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Review Article

The systems and interactions underpinning complex cell wall patterning

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Cell walls can confer amazing properties to plant cells, particularly if they have complex patterns. Complex cell wall patterns in the primary cell wall often lead to complex cell shapes, whereas in the secondary cell wall they lead to advanced material properties that prepare cells for mechanically demanding tasks. Not surprisingly, many of these structures are found in water transporting tissues. In this review, I compare the mechanisms controlling primary and secondary cell wall patterns, with emphasis on water transporting tissues and insights derived from modeling studies. Much of what we know about this is based on complex cell shapes and primary xylem patterns, leading to an emphasis on the Rho-of-plants — cortical microtubule — cellulose microfibril system for secondary cell wall patterning. There is a striking diversity of secondary cell wall patterns with important functional benefits, however, about which we know much less and that may develop in substantially different ways.

Introduction

If plants were man-made machines, their cell walls would be said to consist of fancy metamaterials: materials in which not only the chemical properties of the constituents, like cellulose, but primarily their physical arrangement into microscopic structures determines their physical properties. Through controlling cell wall structure, cell walls can be adjusted to a wide range demands with a relatively small set of chemical constituents. These wonderful material properties, moreover, develop in-place and according to local demands.

In this review, I will discuss some of the most fascinating cell wall structures, their function and what we currently know about how they are formed.

The cell wall

Plant cell walls consist of multiple layers (Figure 1; [2–4]). Typically, the formation of the cell wall starts during cytokinesis with the cell plate, which completes cell division [5]. Subsequently, the wall is modified and thickened through the deposition of additional materials between the plasma membrane and existing wall [5]. From outside to inside, a mature cell wall consists of a pectin rich middle lamella that glues neighboring cells together [6]; a primary cell wall that mainly consists of cellulose microfibrils (CMFs), embedded in a matrix of pectins and hemicelluloses; and, depending on cell type, a secondary cell wall rich in CMFs, hemicelluloses and the more hydrophobic lignin. Plant cells grow through the plastic (i.e., irreversible) extension and simultaneous assembly of the primary cell wall [7]. The much thicker secondary cell walls do not allow for extension, and are typically produced when cells have nearly or completely stopped growing. Additional proteins and polymers like callose and suberin further modify local cell wall properties [2–4]. For more details on the chemical composition of the (primary) cell wall and its dynamic regulation, see [4, 8, 9]. For a detailed review on the structure of secondary cell walls, see [1, 2].

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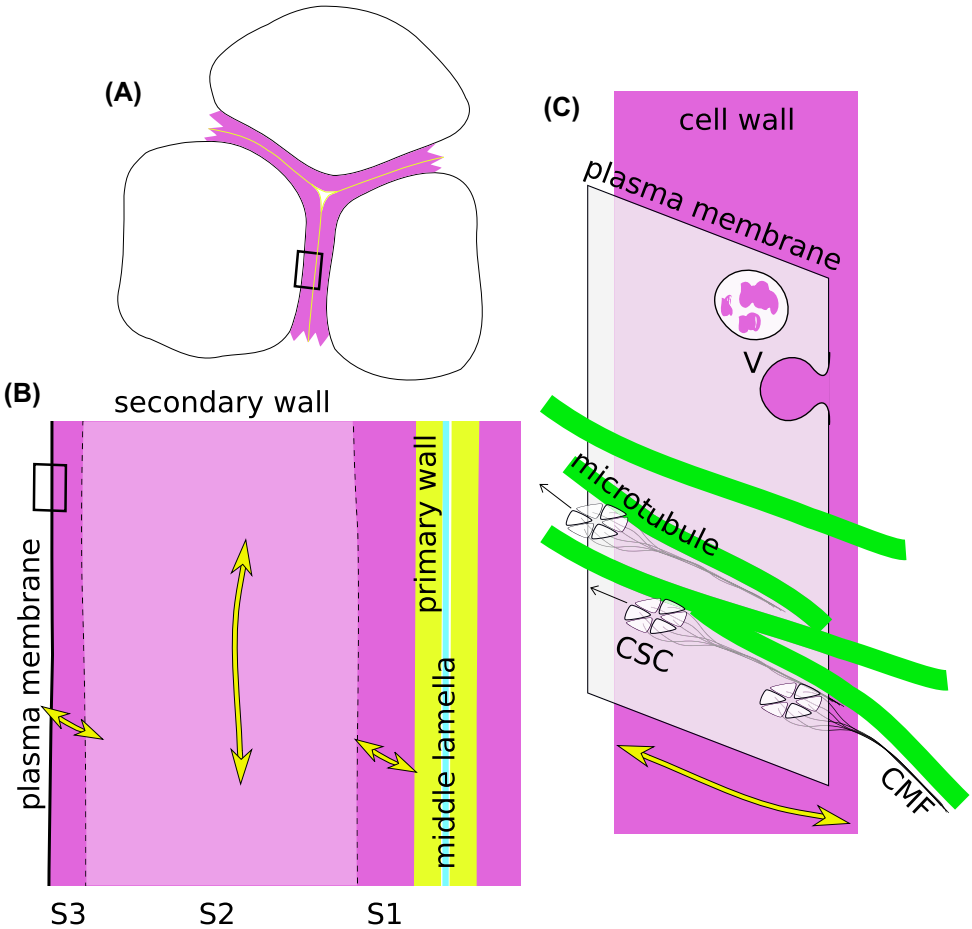

Figure 1. Cell wall structure and construction.

(**A**) Example of a thick (tracheid) cell wall. The cell wall fully surrounds the cell, but only part of it is drawn. (**B**) This cell wall consists of multiple layers. Only a small part of the secondary cell wall of the right cell is depicted. The mature secondary cell wall consists of three layers (S1, S2, S3, indicated with different shades of magenta) that can be recognized using polarized light due to the different main orientation of the cellulose microfibrils (CMFs) (indicated by yellow double headed arrows) [1]. (**C**) Deposition of cell wall materials. Vesicles (V) deliver various materials via exocytosis (dependent on the actin cytoskeleton; not drawn). CMFs add anisotropic mechanical properties to the cell wall and are deposited by cellulose synthase complexes (CSCs). These CSCs are propelled by the polymerization of the CMFs and tend to follow cortical microtubules that reside on the cytoplasmic side of the plasma membrane. CSCs in primary and secondary cell walls are composed of different subunits, and show different CMF polymerization rates [2].

CMFs, the main load-bearing component of the wall, control the direction of anisotropic expansion of primary cell walls if they are deposited in highly aligned layers (Figure 3). In isotropically expanding cell wall sections, however, the CMFs are much less aligned [7]. By varying the cellulose anisotropy and the local degree of (irreversible) cell wall expansion, many complex cell shapes can be formed (Figure 3A; [3, 7]). Where the CMFs are deposited and with which orientation, is primarily guided by the cortical microtubule array (Figure 1C; [2, 10–12]).

The cortical array is found in most interphase cells and consists of dynamic microtubules (Figure 2A) that are attached to the inside of the cell membrane. These microtubules can self-organize into highly aligned structures through the frequent collisions they have among themselves and their angle-dependent outcomes (Figure 2B,C) [14, 15, 20]. Contrary to animal systems, the microtubules of the cortical array are not nucleated from a single microtubule organizing center, but nucleation occurs distributed throughout the array and predominantly from existing microtubules [23, 24]. Theoretical studies have clearly demonstrated that the details

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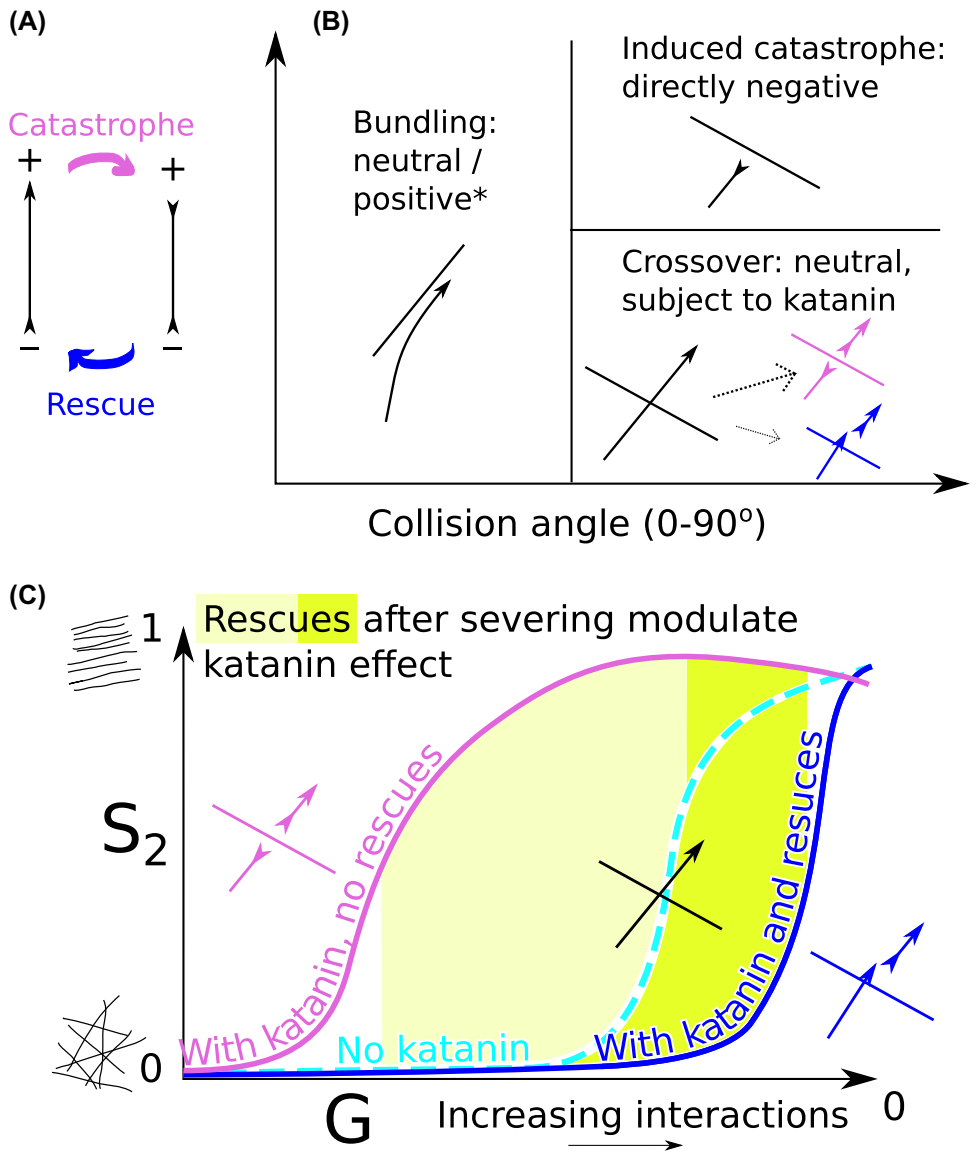

Figure 2. Dynamics of cortical microtubules determines their spontaneous alignment. Part 1 of 2

(**A**) Individual microtubules grow through polymerization at their ends, particularly the plus-end (+). They frequently switch between the growing and shrinking state. These transitions are called 'catastrophe' and 'rescue'. The minus-end (–) is less dynamic and shows net retraction [13] (once released from the nucleation complex by katanin severing). (**B**) Being confined to the cell cortex, cortical microtubules interact via frequent collisions. The outcome of such collisions depends on the relative angle: bundling, continued growth along the obstructing microtubule, for angles less than $\pm 40°$; crossover (continued growth) or induced catastrophe for larger angles [14]. Induced catastrophes confer a direct penalty to microtubules growing against the majority orientation. This reduces the life time of such microtubules and is the main driver of spontaneous alignment. This mechanism is called 'survival of the aligned' [15]. Crossovers are subject to katanin severing. The probability of 'immediate' rescue of the newly formed plus-end can be tuned and determines the impact of katanin [16]. *: With static minus ends, bundling is a neutral interaction [17]. With retracting minus ends, however, it contributes to increased alignment [18]. (**C**) Sketch of bifurcation diagram based on Deinum et al. [16]. *G* on the horizontal axis is a so-called control parameter that collapses the dynamic parameters of individual microtubules to a single number. As *G* increases, the number of interactions per microtubule life time increases (see [19, 20] for more explanation). S_2 on the vertical axis, an order parameter from polymer physics, quantifies the degree of alignment from 0 (isotropic) to 1 (perfectly aligned). With sufficient interactions, the cortical microtubules will spontaneously align (towards the right of the diagram). Crossovers are themselves neutral, but are subject to *crossover severing* by katanin, which primarily affects the latest arriving microtubule. In the common case that the newly

Biochemical Society Transactions (2024) **52** 2385–2398
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Figure 2. Dynamics of cortical microtubules determines their spontaneous alignment. Part 2 of 2

formed plus-end starts in the shrinking state, severing turns crossovers into negative interactions, albeit weaker than induced catastrophes and with a delay. This increases the parameter regime for spontaneous alignment (indicated with light yellow shading between magenta and dashed cyan curve) [16]. If, however, the newly formed plus ends often undergo an 'immediate' rescue (blue curve, e.g., during blue light-induced reorientation [21], which depends on the protein CLASP [22]), the minority direction could actually become amplified, shrinking (or abolishing) the aligned regime and leading to a rapid breakdown of the existing aligned array. The light + dark yellow shaded area between the two katanin curves together indicate the parameter regime in which temporarily increasing the fraction of rescues after severing can be employed to rapidly change the array structure.

of cortical microtubule nucleation have profound influence on array behavior, including its orientation, homogeneity and potential to adopt special patterns [20, 25–28]. The organization of the cortical array can change dramatically while cells develop, e.g., reorient when seedlings emerge above ground [21], or during stomatal guard cell development [29].

Structures in the cell wall

Complex structures in the cell wall have profoundly different effects, depending on whether they occur in the primary or secondary cell wall (Figure 3). Through localized enzyme action and excretion of cell primary wall materials, cells can control where they expand, whereas cell wall stiffening and the aligned deposition of CMFs can locally constrain and orient expansion (Figure 3A). These principles form the basis of many complex cell shapes like puzzle shaped (leaf) epidermal cells [35–39], trichomes with multiple branches [30], or tip growing pollen tubes and root hairs [40–42], the latter of which may branch [31] or curl (in response to nodulation factors) [43, 44]. The dynamic changes in cell shape may feed back on the systems that pattern the secretion of wall materials. Patterns in primary cell wall structure can, moreover, guide turgor driven, reversible cell deformations, enabling, e.g., opening and closing of stomatal guard cells [29, 45, 46] and bending of *Mimosa pudica* leaves/leaflets upon touch, at a specialized organ, the pulvinus (Figure 3B) [32].

In case of patterned secondary cell wall reinforcements (Figure 3C1–C4), cells typically do not change shape during the patterning process and possibly not even grow. The best studied example of this is the formation of primary xylem patterns in angiosperms. While the cells themselves maintain a relatively simple, near cylindrical geometry, their cell walls show banded/spiral (protoxylem: Figure 3C1) or net-like/gapped (metaxylem: Figure 3C2) patterns of cellulosic deposits that are later lignified. These different patterns reflect different mechanical requirements. Protoxylem matures earlier during root development, when the tissue still elongates, whereas metaxylem is inextensible but supports wider vessel diameters [2, 47]. Carlquist [48] presents an interesting overview of functional differences between gymnosperm earlywood and latewood that are formed during different phases of the growth season and how the varying challenges on water transport are solved with adaptations at the vessel level, including different cell wall patterns/structures. Additionally, a variety of structures on the walls of water transporting tissues is thought to enhance drought resistance, from 'pegged rhizoids' in liverworts (Figure 3C5) [34] to small scale surface roughness of various patterns on xylem walls [49]. The latter is called wall sculpturing. Wall sculptures occur more frequently in temperate than in tropical trees [50]. It is thought to increase the resistance to embolism via increasing the wettability of the surface (an opposite 'lotus effect') [49, 51, 52]. Such sculpturing occurs in various patterns, including small 'warty' protrusions and helical thickenings that occur on the inside of pitted vessel walls (Figure 3C3,C4; [52, 53]). Besides these key examples, an even wider diversity in secondary cell wall patterns exist. For a more elaborate review, see [2].

Plasmodesmata: neglected aspect of cell wall mechanics and structure

Almost all cell walls separating neighboring cells contain holes in the form of plasmodesmata (PDs), narrow channels that are critical for intercellular communication/exchange and normal development. The first (primary) PDs are formed when the cell plate is deposited, but additional (secondary) PDs may be formed later on [54]. PDs can have various structures and may be clustered in pit fields, particularly in interfaces that sustain large fluxes through PDs [55, 56]. Cell walls surrounding PDs are enriched in callose, which is (also) used to rapidly regulate PD aperture [56]. The presence of PDs locally disrupts the CMF organization,

Biochemical Society Transactions (2024) **52** 2385–2398
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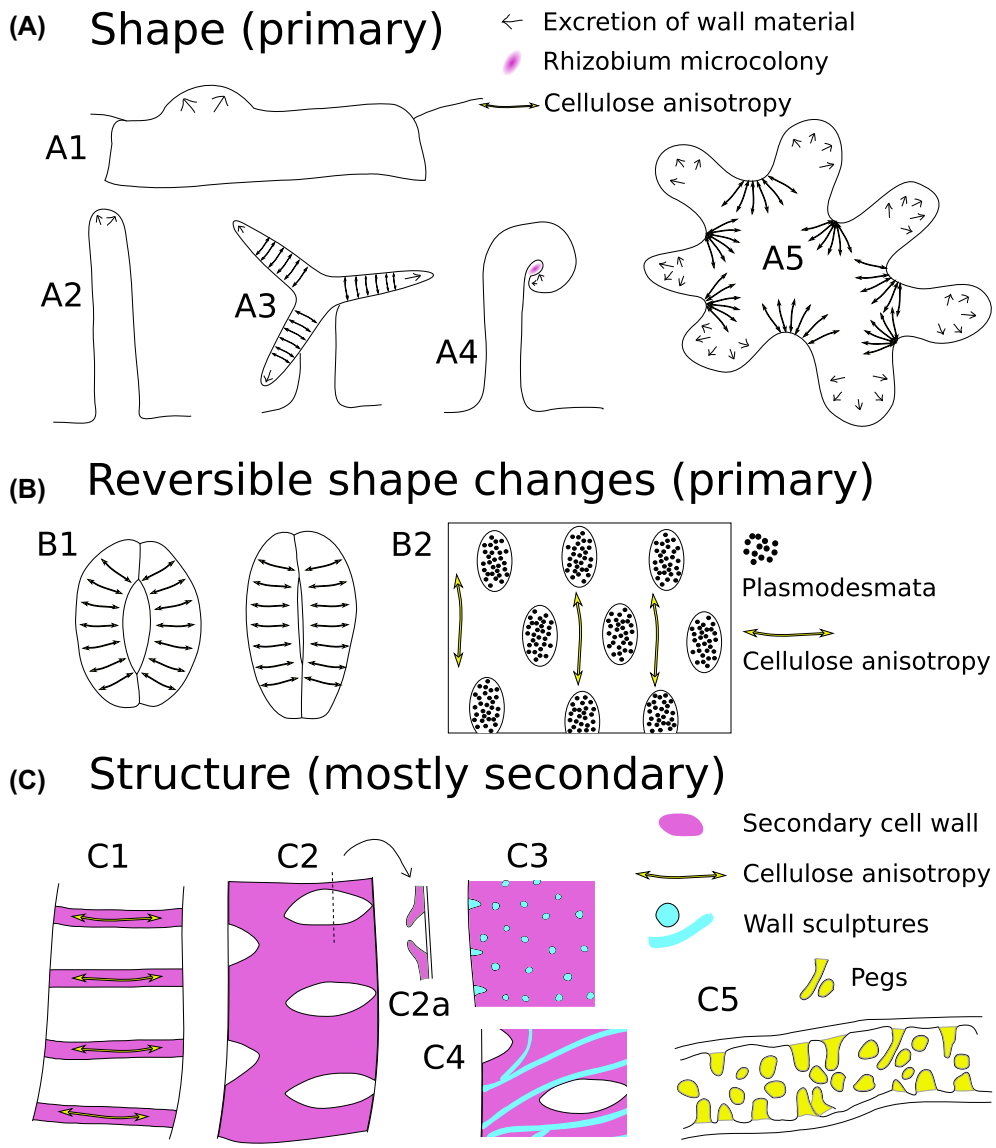

Figure 3. Complex cell wall structures at multiple levels.

(**A**) Localized excretion of primary cell wall material and wall loosening enzymes, combined with local constraints on cell wall expansion, leading to complex cell shapes through localized plastic wall deformation. A1: bulging of a trichoblast is the first step in trichome or root hair formation [30, 31]; A2: tip growing root hair or pollen tube; A3: trichome/mutant root hair with multiple branches; A4: legume root hair curling in response to nodulation factors produced by a *Rhizobium* sp. microcolony (pink dot); A5: puzzle shaped leaf epidermal cell diffusely extending its lobes. (**B**) Structures in the primary cell wall supporting reversible cell shape changes through fully elastic (i.e., reversible) deformations upon changes in turgor pressure. B1: (dicot) stomatal guard cells in open (turgid: left) and closed (flaccid: right) state. Inhomogeneous cell wall thickness and anisotropy of the CMFs facilitate opening and closure. B2: highly anisotropic *Mimosa pudica* pulvinus wall with co-oriented ellipsoid primary pit fields. This structure facilitates rapid bending of the pulvinus. When the turgor pressure increases, the wall extends mainly perpendicular to the CMF orientation [32]. (**C**) Many patterned cell wall structures, often secondary cell wall reinforcements (pink), occur in relation to water transport. All these examples are deposited when cell growth has (almost) ceased. C1: example (angiosperm) protoxylem: ringed pattern. After maturation and programmed cell death, these vessels can still be extended. C2: example (angiosperm) metaxylem: pitted pattern. Mature vessels cannot be extended. Orthogonal detail C2a shows overhanging borders/'arches' of bordered pits [33]. C3,4: so-called sculpturing of xylem walls. Note these patterned depositions are placed on top of potentially patterned cellulosic secondary cell wall material. C3: warted pattern; C4: helical pattern. C5: example of a liverwort's pegged rhizoid. Density and shape of pegs (yellow) is highly variable within and among species [34]. (**A**–**C**) Yellow double headed arrows indicate orientation of CMFs in cases where these are is highly anisotropic.

Biochemical Society Transactions (2024) 52 2385–2398
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particularly in case of pit fields [55]. Cell wall thickness may differ substantially within a cluster of PDs compared to farther away [57]. This variation in thickness may help explain why PDs are often clustered, despite the (mild) reduction of effective wall permeability this brings about compared to evenly spaced PDs [58]. Alternatively, the clustering may arise for developmental reasons [55]. Surprisingly little is known, however, about the regulation of PD and pitfield distributions [59]. Rho-of-plants (ROPs) (see below) might be involved [60, 61], although the (perpendicular) microscopy viewing angle in this study did not allow for appropriate visualization of pit fields.

Although the mechanical impact of isolated PDs on the cell wall as a whole is typically ignored, pathogens like rice blast fungus use PDs as weak spots in the cell wall for their intercellular spreading [62, 63].

Finite element calculations of wall mechanics of the legume pulvinus (Figure 3B2), however, show that PDs clustered in pit fields could substantially impact wall mechanical properties: the ellipsoid primary pitfields, coaligned with highly aligned CMFs, aid in rapid organ bending at the pulvinus [32, 64]. Curiously, these cells are not elongated in shape, suggesting that the strong anisotropy of the CMFs may arise after cells reach their mature size.

ROP system for local cell wall adaptation

ROP proteins[1] are involved in diverse cases of cell wall patterning, with two well studied examples in Figure 4. ROPs form a plant-specific subfamily of the Rho-GTPases that evolved early during streptophyte evolution [65]. They are molecular switches that interact with various effectors when they are in their active state. Additional protein families tune ROP activity: ROP activation and inactivation are promoted by guanine nucleotide exchange factors (GEF) and GTPase-activating proteins (GAPs), respectively, and guanine nucleotide dissociation inhibitor (GDIs) take inactive ROP from the membrane to the cytosol. Many ROP effectors affect the cytoskeleton and, through that, the localized deposition of cell wall materials [66–68]. Intracellular patterns of active ROP can form through a reaction–diffusion mechanism, with diffusion of active ROP at the membrane being slower than diffusion of inactive ROP [68–70]. This way, ROPs control cell shape, e.g., in tip growing pollen tubes and root hairs [31, 42] and puzzle shaped leaf epidermal cells (Figure 4A) [35–37, 67]. Also patterned secondary cell wall deposition in protoxylem and metaxylem is controlled via ROPs (Figure 4B) [71, 72].

Much more can be said about ROPs themselves. For recent reviews, see: ROP and GEF diversity [61], the relation between mathematical models of ROP patterning and the molecular diversity in the different components of the system [68], the role of nano-domains in ROP signaling [66, 67].

Nontrivial question: coexistence of many ROP peaks/clusters

The examples in Figure 4 have many active ROP clusters and pavement cells even develop additional clusters as cell size increases [73, 74]. This is only possible with a ROP system that allows for the stable coexistence of multiple clusters of active ROP [68, 75]. In the simplest mathematical models of small GTPases, however, the stable solution is that all active GTPase ends up in a single cluster [68, 75]. This behavior was no problem in the original context of cell polarization, but turned out problematic in describing occasional yeast cells with multiple simultaneous budding sites and absolutely prohibited proper modeling of pavement cells and xylem patterning. Ultimately, the behavior is independent of the number of ROPs involved [75] and originates from the assumption of mass conservation: ROPs are only interconverted between states, but there is no protein turnover. Much has been written about this problem (e.g., [68, 76]), and multiple solutions have been proposed [69, 75, 77]. These solutions all have in common that the intrinsic competitive advantage of the largest cluster is balanced by a disadvantage that increases with size, in other words: a balance between positive and negative feedback [68, 75].

Complex cell shapes

Much is written about the development of complex cell shapes through the localized deposition of primary cell wall materials and how this is coordinated with help of ROP proteins (see [42, 66, 78, 79] for some excellent reviews).

Figure 4A shows a simplified description of the pavement cell, a widely used model system for cell shape. In short, ROP proteins divide the cell into different zones, which via effectors (here: RIC4, RIC1) recruit different cytoskeletal elements. In the actin zones, the cell wall materials and loosening enzymes are delivered to allow

[1]ROPs are also called Rho-GTPases from plants.

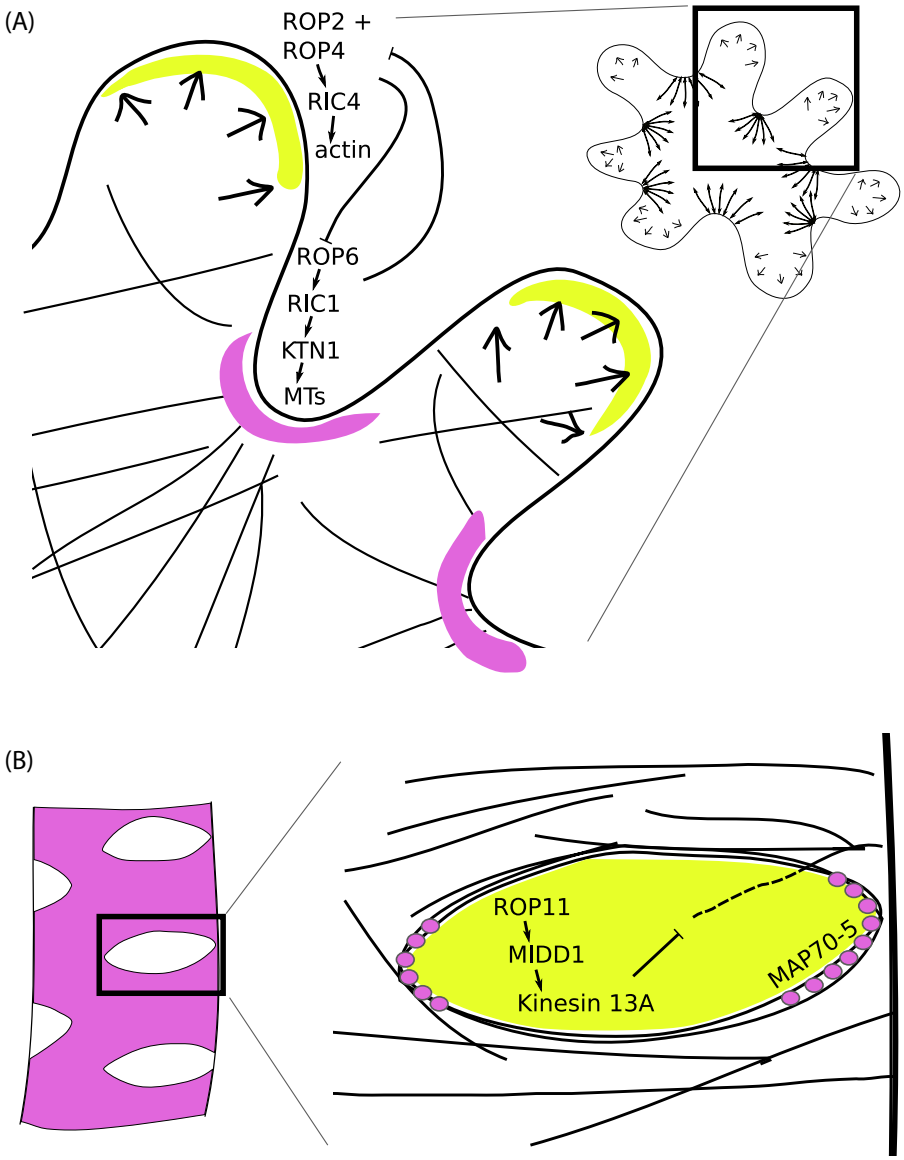

Figure 4. ROPs contribute to complex cell shape and structured cell walls via partitioning the membrane into different zones.

ROP and other protein numbers refer to *Arabidopsis*. (**A**) Consensus model of key players involved in pavement cell shape development. Mutually inhibitory microtubule (MT) friendly (magenta) and actin friendly (yellow) zones are formed, in which the ROP-interactive CRIB motif-containing protein (RIC) effectors promote the polymerization of the respective cytoskeletal elements. The actin cytoskeleton aids the local delivery of primary cell wall materials and wall loosening enzymes, whereas microtubules constrain expansion via controlling the orientation of new CMF deposition. Katanin is required to relay the effect to microtubules (drawn as black lines). (**B**) Consensus model of the key players in metaxylem pit formation. AtROP11 recruits MIDD1 and Kinesin 13A, which locally promote the depolymerization of microtubules. MAP70-5 (magenta circles) decorates the microtubules at the pit boundary and reduces their persistence length.

local expansion. In the microtubule zones, correlated CMF deposition stiffens the wall and may restrict the orientation of cell wall expansion [42, 66, 78, 79]. This zonation in wall structures sustains a mechanical feedback mechanism that exaggerates the lobed cell shape, although recent evidence suggests that lobe initiation itself starts with localized pectin stiffening or even mechanical buckling [38, 80, 81].

Pavement cells have their puzzle shape to increase mechanical stability of the tissue with multiple mechanisms proposed: preventing cell burst [74, 79], preventing tissue crack propagation [39] and reversible

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accommodation of mechanical stretching [82]. For this, the coordination of lobes and indents between neighboring cells is at least as important as the shape of individual cells. This calls for a new generation of multi-level models, adding auxin and interacting receptors TMK1 and ABP1/ABL1/ABL2, as well as mechanical coupling between cells [66, 79, 83, 84].

Spotlight: the role of katanin in pavement cell morphology

Locally aligning cortical microtubules in lobes (downstream of ROP6 and RIC1) genetically depends on katanin (KTN1 in Figure 4A) [85]. Katanin is also required for hyperalignment of the cortical array in pavement cells under artificially increased mechanical stress [86, 87]. This microtubule severing protein [88, 89], however, can act in different ways. Katanin preferentially severs at microtubule crossovers (Figure 2B), with a preference for the later arriving microtubule, which most likely is on the exposed, cytoplasmic side of the crossover [21, 90]. Theoretical work shows that crossover severing increases the parameter regime of spontaneous alignment, provided a large majority of the plus-ends formed through severing appears in the shrinking state (Figure 2C). This difference creates a regime where loss of katanin severing results in reduced microtubule alignment [16]. The fraction of rescues immediately following crossover severing can be increased by the protein CLASP [22], to the point that microtubules of the minority orientation are amplified [91, 92] and alignment breaks down (Figure 2C). In this state, katanin actively contributes to array reorientation [21, 22, 91–93]. Indeed, array orientation is more stable in the *clasp-1* mutant [22, 94]. Finally, the animal/*in vitro* literature suggests that katanin may increase microtubule stability via making incomplete cuts that subsequently heal with GTP-tubulin, leaving behind transient 'rescue-sites' [95–97]. This mechanism could potentially enhance a specific subset of microtubules, e.g., those under tensile stress, but this needs further investigation.

Notably, the *clasp-1* mutant shows hyperaligned arrays in pavement cells, with less deep indents/less pronounced lobes, and their orientation more tightly aligned with the likely orientation of supracellular wall mechanical stresses [87]. With the above mechanisms in mind, this suggests that katanin's contribution to pavement cell morphology depends on the local induction of alignment via crossover severing, and that CLASP normally dampens this effect by promoting rescues after severing (see also: [79]). Following frequent rescues after severing, the final orientation must be selected via some bias in microtubule stability [92]. Simulations by Smithers [98] also demonstrate the importance of selectively stabilizing microtubules by orientation for reproducing the typical pavement cell pattern.

Primary xylem

The influence of ROPs in primary xylem is best established for metaxylem (see also Figure 4B, [71, 99, 100]). Much of what we know of the process is discovered via induction of the VND6 and VND7 transcriptional master regulators, which induce ectopic metaxylem and protoxylem formation, respectively [101], and allow for detailed study of the proteins involved in the outer surface of the epidermis or cell culture. In Arabidopsis metaxylem, patches of active AtROP11 form, which recruit MIDD1 and Kinesin-13A, which in turn lead to local depolymerization of microtubules [71, 99]. Simulations in the protoxylem context show that such local 'hostile zones' subsequently translate to an array with dense and sparse regions in accordance with the ROP pattern [28, 102]. After the pattern is established in the cortical microtubule array, subsequent cellulosic secondary cell wall deposits occur in the microtubule dense regions, establishing the typical xylem cell wall structures [102].

Although it is intuitively assumed unlikely that protoxylem patterning is governed by completely unrelated proteins, and striated ROP patterns have been observed in developing protoxylem [72, 103], protoxylem patterning is genetically much harder to perturb, as, e.g., even the *Atrop7/8/9* triple knockout still shows a banded pattern in the protoxylem cell wall, albeit with an altered band–band spacing [72].

Whether ROPs also play a role in pegged rhizoid (Figure 3C5) wall structuring, however, is an open question. Peg formation precedes cell death [104], but compared to the above understanding of xylem patterning, there are several differences (based on *Marchantia polymorpha* [104]): (1) the pattern appears base-to-tip [104], rather than simultaneously per cell as in protoxylem [102]; (2) the transcriptional regulation is not via MpNAC5, the sole ortholog of the VND genes in the *M. polymorpha*, but ZHOUPI and ICE [104], which in *Arabidopsis* regulate endosperm breakdown to make space for the developing embryo on the seed [105]; (3) RNAseq including *Mpzou1* and *Mpice1* mutants, which lack pegged rhizoids [104], and TEM analysis [34] suggests a different balance of wall components including much less cellulose in pegs. Whereas the last point

suggests the cortical microtubule/CMF module is not involved, the involvement of ROPs in peg positioning remains an open question.

Similarly, little is known about the involvement of ROP patterning in wall sculpturing. The phenomenon is widespread among gymnosperm and angiosperm woody species (e.g., [50, 53, 106]) and, hence, little studied genetically. Enzymatic digestions of the warty layer in *Cryptomeria japonica* show that they consist of a (xylanase digestable) arabino-4-*O*-methylglucuronoxylan core coated by lignin [107]. It is unclear to what extend this result generalizes. The warts in this particular species are small, with a 200–300 nm spacing (both larger, micrometer spaced warts and smaller warts also exist [52, 106]). From a geometrical consideration, such a pattern seems ill compatible with what we know about CMF deposition. The formation of long, thin stripes for helical sculpturing patterns, however, seems possible or even most easily executed with help of long fibrils. To my knowledge, however, nothing is known about the chemical composition of helical sculptures. Overall, the range of shapes and sizes of wall sculptures could imply that multiple different patterning mechanisms are employed across species.

Linking ROPs and microtubules

The above summary of ROP patterning in primary xylem may suggest that microtubules merely follow the ROP pattern. Theoretical work shows, however, that a well oriented and well aligned cortical array is important for the formation of straight protoxylem bands or spirals [108]. Also various experimental observations show that the microtubules themselves feed back on the developing ROP pattern [26]. The katanin loss-of-function mutant, which typically leads to less aligned arrays [16, 88, 89], shows slower band formation as well as less straight bands and more remaining connections between bands [102]. Also mutations in microtubule related proteins like MAP70-5, IQD13 and CORD1/2 (see below) affect xylem patterns.

Metaxylem pits have well rounded boundaries, which at some stage during their development are lined by a microtubule bundle [109]. Such high curvature, however, is at odds with the *in vitro* persistence length of microtubules in the millimeter range [17, 110]. MAP70-5 is found specifically at the boundaries of developing metaxylem pits [111, 112]. *In vitro*, this protein reduces the microtubule persistence length and promotes the formation of circular microtubule bundles [110]. Tuning of the persistence length is important for array behavior, as a moderate persistence length (hundreds of micrometers) allows microtubules to more easily adjust to a specific ROP pattern, including straight protoxylem bands, but too low persistence length (e.g., the 26 μm used in [113, 114]) jeopardizes array alignment [28]. Also CORD proteins, which promote local detachment of microtubules, contribute to the oval shape of metaxylem pits [109]. They also reduce the overall degree of alignment outside the gaps [109]. IQD13, which promotes microtubule attachment to the membrane, has the opposite effect, with loss of function leading to more circular metaxylem gaps and overexpression leading to more elongated ones [100]. The opposite effects of CORD and IQD13 mutations on pit shape could be explained in a unified way via their effect on how strongly microtubules hinder ROP diffusion at the membrane and how anisotropic they thus make ROP diffusion. This view is supported by the ROP patterning model of Jacobs et al. [108], in which increasingly anisotropic ROP diffusion leads to more elongated gaps and, in the extreme case, even to banded patterns like those observed with strong IQD13 overexpression [100].

Given the mutual feedbacks between ROP and microtubule patterns, it is imperative that the next generation of models will dynamically couple both systems: stochastic microtubules and dynamic ROPs. Currently, two models make a start at combining both systems. Jacobs et al. [108] includes both ROPs and microtubules in a xylem patterning model, but the microtubules are included as density fields that affect ROP diffusion anisotropy, not as dynamic discrete obstacles. The anisotropic ROP diffusion has a profound impact on the resulting ROP patterns [108]. The pavement cell model by Smithers [98], on the other hand, contains explicit stochastic microtubules (albeit without katanin severing at crossovers, finite persistence length and realistic microtubule-based nucleation, which are known to affect array alignment and how well the array responds to local vs. global cues [16, 26–28]), but these do not influence ROP diffusion anisotropy. Responsiveness to mechanical stress is included via an elegant proxy: when microtubules hit the edge, they may undergo a catastrophe depending on the local edge curvature and angle of incidence. The partial differential equation model of ROP2, ROP6 and RIC1 shows high RIC1 activity at indent regions and only close to the cell edges. The different rules used for coupling the stabilizing effect of RIC1 microtubule dynamics show that the typical patterns of microtubule organization (Figure 4A) [86, 87] can be reproduced if the RIC1 effect is picked up mostly locally and enough microtubules become long enough to sample the edge regions. See also the discussion about how cell

Biochemical Society Transactions (2024) **52** 2385–2398
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dimension and average non-interacting microtubule length determine the sensitivity of array orientation to edge-based cues in [27, 115].

These results so far show that we can expect more interesting discoveries when modelers continue to investigate the mutual feedbacks between cortical microtubules and the ROP system on cell shape and secondary cell wall patterning.

We should not lose sight, however, of other wall components. The wall around the (Arabidopsis) metaxylem pit is further modified by the production of overhanging cell wall borders or 'arches' (Figure 3C2a) which reduce pit aperture but not pit membrane area. Boundary of ROP domain1 and WALLIN recruit a ring of actin at the pit boundary where the arch material is deposited [33]. In rice, the hemicellulose xylan is deposited at the pit boundaries [116]. Mutants affected in xylan biosynthesis have larger metaxylem pit sizes, reduced secondary cell wall thickness and less aligned CMFs near pits [116, 117].

Perspectives

- Complex cell wall patterns are vital for a wide range of plant functions. They occur both in primary cell walls, facilitating complex cell shapes and reversible shape changes, and secondary cell walls, resulting in 'metamaterials' optimally supporting a range of mechanically demanding functions including water transport.
- The currently used model systems lead to a strong focus on the common factor: the interaction between ROP patterning and (microtubule) cytoskeleton and correlated structured deposition of CMFs. Far less is known about the regulation of other cases of complex cell wall patterning, which may involve substantially different components and systems.
- The degree of overlap between the patterning systems of (initially) cellulosic secondary cell wall reinforcements in primary xylem on the one hand and on the other hand other xylem cell wall sculpturing in various woody species and pegged rhizoids in liverworts remains a wide open question. Simulation models, meanwhile, remain ideal tools for understanding the consequences of mutual interactions between different key systems involved in the model systems of cell wall patterning.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

CMFs, cellulose microfibrils; CSCs, cellulose synthase complexes; PDs, plasmodesmata; ROPs, Rho-of-plants.

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