments, resulting in an uncapping of actin filament ends, and therefore to the promotion of actin polymerization. Consistent with these observations, treatment of wild-type cell with exogenous PA phenocopied the actin filament end behavior in *cp* mutant cells, such as elevated free actin filament ends indicated by higher annealing frequency (Li et al. 2012a). Moreover, the activity of PLDβ is in turn modulated by actin binding in a polymerization-dependent manner, i.e., filamentous actin activates PLDβ, while monomeric actin inhibits its activity (Pleskot et al. 2010). This positive feedback mechanism could facilitate signal amplification and provide an important mechanism to locally increase membrane-F-actin dynamics at the cortex of plant cells (Pleskot et al. 2010).

CONCLUSION AND PERSPECTIVES

Recent studies have revealed that cell wall production relies on a close interaction between the plasma membrane and the underlying cytoskeleton in plants. The cell wall, plasma membrane and cytoskeleton nexus could be mediated by a single bridging protein or through several proteins that form a scaffolding structure or that may participate in signaling pathways. However, the roles of the already identified candidate proteins are not clarified and further studies in this field are needed. It may be anticipated that high-end proteomics will aid in the identification of additional proteins that link the cytoskeleton and the plasma membrane and that *in vitro* and *in vivo* work using high-end cell biology techniques will reveal their functions. These data should significantly advance our understanding for how the cytoskeleton is tethered to the plasma membrane and will likely reveal insights into differences in cytoskeletal arrangements compared to yeast and animal cells.

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