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Plant cell polarity as the nexus of tissue mechanics and morphogenesis

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How reproducible body patterns emerge from the collective activity of individual cells is a key question in developmental biology. Plant cells are encaged in their walls and unable to migrate. Morphogenesis thus relies on directional cell division, by precise positioning of division planes, and anisotropic cellular growth, mediated by regulated mechanical inhomogeneity of the walls. Both processes require the prior establishment of cell polarity, marked by the formation of polar domains at the plasma membrane, in a number of developmental contexts. The establishment of cell polarity involves biochemical cues, but increasing evidence suggests that mechanical forces also play a prominent instructive role. While evidence for mutual regulation between cell polarity and tissue mechanics is emerging, the nature of this bidirectional feedback remains unclear. Here we review the role of cell polarity at the interface of tissue mechanics and morphogenesis. We also aim to integrate biochemistry-centred insights with concepts derived from physics and physical chemistry. Lastly, we propose a set of questions that will help address the fundamental nature of cell polarization and its mechanistic basis.

Marvelling at the astonishing diversity of living forms today, we find ourselves asking the same question that Charles Darwin, D'Arcy Thompson and Alan Turing asked throughout the nineteenth and twentieth centuries[1–3]: how do reproducible body patterns and shapes emerge from interactions between individual cells? Morphogenesis of any multicellular organism relies on growth rate and direction. As plant cells are encapsulated in their cellulosic walls and unable to migrate, directional growth of plant tissues and organs is achieved through anisotropic cell expansion and division plane positioning. Both processes are preceded by the establishment of polarity at the cellular and tissue levels in a number of developmental contexts[4–6]. Cellular polarity is manifested by the segregation of subcellular components, such as RNAs, proteins, organelles and hormones. The polarity of cells can be coordinated at the tissue level, also known as planar polarity. The establishment of cell polarity biases many key processes in plant growth and development and therefore has to be tightly regulated[7].

In the past few decades, deep insight has been gained into the establishment of polarity in yeast and animal models, highlighting the role of polar cortical protein domains in this process. The key elements of the polarity machinery in animals (for example, PAR polar proteins) and the crucial role of their interactions with the cytoskeleton have been revealed using *Caenorhabditis elegans* and *Drosophila* models[8,9]. A number of polar proteins associated with specific domains of the cellular cortex have also been identified in plants. Among these are BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL), POLAR LOCALISATION DURING ASYMMETRIC DIVISION AND REDISTRIBUTION (POLAR) and BREVIS RADIX (BRX) in stomatal development; PIN proteins enabling polar auxin transport; and SOSEKI family members that can potentially constitute a coordinate system, integrating apical–basal and lateral polarities[4,6,7,10]. However, the machinery driving polarity establishment in plants remains poorly elucidated, and the primary cues that guide proteins towards their polar domains are not well understood.

Cell polarity and other aspects of plant development have long been proposed to be regulated by biochemical signals[4,7]. However, it is becoming evident that mechanical cues are also critical in instructing polarity establishment and other crucial processes in plant development, such as differentiation, expansion, cell division and cell-fate specification[11–17]. This implies that mechanical forces are intimately intertwined with biochemical pathways in orchestrating plant development and morphogenesis[16,18–21], akin to insights gained from animal models[22,23].

It is, however, not clear how mechanical signals are perceived and translated into such cellular responses. The cell wall probably plays a key role in the perception of mechanical signals. Anisotropy of its expansion determines cellular growth direction and is in fact regulated by subcellular structures, such as cortical microtubules (CMTs) that are associated with the cellular cortex and guide the deposition of the key component of the cell wall, cellulose fibrils[24]. Polar protein domains, in turn, adjust the organization of CMTs[12,25–28]. This suggests an interesting connection and mutual regulation between the cell wall, polar cortical domains and subcellular structures. In this Review, we focus on the workings of this continuum and address its role in the mechanical control of plant morphogenesis.

Mechanical forces in plant cells and tissues

Small mechanical stresses acting on a solid body, such as a cell wall, result in small elastic (reversible) deformations, whose magnitude is proportional to the ratio of the stress amplitude over the material stiffness. For larger stresses that exceed the elastic yield limit of the material, irreversible (plastic) deformations occur due to yielding of the solid walls. Directional deformations, such as those involved in polar cell growth (for example, elongation or tip growth), require either anisotropic stresses or anisotropic mechanical properties.

Internal mechanical forces in plant tissues are largely of osmotic origin (Fig. 1a) and are isotropic at the cell level. Turgor pressure pushes the plasma membrane (PM) against the cell wall; the resulting elastic expansion generates tensile stresses in the wall,

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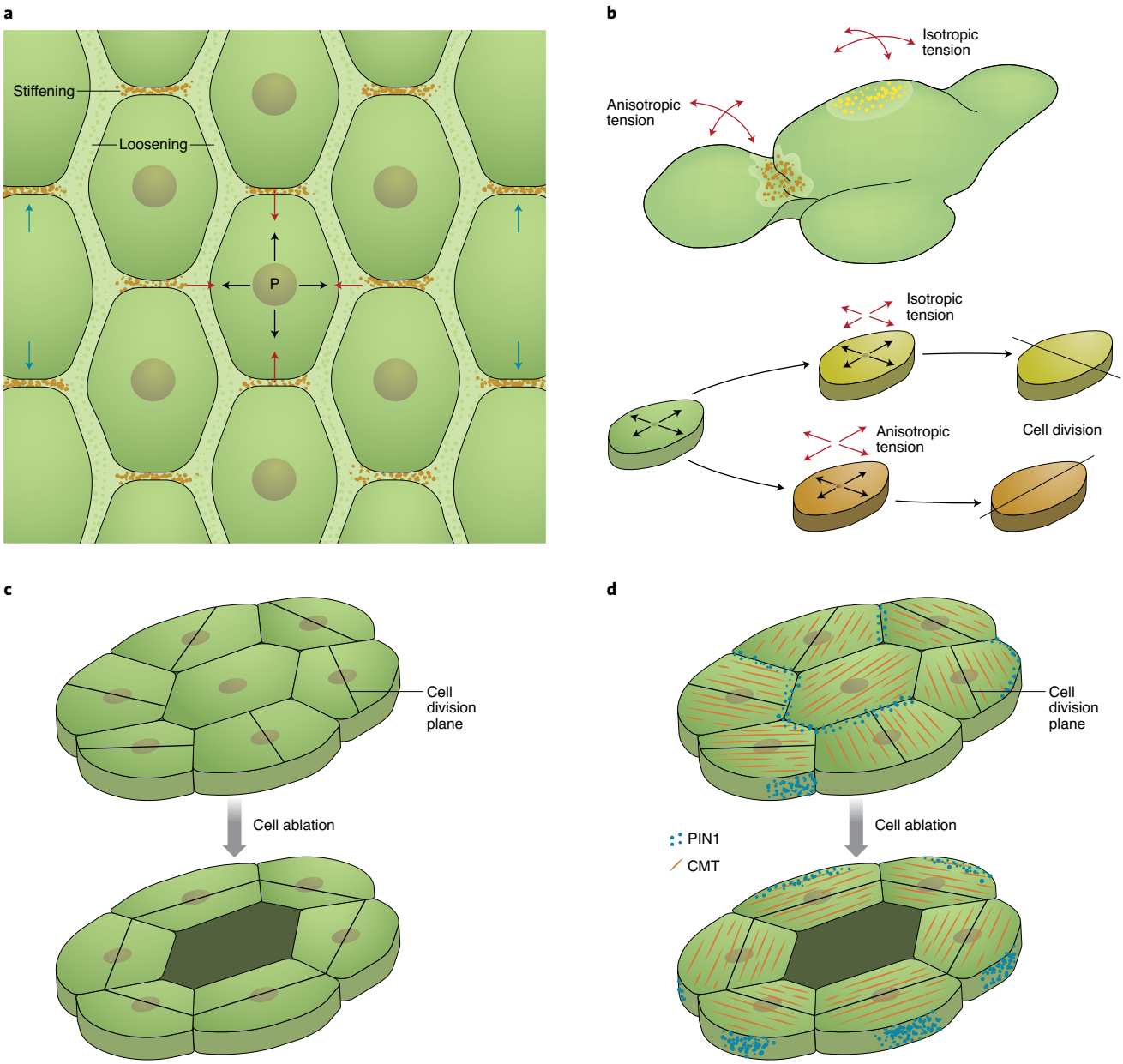

Fig. 1 | Mechanical forces in plant cells and tissues. a, The origin of mechanical forces in plant cells and tensegrity of plant tissues. In the central cell, turgor pressure (P, black arrows) pushes the PM (black) against the cell wall (pale green), generating tension. The cell wall responds to the tensile stress with an equal and oppositely signed force (red arrows). Anisotropy of cellular growth (blue arrows in lateral cells) is achieved by the activity of cell-wall-modifying enzymes, resulting in wall loosening (green pattern) or stiffening (orange pattern). **b**, Direction of maximal tension (red arrows) in the SAM (top) instructs the positioning of division planes (bottom, black lines). Cells where tissue tensile stress is isotropic (top, yellow area of the SAM) divide along the shortest path (bottom, yellow cells), while cells where tissue stress is anisotropic (top, orange area of the SAM) divide along a longer path (bottom, orange cells). The black arrows on the cells in the lower panel indicate turgor pressure. **c**, Tensile pattern resulting from cell ablation instructs cell division in the SAM. In the intact SAM (top), tensile stress is isotropic, and cells divide along the shortest path. Cell ablation (bottom, black zone) results in an anisotropic tensile pattern (circumferential) and reorientation of the division planes. **d**, The role of tensile stress in the organization of CMT arrays and polar protein domains. Anisotropic tensile stress resulting from cell ablation causes reorientation of CMTs and PIN1 polar domains in the SAM (bottom).

which responds with an equal and oppositely signed force, providing a counterbalance to the osmotic stress[29]. This force balance of outward-directed turgor and inward-directed stress due to wall stretching, known as a tensegrity (tensional integrity) balance, provides walled cells with their rigidity, which is key for plant morphogenesis[30–32]. The balance between the two opposing forces sets the whole cell wall network of an organ to a state of pre-stress (pre-existing tension), making it susceptible to responding globally to local mechanical stimuli[32]. One can envision an analogy to the mechanical network of a spider web, where fibres are interconnected in such a way that disturbing the force balance locally (for example, by pulling a single thread) sets in motion stress redistribution across the whole network. This principle offers a picture of morphogenesis as a self-sustained mechanical system that regulates its mechanical state through local mechanical signals. Information in a mechanical network system can be relayed rapidly, since the propagation of mechanical signals in theory can occur at the speed of sound, much faster than the diffusion or active transport of

chemical cues. Initially, the principle of tensegrity was applied to animal cells, where specific architectures are achieved through the interplay between cytoskeletal elements that act as both compression-bearing and tension-generating elements[32,33].

The balance between turgor and cell wall tensile resistance determines plant growth rate and direction[29]. Turgor pressures and cell wall stiffness vary widely among different cell types, resulting in a broad range of stresses and deformation amplitudes within a single organ[34,35]. We note that turgor magnitude alone does not dictate the tensile stress in the walls, whose amplitude is determined also by the thickness and local geometry of the wall and its mechanical response. Moreover, the mechanical pattern of an organ is influenced by its shape, its rates of cell division, its structure and the topology of its tissues. At the cellular level, the combination of three factors (namely, turgor pressure, biochemical heterogeneity of the cell wall and cellular geometry) determines the cell-autonomous (subcellular) stress pattern. Since stresses are locally integrated, cell-autonomous mechanical stress can be overridden by tissue-wide stress (supracellular) of a greater magnitude[14].

Because mechanical interactions are long-ranged in an elastic network structure such as the plant cell wall, the local force balance on the wall of a single cell is governed by a complex mechanical interplay spanning beyond its direct neighbours. As a result, different walls of the same cell can bear different stresses[13,36,37]. Tissue topology also contributes to the mechanical heterogeneity of plant cells. In many cases, the outer cell layers are under tension, while inner cells are under compression—for example, as demonstrated by the outward curling of peeled epidermal cell layers, where the outer wall is under higher tensile stress than the inner wall, leading to tensile deformations on the exterior and compressive deformations on the interior of the curl[29,34]. The mechanical state of walls in a tissue is thus highly heterogeneous in both space and time, caused by the complex network topology, difference in turgor pressures between cells and local variations in wall stiffness and yield stress. Wall mechanics are also inhomogeneous in space and time due to biochemical remodelling of the cell wall through the differential deposition of cell wall components and local activity of wall-modifying enzymes[38] (Fig. 1a).

While cell growth is driven by osmotic pressure, the direction of growth is determined by a combination of elastic interactions with neighbours and an inhomogeneous yield resistance of the wall[29]. Local modifications to cell wall yield resistance are thus critical to polarized growth. Moreover, the force equilibrium at the tissue scale, through balancing the tensegrity of individual cells across the tissue, results in complex mechanical patterns that can instruct division plane positioning, as evidenced in the shoot apical meristem (SAM)[11]. In the central SAM region, where stress on individual cells is isotropic, division follows the shortest path. By contrast, in the boundary region, where cells are under highly anisotropic stresses, cells instead divide along the axis of maximum tension, which is a longer path (Fig. 1b)[11]. In line with the notion that cell divisions are under mechanical control, in one study, cell ablation in the SAM led to the rearrangement of stress patterns and resulted in the reorientation of cell divisions in the shoot apex (Fig. 1c)[12]. The mechanical stimulus in this experiment also triggered the repolarization of auxin transporter PIN1 and the reorganization of CMTs[12] (Fig. 1d). This peculiar behaviour of the subcellular structures instructed by mechanical patterns provides a different perspective on how plant polarity is established.

Polar protein domains and membrane heterogeneity

The development of different tissue-specific cellular morphologies is often preceded by the establishment of polar domains within cells, marked by the localization of unique (polarity) proteins[4,5]. Here we will distinguish three types of polarity establishment: (1) polarity formation during localized cellular growth, (2) transient polarity establishment during asymmetric divisions and (3) stable polarity of polyhedral cells, which are characterized by flat, angular faces with sharp edges and corners (axial polarity; this type of polarity will gain the main focus in this manuscript). The first polarity type is encountered in tip-growing cells, such as root hairs and pollen tubes, or during the formation of jigsaw-puzzle shapes of leaf epidermal pavement cells, where it is marked by the formation of PM domains decorated by Rho of Plants (ROP) GTPases[5,39]. An example of transient polarity is found in stomatal development, where asymmetric divisions require the formation of polar domains of BASL, POLAR and BRX[4,6,7]. An example of stable polarity is found in polyhedral root cells, where apical and basal (apical–basal polarity) or inner and outer lateral membrane domains (lateral polarity) are uniquely marked. The former is characteristic of transmembrane PIN-FORMED (PIN1-4 and PIN7) auxin transporters[40], as well as of membrane-associated BRX and OCTOPUS (OPS) required for phloem development (Fig. 2a)[41,42]. The lateral polarity is manifested by transmembrane nutrient transporters, such as REQUIRES HIGH BORON1 (BOR1) and NOD26-LIKE INTRINSIC PROTEIN 5;1 (NIP5;1) facilitating boron transport, as well as transmembrane kinase receptor INFLORESCENCE AND ROOT APICES RECEPTOR KINASE (IRK) (Fig. 2a)[43–45]. Interestingly, the preference for specific cell faces of the proteins is often cell type dependent. PIN2 thus localizes to the basal face of cortex cells but shows apical localization in the epidermal cell file, while IRK localizes to the outer and inner cell faces in the endodermis and cortex, respectively (Fig. 2a)[40,45]. Importantly, face localization of the abovementioned polar proteins is maintained throughout a cell file and is thus an example of planar polarity that suggests the presence of polarity fields in plant bodies.

Apart from cell faces, protein domains can also be associated with cell edges. Examples include GAMMA TUBULIN COMPLEX PROTEINS2 and 3 (GCP2 and GCP3)[27], CLIP-ASSOCIATED PROTEIN (CLASP)[25,46] and Rab-A5c[26] (Fig. 2b), which are enriched at cellular edges. The edges of root cells can also be decorated by members of the SOSEKI protein family, which demonstrate a preference for specific edges and tissue types[10]. Unlike those of GCPs and CLASP, which are restricted to edges, the domains of SOSEKI also spread to cell faces (apical/basal and inner/outer lateral) that converge on a specific edge (Fig. 2b). The edge preferences of SOSEKI proteins are conserved throughout specific cell files, suggesting yet another case of planar polarity. Surprisingly, the PM association of SOSEKI proteins can be easily perturbed by both osmotic shock and the digestion of the cell wall, pointing to the dependence of these polar protein domains on either properties of the cell wall or cellular geometry imposed by the intact cell wall. Integrity and the connection of the cell wall to the PM have also been shown to be crucial for the maintenance of PIN polar domains[47].

Specific preferences of polar proteins for certain faces or edges within a given cell and their differential localization in different tissue types illustrate the complex nature of the cellular polarity machinery in plants and emphasize the contribution of PM heterogeneity to its establishment. Heterogeneity is an intrinsic property of eukaryotic plasmatic membranes, which are highly compartmentalized structures, composed of lipids and proteins that segregate into domains with specific properties (Fig. 2c)[48,49]. The domains of PMs vary strongly in composition and size. The smallest domains, called membrane rafts (2–20 nm in diameter), have been described as liquid-ordered protein–lipid aggregates enriched in sterols and sphingolipids[50]. The binding of proteins to these nanodomains through protein post-translational modifications, such as the addition of glycosylphosphatidylinositol and S-acyl (palmitoyl) moieties[51,52], or via electrostatic interactions[53] might lead to the fusion of membrane rafts and their aggregation into higher-order structures, called membrane microdomains (from 40–300 nm to micrometres in diameter)[54].

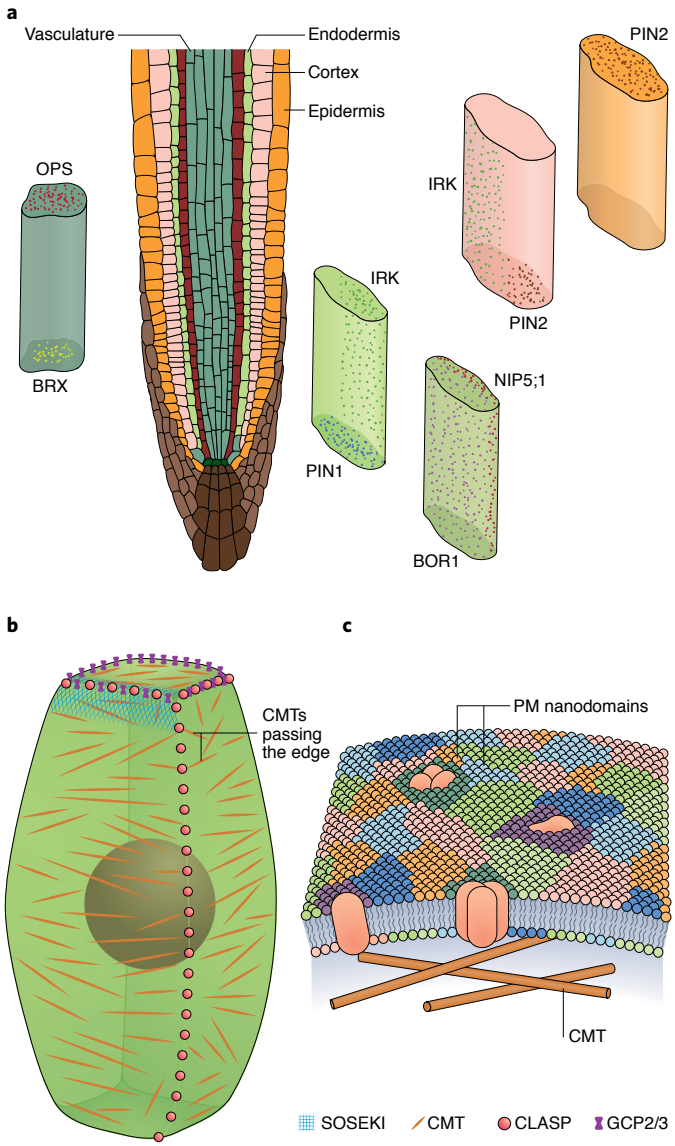

Fig. 2 | Polar domains and PM heterogeneity. a, Distribution of polar protein domains in the *Arabidopsis* root. The main diagram illustrates tissue organization in the *Arabidopsis* root, and the surrounding illustrations show the face localization of different polar proteins in specific cell types. The colours of the cells match the colours of the corresponding tissue layers in the root diagram. **b**, Polar domains in polygonal root cells, including the localization of SOSEKI protein at cellular edges and adjacent faces and the targeting of GCP2/3 and CLASP proteins to cellular edges. CLASP stabilizes CMT polymerization, thereby enabling CMT localization at sharp cell edges. **c**, Lateral segregation of PM components into nanodomains. Nanodomains formed within the PM are indicated by patches of different colours; CMTs are represented by orange rods beneath the PM.

Well-known examples of such domain-associated proteins include flotillins and remorins, which decorate distinct microdomains at the cytosolic leaflet of the PM in animal cells and whose plant homologues also associate with PM microdomains[55,56]. Analysis of the distribution of these markers in *Arabidopsis* and tobacco cells suggests that different sides of a single cell might possess specific temporally stable microdomain signatures[57]. The advancement of microscopy techniques has allowed further study of the behaviour of PM microdomain arrays in vivo in plants and has demonstrated that the horizontal movement of these arrays within a single leaflet of the lipid bilayer (known as lateral diffusion) is restricted, and they remain stable on timescales of several minutes[47,57–62]. It is still unclear how this lateral segregation of PM components is maintained. Studies analysing microdomains in animals and yeast have demonstrated their association with the cellular cortical cytoskeleton, which rendered the microdomains immobile for minutes to hours (Fig. 2c)[48,63,64]. A similar organizing role of the cytoskeleton has been reported in plants in the context of the leaf and root epidermis[57,62,65,66]. The role of CMTs in restricting lateral diffusion has also been demonstrated for a special case of polar growth in leaf trichomes, where cortical CMTs restrict the movement of PM-associated SPK1, a member of the guanine nucleotide exchange factor family[67].

Another prominent stabilizer of nano- and microdomain structure of the PM in plants is the cell wall. This was suggested a decade ago by a study reporting on the mobility of a set of PM proteins bearing a minimal PM anchoring domain and a fluorescent tag. The study demonstrated that the cell wall was important to temporally immobilize the tagged proteins[68]. This notion was corroborated by more recent reports showing cell-wall-dependent stabilization of PM domains associated with the polar auxin transporter PIN3, the pathogen receptor FLAGELLIN SENSING2 (FLS2), flotillin and hypersensitive induced reaction proteins. These studies also suggested involvement of the CMT cytoskeleton in preventing lateral diffusion of these domains within the PM[69,70].

Altogether, it emerges that the PMs of living cells are highly heterogeneous, and different PM sides of a single cell possess specific temporally stable signatures of nano- and microdomains. An important question is whether and how these nanodomain signatures determine cellular polarity domains decorated by known polar proteins. Can polar proteins directly recognize the areas with specific nanodomain signatures? A recent study using the model of leaf epidermis pavement cells demonstrated that this is a plausible scenario by showing that membrane nanoclustering underlies cell polarity establishment[71]. The study demonstrated that auxin-mediated nanoclustering of cell surface transmembrane receptor-like kinase 1 (TMK1) initiated the establishment of ROP6 polar domains during morphogenesis of pavement cells and highlighted the interdependence of this polar domain formation and cytoskeleton organization. As this context is an example of polar growth, it remains to be addressed whether such phenomena also guide the formation of polar domains in general and whether planar polarity markers (such as SOSEKI) follow PM microdomain signatures. Another important point that lacks sufficient insight is the nature of the cues orchestrating the targeting of proteins to their polar PM domains. The emerging importance of mechanical cues in development and morphogenesis makes one wonder whether these cues can instruct the polar protein localization and what the mechanistic basis of such regulation could be.

Mechanosensing and transduction

The cell wall–PM–cytoskeleton continuum has long been hypothesized to represent the cornerstone of plant mechanical responses, although the mode of action and regulation of this continuum remains unclear, largely due to the complexity of responses. For example, plant cells have to be able to accurately perceive information on the strength and direction of mechanical signals, if these are to be used as developmental cues. Furthermore, the signalling between the cell wall and the interior is expected to be bidirectional.

The remarkable biochemical and mechanical heterogeneity of plant tissues discussed above should be regarded as an important premise for mechanosignalling. While insight into how mechanical heterogeneity is established and maintained is rather advanced[38,72], it is less clear how it is first translated into PM properties and to other subcellular responses. Stresses that are experienced by the cell wall are first relayed to the PM, which 'gates' mechanical information

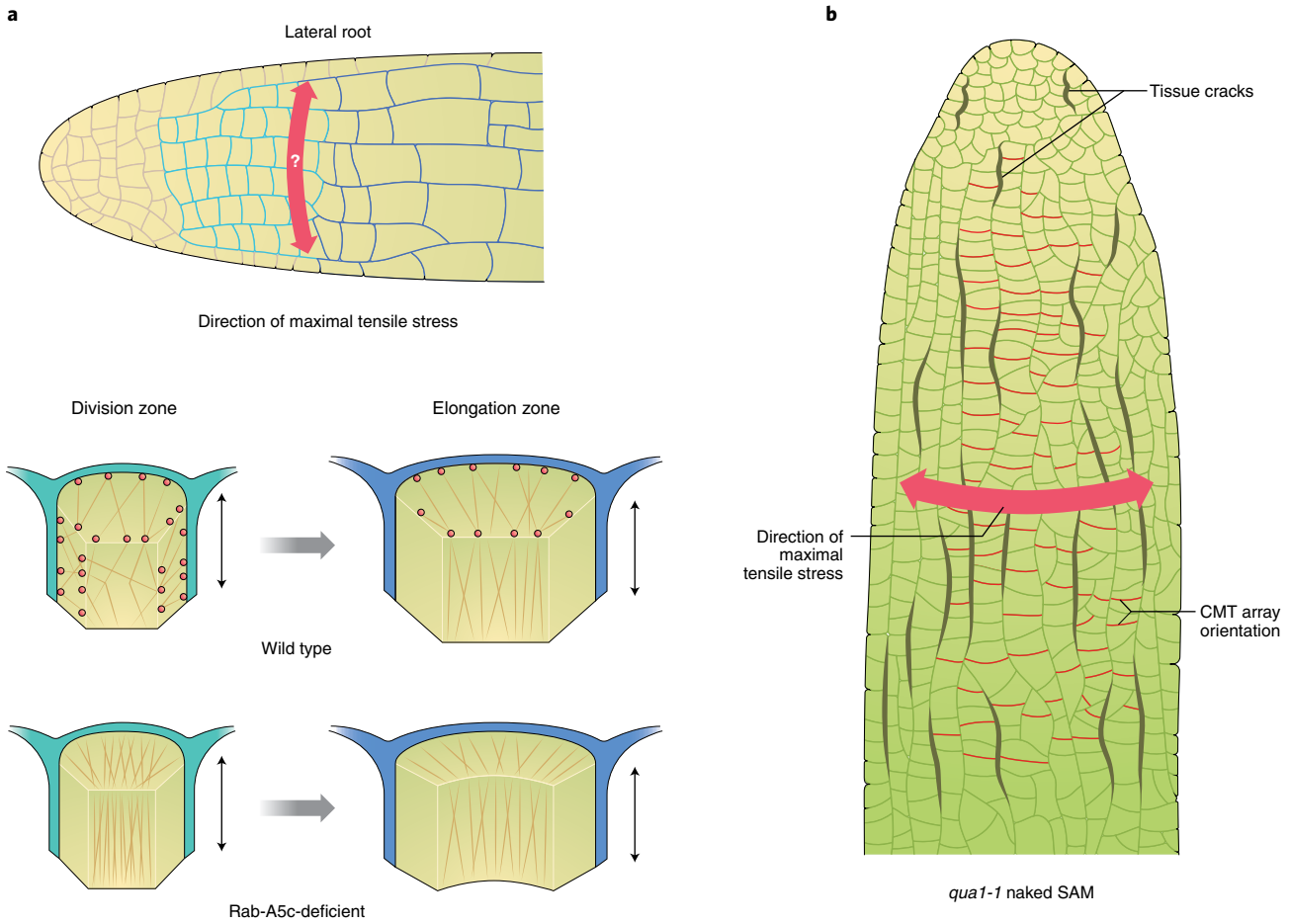

Fig. 3 | The link between polar protein domains and morphogenesis. a, Function of Rab-A5c domains. Top, division (light blue) and elongation (dark blue) zones of the lateral root. Bottom, CMT alignment (orange lines), Rab-A5c localization (red dots) and cellular growth anisotropy in wild-type and Rab-A5c-deficient plants. The lateral root epidermal cells of wild-type plants display isotropy of CMT arrays in the division zone and anisotropy of cellular expansion in the elongation zone. In Rab-A5c-deficient plants, CMTs align with the presumptive direction of maximal tensile stress (black arrows) in the division zone, and cells in the elongation zone undergo isotropic expansion. **b**, Schematic representation of the cell adhesion defects and orientation of CMT arrays in the naked SAM of *qua1-1* mutants. Horizontal tension pulls cells apart, creating vertical gaps between cells, which appear as tissue cracks (olive green) perpendicular to the maximal tensile stress (circumferential, indicated by the red arrow). CMTs align horizontally, parallel to the maximal tensile stress.

and transduces it to the cellular interior. It has long been established that the PM 'senses' mechanical signals through mechanosensitive ion channels that control the passage of ions across the PM upon stimulation[73]. It is, however, not well elucidated whether and how mechanosensitive ion channels read the mechanical heterogeneity of the cell wall and whether they could play a role in maintaining it.

CMTs are well known to play an important role in controlling cellular mechanical anisotropy, through regulating the deposition of cellulose fibrils and thereby determining cellular shape and growth direction[74,75]. Mutants affected in CMT organization demonstrate morphological defects reflected in severe distortion of cell shapes and a switch to isotropic growth[76]. Cell geometry has an important influence in organizing CMT arrays: in single cells, arrays are typically aligned along the longest cellular axis[77,78]. However, in the context of plant tissues, this rule is often disobeyed, suggesting that geometry-determined CMT organization can be regarded as the 'default' that is adjusted by tissue-borne cues. But how do CMTs perceive wall and membrane mechanics? Knowledge on how microtubules are anchored to the PM is rather limited. The only available reports suggest that phospholipase and CELLULOSE SYNTHASE INTERACTIVE PROTEIN 1 (CSI1) are involved in this process; however, no mechanism has been proposed, and mutant phenotypes suggest the involvement of additional factors[79–81]. The control of CMT stability and dynamics is understood in more detail. These two processes involve microtubule-associated proteins, which, as the name suggests, bind CMTs[82]. A prominent example is KATANIN, which severs CMTs and is therefore crucial for the reorganization of CMT arrays[16,83]. Another important microtubule-associated protein is CLASP, which specifically localizes at cell edges and stabilizes the polymerization of CMTs (Fig. 2b). In the cells of *clasp* mutants, CMTs are not stabilized at sharp edges and are therefore more likely to undergo a catastrophe and depolymerization[25,46].

Apart from CLASP, the organization of CMTs at edges depends on edge-localized GCP2 and GCP3 (Fig. 2b)[27]. Interestingly, CLASP and GCP2/3 proteins do not decorate all cellular edges but have rather specific preferences that change as cells progress through developmental zones. Thus, in root tips, GCP2/3 is found at transverse edges but is absent from longitudinal ones[27]. CLASP is mainly present at transverse edges of cells in the root apical meristem but is enriched at longitudinal edges of cells in the elongation zone[25]. This shows that CLASP and GCP2/3 polar protein domains determine the heterogeneity of the PM cortex and modulate CMT organization and that cellular biochemistry allows the overriding of physical constraints dictated by the intrinsic properties of CMTs. How

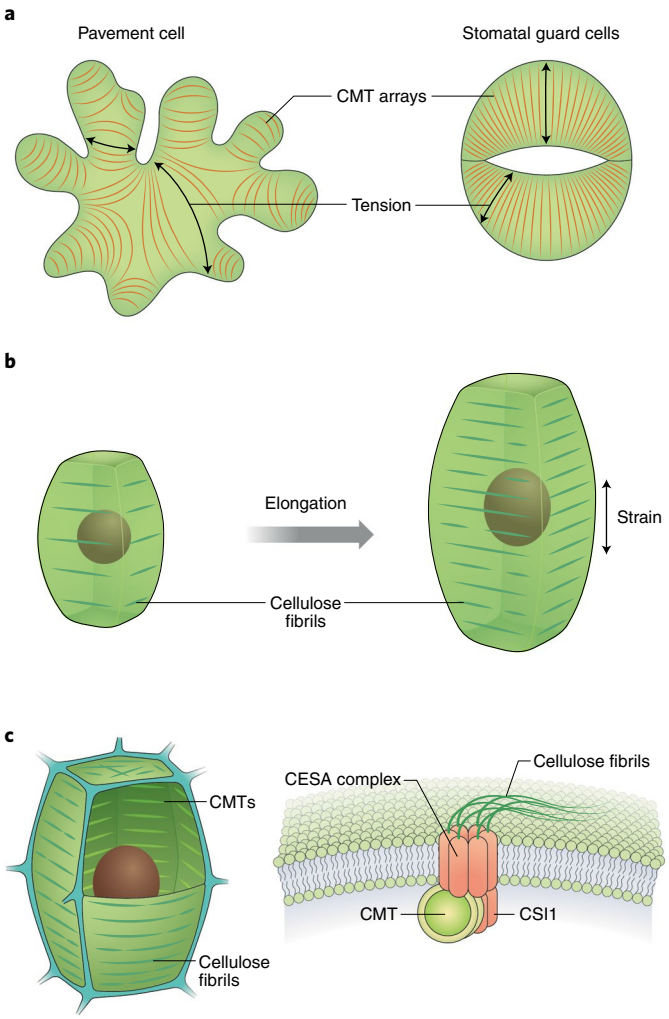

Fig. 4 | Sensitivity of CMTs to tensile stress and their role in growth anisotropy. a, CMT organization follows the cellular tensile pattern (examples of a leaf epidermal pavement cell and stomatal guard cells are shown). The orange lines represent the alignment of CMTs, and the black arrows indicate maximal tension. **b**, CMTs orient perpendicularly to the principal direction of strain, enabling anisotropic growth in root polygonal cells. **c**, The role of CMTs in cellulose synthesis (left, localization of CMTs at the PM and deposition of cellulose fibrils in the cell wall of a polygonal cell; right, CMTs are connected to the cellulose synthase (CESA) complex through CSI1, thereby directing the deposition of cellulose fibrils).

far can we extrapolate this phenomenon? It is tempting to speculate that PM-associated polar proteins in general could contribute to the modulation of CMT behaviour. Indeed, proteins that associate with the PM form an intricate patchwork on its surface and might thereby locally adjust its characteristics, such as electrostatics and shape. One can assume that such adjustment of PM properties might enable a fast and local cellular response to external stimuli that would allow the level of complexity required for a cell to 'sense' mechanical anisotropy within plant tissues.

The modulation of CMT organization is not restricted to edge PM domains, as CMT arrays have also been shown to be coupled with domains of PIN and ROP proteins at cell faces[28,84]. The role of polar domains in the CMT network has also been supported by computational studies[25,85]. However, does such regulation of CMT arrays by polar domains have an effect on morphology? Considering the crucial role of CMTs in the deposition of cellulose fibrils that determine cellular growth direction, one would expect this to take place. Indeed, the root and hypocotyl cells of *clasp* mutants mainly expand isotropically, and their leaf pavement cells exhibit simpler shapes[86]. Similarly, plants mutant for the edge-localized trafficking regulator Rab-A5c exhibit disturbance in CMT arrangement that leads to severe morphological defects[26]. Specifically, in the absence of Rab-A5c polar domains, the normal predominantly isotropic CMT organization is reverted to strongly anisotropic transverse (circumferential at the organ scale) in epidermal root tip cells of the mutants (Fig. 3a). While such transverse alignment could be a compensatory response to preserve mechanical anisotropy, it is also conceivable that, in the absence of Rab-A5c function at edges, the CMT array orients by default according to tissue tensile stress, which is presumably circumferential. Analysis of NPA-induced naked inflorescences of the pectin synthesis mutant *quasimodo* (*qua1-1*)[87] allowed researchers to address this question. These mutants show cell-to-cell adhesion defects that appear as cracks in the epidermal cell layer, where tensile stress is the highest, although heterogeneity in the strength of the middle lamella may also contribute to crack patterns. Mutant stems showed longitudinal cracks, suggesting a circumferential direction of the maximal tensile stress (Fig. 3b). Interestingly, the predicted stress pattern was accurately aligned with CMT arrays. This peculiar behaviour of CMTs pointed to their possible role as indicators of tensile stress patterns at the tissue level. The semblance of shape between the inflorescence and the root tip, as well as their similarly slow longitudinal growth that mainly occurs through cell division, allow us to draw a parallel and assume that the maximal tensile stress in the root tip might also be circumferential.

Interestingly, cellular force microscopy analysis of the onion epidermal layer has demonstrated that cellulosic walls at cell edges might be softer, which might make them less capable of preserving curvature[88]. It is therefore tempting to speculate that RAB-A5c might have a role in modulating mechanical properties at edges. This is consistent with the highly isotropic cellular expansion of Rab-A5c-deficient plants, where cells swell and obtain spherical rather than polyhedral shapes[26]. This observation raises a set of interesting questions. Does Rab-A5c locally modulate tension at cell edges? If so, does this allow the mechanical properties of the PM to be uncoupled from those of the cell wall and thereby allow CMT arrays to disobey organization according to the direction of maximal tension? Can Rab-A5c protein domains instruct the reverse mechanical signalling (from the PM to the cell wall)? Hopefully, these questions will soon be addressed by future studies.

Tension as an instructive cue in plants

The notion that cellular geometry is not the sole determinant of CMT behaviour has been corroborated by studies analysing CMT arrays in root and hypocotyl epidermal cells, showing that the organization of CMTs differs between outer and inner periclinal cell faces[89,90]. This discrepancy was attributed to the difference in tensile stresses between cell faces—the outer face is under tension, while the inner face is under compression[34,36]. Particularly, there was little or no CMT anisotropy observed at the outer face, while CMT arrays at the inner face were in perfectly transverse orientation. The differential CMT orientation was consistent with the observed arrangement of cellulose fibrils in the hypocotyl[89,90]. CMT arrays sensitively responded to rearrangement of the tensile stress pattern in a study employing longitudinal compression of hypocotyls. Upon such compression, the direction of maximal tension changed from longitudinal to transverse, and CMT arrays at the outer face conformed, shifting orientation from longitudinal to transverse[91]. Tension could thus be an instructive cue for CMT organization, as reviewed in detail elsewhere[75].

CMTs are indeed very sensitive to mechanical stimuli in ablation experiments in the SAM or wounding of root tissues[12,13,92]. The rearrangement of CMTs in cells around an ablation site (Fig. 1d) was not

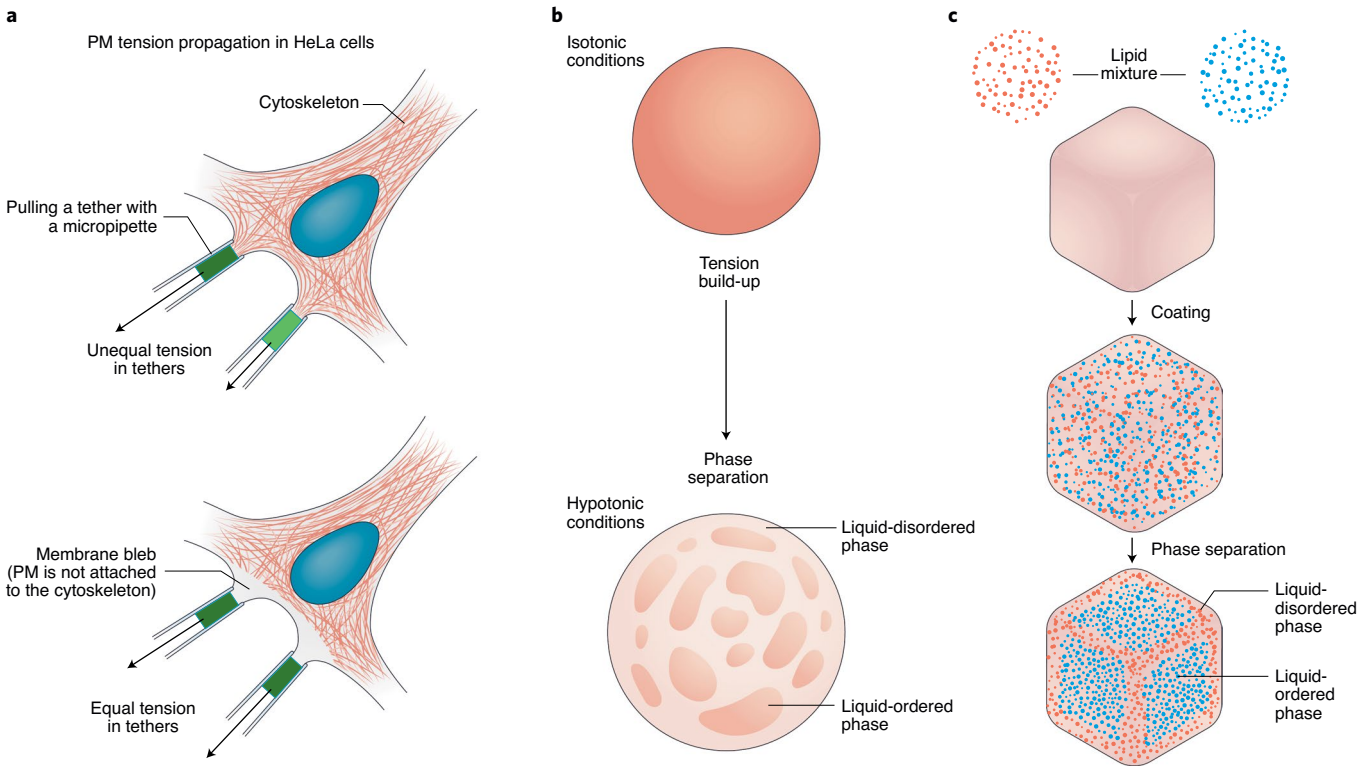

Fig. 5 | Physical control over PM organization and behaviour. a, The role of the cytoskeleton in the propagation of tensile stresses in the PM of HeLa cells (top, an intact HeLa cell; bottom, a HeLa cell with a bleb, a region of the PM not attached to the cytoskeleton). On each cell, two tethers are pulled using micropipettes, and PM tension is read through detecting the fluorescence intensity of a PM-localized reporter (dark green indicates higher tension; light green indicates lower tension). In the intact cell, stronger pulling by one tether does not cause any build-up of tension in the other, while in the cell with a bleb, stronger pulling by one tether results in equal PM tension in both. **b**, Lipid phase separation in artificial vesicles triggered by an increase in surface tension caused by hypotonic shock. **c**, Lipid coating of cubic particles. The lipid bilayer undergoes phase separation into liquid-disordered and liquid-ordered phases, schematically represented by red and blue dotted patterns, respectively.

accompanied by obvious alteration of the shapes of these cells, further corroborating the notion that CMT organization cannot rely solely on geometry cues[13,84]. In addition, CMTs have been shown to accurately follow the tissue mechanical pattern and have been proposed to be a proxy for tensile stress[11,13,18,75]. It is known that CMTs are involved in the reinforcement of the cell wall, enabling plant cells and tissues to resist the stress as they align along the maximal tensile stress, and in the direct deposition of cellulose fibrils (Fig. 4a,c)[13,15,37,74,93,94]. It has also been shown that CMT arrays can be perpendicular to maximal strain (and therefore support growth anisotropy; strain is a measure of deformation) (Fig. 4b) and are parallel to maximal tensile stress (and therefore allow cells to indirectly resist stress though coupling with cellulose synthesis; Fig. 4c)[13,14,16,18,91,93,94]. It should be noted that CMTs are not always perpendicular to maximal strain but rather align with it, as in the boundary region of the SAM[11]. This observation does not contradict the notion that CMT arrays are guided by tension, since strain and maximal tension co-align in this region.

It is important, however, to understand what sort of stresses CMTs can sense. On the one hand, in the model of the SAM, it has been demonstrated that CMT organization is instructed by tissue shape-derived tension[13]. On the other hand, a study using leaf pavement cells suggested that CMT arrays are orchestrated by subcellular mechanical stresses, which, however, can be overridden by tissue-wide mechanical stresses of a greater magnitude[14]. These insights suggest that one has to consider the interplay between the subcellular and supracellular stresses while studying CMT organization.

Responsiveness of CMTs to tensile stress was observed in several studies using artificial systems[75], as well as in a recent report using *Arabidopsis* protoplasts[95]. Tension has also been reported to instruct the localization of PIN1, intriguingly, in a CMT-independent manner. This allows one to assume that the localization of polar protein domains can be instructed by mechanical stimuli independently of CMTs. However, one has to be careful in ruling out the interaction between CMTs and polar protein domains in response to mechanical stimulation. As we discussed above, these two elements exist in intimate connection and demonstrate mutual regulation. Another type of cytoskeletal element, actin, has emerged as a prominent tension-responsive as well as tension-regulating structure in animal cells[96–99]. While it is less clear whether a similar function can be carried out by actin in plant cells, one might envision such a scenario taking into account the role of actin in membrane trafficking, which is crucial for membrane restructuring and polar protein localization[100].

The responsiveness of subcellular structures such as cytoskeletal elements and polar protein domains to tension aligns with the perception of the developing plant organs as pre-stressed networks of interconnected elements, where the progression of morphogenesis via cell division and expansion causes the redistribution of tensile stresses. This rearrangement has to be accurately sensed by individual cells, and both the magnitude and direction of tensile stress have to be perceived. How this is executed at the subcellular level, however, remains a conundrum. To answer this question, one has to understand what actually determines the behaviour of polar protein domains and cytoskeletal elements. One might intuitively assume that the alteration of PM properties under tension might play a role. However, it remains an open question as to which PM properties are involved and how a purely physical phenomenon such as tensile stress would translate into cellular responses.

Cellular structures under physical control

PM tension has a profound effect on cellular processes in animals. It has been demonstrated to affect cell migration[101], vesicle fusion and recycling[102,103], the progression of the cell cycle[104], cell signalling[101,105] and mechanosensation[106]. If PM tension is to be an instructive cue, it should be localized in specific PM domains without dissipating throughout the cell on timescales relevant for its perception. It should inflict a local impact and also scale with the magnitude of mechanical stimulation at specific parts of the cell. Until very recently, the PM was viewed as a "fluid-mosaic"[107], behaving as a fluid-like structure. It was, however, unclear at what speed and to what degree local changes in PM tension propagate in cells[108]. In line with the fluid-mosaic hypothesis, studies using artificial lipid bilayers demonstrated that changes in membrane tension propagate across a cell-sized region in milliseconds[109,110]. In recent years, it has been proposed that temporally stable tension gradients might be present in the PMs of living cells[105,111–113], where additional complexities exist that may change membrane behaviour relative to that in minimal synthetic systems.

One example came from a conceptually simple but technically challenging experiment of pulling two membrane tethers simultaneously with two micropipettes and monitoring the signal intensity of PM protein marker in these tethers in HeLa cells[114] (Fig. 5a). This experiment convincingly demonstrated the existence of localized mechanosignalling within living cells and the possibility of strong mechanical heterogeneity within the PMs of living cells. This notion aligns with the finding that PM tension might not be equal at all sides of plant cells[13,89,93,94]. Several key questions emerge. How is tension perceived by subcellular structures, such as CMTs and polar protein domains? Can tension be translated into PM heterogeneity? Can it be a stress-magnitude-dependent response?

The principles of membrane organization are largely unexplored. For example, it remains unclear whether physical characteristics of a membrane, such as tension, actually determine and modulate its heterogeneity. Studies using artificial lipid vesicles have demonstrated spontaneous segregation of multicomponent membranes into separate phases—namely, a liquid-ordered phase enriched in sphingolipids and cholesterol and a liquid-disordered phase[48,115]. These artificial membrane systems allowed researchers to study the behaviour of membrane domains and gain insight into physical control over their formation. The effect of lateral membrane tension was first observed in artificial membrane models, such as giant lipid vesicles, which represent closed semi-permeable compartments consisting of phase-separating lipid mixtures[116]. Placing such vesicles into hypotonic conditions that cause an influx of water and thereby result in an increase in membrane lateral tension induces the formation of lipid domains (Fig. 5b). More precedents of a similar phenomenon are known from earlier reports[117–119] and seem to be consistent with observations in living cells[119]. In yeast, a decrease in tension triggers PtdIns(4,5)P2 phase separation into invaginated membrane domains, which cluster and deactivate the target of rapamycin complex 2 (TORC2), triggering a signalling cascade[120]. The recent development of sensitive plant-cell-permeable mechanoprobes provides an excellent opportunity to visualize the stress patterns of inner tissues of plant organs at the subcellular scale[121]. Using these probes in live cells, one can envision addressing whether mechanical anisotropy of the cell wall is translated into mechanical properties of the PM (tension) that could underlie the formation of specific nanodomain signatures, which in turn might be recognized and targeted by PM-associated polar proteins. As the nanodomain signatures would feature specific assortments of both lipids and transmembrane proteins, this scenario would account for the targeting of a wide range of perimembrane proteins, such as those associating with the PM through interaction with lipids, via transmembrane protein partners or by anchoring the PM with their fatty-acid tails gained through post-translational modification.

Tensile strain is known to also affect PM composition by altering membrane trafficking in plant cells. Both exocytosis and endocytosis have been shown to be influenced by PM tension[122–126]. Interestingly, the modulation of membrane trafficking by tensile strain affects the abundance of PIN1 in the tomato SAM[17]. The same study demonstrated that another PM-localized protein, H^+-ATPase, responds to changes in PM tension in a similar manner, albeit with lower sensitivity. The basis of the differential sensitivity of these two PM-localized proteins to mechanical stimuli remains unknown.

Apart from tension, phase behaviour within lipid membranes can be instructed by curvature. This notion comes from experiments using artificial multicomponent lipid bilayers that were pulled into highly curved shapes, placed into shaped environments or assembled on shaped substrates[127–130]. All these approaches of adding curvatures to lipid bilayers resulted in lateral demixing of lipids in an interesting fashion: liquid-ordered and liquid-disordered domains occupied regions of low and high curvature, respectively (Fig. 5c). It is conceivable that these insights gained in artificial systems might be applicable to living systems as well. If true, the phenomenon of lateral phase separation in the PM might underlie the interplay between membrane geometry and its composition and represent a potential pathway for translating cellular geometry into biochemical response. The effect of PM geometry on symmetry breaking has been previously observed in living systems. The contact of a neutrophil with a surface or substrate has been demonstrated to result in a local increase in PM curvature that in turn serves as an instructing cue for binding SRGAP2 through its curvature-sensitive F-BAR domain. This binding activates PI4KA and results in PM PtdIns4P polarization, which triggers a cascade reinforcing neutrophil polarity and further increasing curvature, thereby reinforcing its attachment to endothelium[131]. Moreover, the binding of BAR proteins consolidates PM microdomains through impeding their lateral diffusion.

This mechanism of self-reinforcing polarity in animals triggered by a cue relies on basic physical principles. A recent study showed an interesting case of symmetry breaking in tobacco protoplasts that are initially spherical and possess a polar BASL domain that migrates around the PM before it becomes fixed. The fixation of the polar domain in one position at the PM is accompanied by growth anisotropy of the protoplasts[132]. It will be interesting to learn whether this intrinsic polarity is instructed by PM heterogeneity that was present before the cell wall lysis (PM memory). Likewise, it will be important to understand whether this polarization involves feedback of BASL on PM curvature, thereby triggering anisotropic growth and the reinforcement of polarity. Here we can draw a parallel with artificial vesicle models and speculate how impactful the effect of curvature on PM organization can be in plant cells, which are encapsulated in the rigid cell wall, imposing specific cellular geometry. An important notion to be kept in mind while determining the effect of PM tension and geometry on polar protein localization is the intimate connection and interdependence between these two processes. The recently developed molecular mechanoprobes hold great promise in understanding the individual effects of tension and geometry on the establishment of PM heterogeneity and the formation of polar protein domains[121].

Polar domains in the cross-talk between the cell wall and cellular interior

Similarly to mammalian tissues, where mechanical stimuli are actively transduced by the extracellular matrix and then perceived at the PM, in plants, these instructing cues are first transmitted by the cell wall to the PM, where known mechanosensory molecules are located. It remains unknown whether the wall itself features active mechanoperception molecules in plants.

Along with the cytoskeleton, the cell wall has been demonstrated to regulate both the dynamics and size of PM nanodomains in *Arabidopsis*[60,69,70]. This follows from the intimate connection

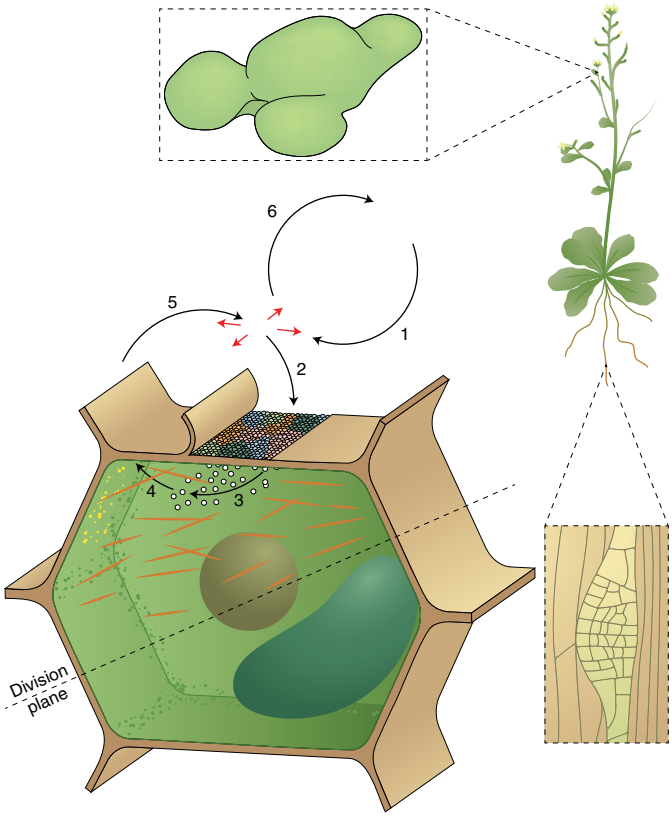

Fig. 6 | The role of polar protein domains in the mechanical regulation of plant morphogenesis. Mechanical signals that originate from changes in the tissue tensile stress during morphogenesis (for example, during SAM organogenesis or lateral root development) alter the mechanical pattern of the cellulosic wall at the subcellular level (1, red arrows). The PM conforms and changes its tensile pattern (2). The alteration of the tensile pattern of the PM results in its reorganization through phase separation of its components or through membrane trafficking (3, white circles). The new membrane organization (the signatures of nanodomains are indicated by coloured areas in the PM) instructs the localization of polar proteins (4, yellow and green dotted patterns), which, through interactions with the cytoskeleton, further reinforces PM heterogeneity. This consolidates polarity, which is a prerequisite for numerous cellular responses (such as division plane positioning). PM heterogeneity also affects the composition of the cell wall by modulating the activity of cell-wall-modifying enzymes, thereby facilitating the establishment of its biochemical and mechanical heterogeneity at the subcellular level (5). Cellular responses to the mechanical cues, such as local alteration of cell wall properties or deviation of division plane orientation, will affect the mechanical pattern at the tissue level and ultimately influence the course of morphogenesis (6).

between the cell wall and the PM, with a wealth of transmembrane proteins that anchor the PM to the cell wall. Nanodomains containing such cell-wall-interacting transmembrane proteins would directly depend on and respond to minute changes in cell wall mechanics and expansion (Fig. 6). The distribution of nanodomains can be instructive for the formation of higher-order protein domains, such as those decorating individual cell faces and edges (BASL, SOSEKI and so on). Cues coming from the cell wall in the form of altered tension or curvature can thus be translated into heterogeneity of the PM and eventually into the establishment of higher-order polar domains that would serve as a scaffold for subcellular elements, including proteins and the cytoskeleton (Fig. 6). Some of the recruited elements of the subcellular interior would then play a role in sustaining the formed PM heterogeneity or further reinforcing it[131,133]. The established cellular polarity would further underlie growth anisotropy and morphology changes[132] (Fig. 6).

In addition to a protoplast system, anisotropy of growth accompanied by the formation of PM polar protein domains has also been observed in a tissue context. Dong and colleagues observed bulging of hypocotyl epidermal cells upon overexpression of BASL protein in *Arabidopsis*[6]. The exclusive localization of BASL domains in the formed bulges hints at a cause-and-effect relationship and allows us to speculate that BASL domains trigger the alteration of cell wall properties, leading to the bulge formation. Polar protein domains that are in contact with both the PM (in turn directly connected to the cell wall) and the cellular interior might thus operate at the interface between mechanics and morphogenesis, enabling bidirectional signalling.

Acting as a lateral diffusion barrier for PM components, polar domains can also enable tension build-up, thereby modulating membrane mechanical anisotropy. This phenomenon has been demonstrated in vitro using membrane nanotubules, where BAR protein scaffolds created a frictional barrier to lipid diffusion[134]. As discussed above, the cytoskeleton also plays an important role in constraining membrane lateral diffusion. Both polar protein domains and the cytoskeleton are known to be linked to morphogenesis. For example, mutants affected in microtubule dynamics exhibit pronounced morphological defects. Mutations in genes encoding the microtubule-associated proteins SPIRAL2 and KATANIN, which modulate CMT severing, resulted in the formation of narrow and blunt sepal tips, respectively[18]. Moreover, CMTs can direct the deposition of cellulose fibrils, thereby determining the direction of cell wall expansion. CMTs might also be part of a feedback mechanism leading to cellulose-mediated rigidification of the cell wall where local stresses arise[135,136]. Correct localization of the polar auxin transporter PIN1 is crucial for the establishment of auxin flow and organogenesis at the SAM through cell wall loosening[137]. The organization of cytoskeletal elements, in turn, is adjusted by polar protein domains[25–27]. This scenario suggests that all elements (namely, the cell wall, the PM, polar protein domains and the cytoskeleton) are intimately connected, linked in a mutual feedback, and act as a composite material rather than individual components. Polar proteins in this composite structure might act at the interface between the PM (which perceives mechanical stimuli from the cell wall) and subcellular structures (Fig. 6).

The mechanical regulation of morphogenesis allows cells to integrate shape with function, something that could not be achieved by biochemical regulation alone. Its long-range signals convey information rapidly and instruct on both magnitude and directionality. However, the processing of the information and the feedback to mechanics are inconceivable without biochemical regulation that steps in to provide the complexity of responses to mechanical cues and ultimately creates the wealth of shapes and forms observed in nature.

Conclusion

It is becoming evident that mechanical forces act as instructive cues in plant development and morphogenesis. However, we still lack key insights into how mechanical signals are perceived, relayed and translated into cellular responses. The central role in this process is conventionally attributed to the cell wall–PM–cytoskeleton continuum. However, growing knowledge of polar protein domain functions and their interactions with every element of the above continuum suggests that polar proteins might be an intrinsic part of the cell mechanical machinery. Considering the specificity of polar protein domains for certain tissue types and developmental stages and their responsiveness to mechanical stimuli, one can speculate that they could allow for a tailored adjustment of the cellular response to mechanical stimuli and underlie the complexity of this response. We emphasize that these intimately connected elements

should be regarded as a composite material rather than individual structures. We draw attention to the bidirectional feedback between plant morphology and biochemistry mediated by mechanical signalling and ask how this feedback is regulated. The idea of mechanical forces serving as instructive cues in development and morphogenesis, processes that for decades have been approached exclusively from the biochemical standpoint, makes one wonder how far basic physical principles can be extrapolated to explain these processes in living systems. By analogy, it is becoming clear that another physical process, phase separation, can be highly relevant for many cellular and organismal processes.

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References

1. Thompson, D. W. *On Growth and Form* (1942).
2. Darwin, C. *The Origin of Species* (PF Collier & Son, 1909).
3. Turing, A. M. The chemical basis of morphogenesis. *Bull. Math. Biol.* **52**, 153–197 (1990).
4. Pillitteri, L. J., Guo, X. & Dong, J. Asymmetric cell division in plants: mechanisms of symmetry breaking and cell fate determination. *Cell. Mol. Life Sci.* **73**, 4213–4229 (2016).
5. Yang, Z. & Lavagi, I. Spatial control of plasma membrane domains: ROP GTPase-based symmetry breaking. *Curr. Opin. Plant Biol.* **15**, 601–607 (2012).
6. Dong, J., MacAlister, C. A. & Bergmann, D. C. BASL controls asymmetric cell division in *Arabidopsis*. *Cell* **137**, 1320–1330 (2009).
7. Muroyama, A. & Bergmann, D. Plant cell polarity: creating diversity from inside the box. *Annu. Rev. Cell Dev. Biol.* **35**, 309–336 (2019).
8. Goehring, N. W. PAR polarity: from complexity to design principles. *Exp. Cell Res.* **328**, 258–266 (2014).
9. Hoege, C. & Hyman, A. A. Principles of PAR polarity in *Caenorhabditis elegans* embryos. *Nat. Rev. Mol. Cell Biol.* **14**, 315–322 (2013).
10. Yoshida, S. et al. A SOSEKI-based coordinate system interprets global polarity cues in *Arabidopsis*. *Nat. Plants* **5**, 160–166 (2019).
11. Louveaux, M., Julien, J.-D., Mirabet, V., Boudaoud, A. & Hamant, O. Cell division plane orientation based on tensile stress in *Arabidopsis thaliana*. *Proc. Natl Acad. Sci. USA* **113**, E4294–E4303 (2016).
12. Heisler, M. G. et al. Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. *PLoS Biol.* **8**, e1000516 (2010).
13. Hamant, O. et al. Developmental patterning by mechanical signals in *Arabidopsis*. *Science* **322**, 1650–1655 (2008).
14. Sampathkumar, A. et al. Subcellular and supracellular mechanical stress prescribes cytoskeleton behavior in *Arabidopsis* cotyledon pavement cells. *eLife* **3**, e01967 (2014).
15. Sampathkumar, A., Yan, A., Krupinski, P. & Meyerowitz, E. M. Physical forces regulate plant development and morphogenesis. *Curr. Biol.* **24**, R475–R483 (2014).
16. Uyttewaal, M. et al. Mechanical stress acts via katanin to amplify differences in growth rate between adjacent cells in *Arabidopsis*. *Cell* **149**, 439–451 (2012).
17. Nakayama, N. et al. Mechanical regulation of auxin-mediated growth. *Curr. Biol.* **22**, 1468–1476 (2012).
18. Hervieux, N. et al. A mechanical feedback restricts sepal growth and shape in *Arabidopsis*. *Curr. Biol.* **26**, 1019–1028 (2016).
19. Landrein, B. et al. Mechanical stress contributes to the expression of the STM homeobox gene in *Arabidopsis* shoot meristems. *eLife* **4**, e07811 (2015).
20. Bringmann, M. & Bergmann, D. C. Tissue-wide mechanical forces influence the polarity of stomatal stem cells in *Arabidopsis*. *Curr. Biol.* **27**, 877–883 (2017).
21. Hong, L. et al. Variable cell growth yields reproducible organ development through spatiotemporal averaging. *Dev. Cell* **38**, 15–32 (2016).
22. Dalous, J. et al. Reversal of cell polarity and actin–myosin cytoskeleton reorganization under mechanical and chemical stimulation. *Biophys. J.* **94**, 1063–1074 (2008).
23. Aigouy, B. et al. Cell flow reorients the axis of planar polarity in the wing epithelium of *Drosophila*. *Cell* **142**, 773–786 (2010).
24. Paredez, A. R., Somerville, C. R. & Ehrhardt, D. W. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**, 1491–1495 (2006).
25. Ambrose, C., Allard, J. F., Cytrynbaum, E. N. & Wasteneys, G. O. A CLASP-modulated cell edge barrier mechanism drives cell-wide cortical microtubule organization in *Arabidopsis*. *Nat. Commun.* **2**, 430 (2011).
26. Kirchhelle, C., Garcia-Gonzalez, D., Irani, N. G., Jérusalem, A. & Moore, I. Two mechanisms regulate directional cell growth in *Arabidopsis* lateral roots. *eLife* **8**, e47988 (2019).
27. Ambrose, C. & Wasteneys, G. O. Cell edges accumulate gamma tubulin complex components and nucleate microtubules following cytokinesis in *Arabidopsis thaliana*. *PLoS ONE* **6**, e27423 (2011).
28. Fu, Y., Xu, T., Zhu, L., Wen, M. & Yang, Z. A ROP GTPase signaling pathway controls cortical microtubule ordering and cell expansion in *Arabidopsis*. *Curr. Biol.* **19**, 1827–1832 (2009).
29. Hamant, O. & Traas, J. The mechanics behind plant development. *N. Phytol.* **185**, 369–385 (2010).
30. Fuller, B. Tensegrity. *Portf. Art News Annu.* **4**, 112–127 (1961).
31. Stamenović, D. & Ingber, D. E. Tensegrity-guided self assembly: from molecules to living cells. *Soft Matter* **5**, 1137–1145 (2009).
32. Ingber, D. E. Tensegrity-based mechanosensing from macro to micro. *Prog. Biophys. Mol. Biol.* **97**, 163–179 (2008).
33. Hernández-Hernández, V., Rueda, D., Caballero, L., Alvarez-Buylla, E. R. & Benítez, M. Mechanical forces as information: an integrated approach to plant and animal development. *Front. Plant Sci.* **5**, 265 (2014).
34. Kutschera, U., Bergfeld, R. & Schopfer, P. Cooperation of epidermis and inner tissues in auxin-mediated growth of maize coleoptiles. *Planta* **170**, 168–180 (1987).
35. Hernández-Hernández, V., Benítez, M. & Boudaoud, A. Interplay between turgor pressure and plasmodesmata during plant development. *J. Exp. Bot.* **71**, 768–777 (2020).
36. Kutschera, U. Tissue stresses in growing plant organs. *Physiol. Plant.* **77**, 157–163 (1989).
37. Williamson, R. E. Alignment of cortical microtubules by anisotropic wall stresses. *Funct. Plant Biol.* **17**, 601–613 (1990).
38. Peaucelle, A., Wightman, R. & Höfte, H. The control of growth symmetry breaking in the *Arabidopsis* hypocotyl. *Curr. Biol.* **25**, 1746–1752 (2015).
39. Denninger, P. et al. Distinct RopGEFs successively drive polarization and outgrowth of root hairs. *Curr. Biol.* **29**, 1854–1865.e1855 (2019).
40. Michniewicz, M., Brewer, P. B. & Friml, J. Polar auxin transport and asymmetric auxin distribution. *Arabidopsis Book* **5**, e0108 (2007).
41. Scacchi, E. et al. Dynamic, auxin-responsive plasma membrane-to-nucleus movement of *Arabidopsis* BRX. *Development* **136**, 2059–2067 (2009).
42. Truernit, E., Bauby, H., Belcram, K., Barthélémy, J. & Palauqui, J.-C. OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in *Arabidopsis thaliana*. *Development* **139**, 1306–1315 (2012).
43. Miwa, K. et al. Plants tolerant of high boron levels. *Science* **318**, 1417 (2007).
44. Takano, J. et al. Polar localization and degradation of *Arabidopsis* boron transporters through distinct trafficking pathways. *Proc. Natl Acad. Sci. USA* **107**, 200910744 (2010).
45. Campos, R., Goff, J., Rodriguez-Furlan, C. & Van Norman, J. M. The *Arabidopsis* receptor kinase IRK is polarized and represses specific cell divisions in roots. *Dev. Cell* **52**, 183–195.e184 (2020).
46. Le, P. Y. & Ambrose, C. CLASP promotes stable tethering of endoplasmic microtubules to the cell cortex to maintain cytoplasmic stability in *Arabidopsis* meristematic cells. *PLoS ONE* **13**, e0198521 (2018).
47. Feraru, E. et al. PIN polarity maintenance by the cell wall in *Arabidopsis*. *Curr. Biol.* **21**, 338–343 (2011).
48. Kusumi, A. et al. Dynamic organizing principles of the plasma membrane that regulate signal transduction: commemorating the fortieth anniversary of Singer and Nicolson's fluid-mosaic model. *Annu. Rev. Cell Dev. Biol.* **28**, 215–250 (2012).
49. Malinsky, J., Opekarová, M., Grossmann, G. & Tanner, W. Membrane microdomains, rafts, and detergent-resistant membranes in plants and fungi. *Annu. Rev. Plant Biol.* **64**, 501–529 (2013).
50. Boutté, Y. & Grebe, M. Cellular processes relying on sterol function in plants. *Curr. Opin. Plant Biol.* **12**, 705–713 (2009).
51. Levental, I., Grzybek, M. & Simons, K. Greasing their way: lipid modifications determine protein association with membrane rafts. *Biochemistry* **49**, 6305–6316 (2010).
52. Levental, I., Lingwood, D., Grzybek, M., Coskun, Ü. & Simons, K. Palmitoylation regulates raft affinity for the majority of integral raft proteins. *Proc. Natl Acad. Sci. USA* **107**, 22050–22054 (2010).
53. Van Den Bogaart, G. et al. Membrane protein sequestering by ionic protein–lipid interactions. *Nature* **479**, 552–555 (2011).
54. Lingwood, D. & Simons, K. Lipid rafts as a membrane-organizing principle. *Science* **327**, 46–50 (2010).
55. Daněk, M., Valentová, O. & Martinec, J. Flotillins, erlins, and HIRs: from animal base camp to plant new horizons. *Crit. Rev. Plant Sci.* **35**, 191–214 (2016).
56. Raffaele, S., Mongrand, S., Gamas, P., Niebel, A. & Ott, T. Genome-wide annotation of remorins, a plant-specific protein family: evolutionary and functional perspectives. *Plant Physiol.* **145**, 593–600 (2007).

57. Jarsch, I. K. et al. Plasma membranes are subcompartmentalized into a plethora of coexisting and diverse microdomains in *Arabidopsis* and *Nicotiana benthamiana*. *Plant Cell* **26**, 1698–1711 (2014).
58. Demir, F. et al. *Arabidopsis* nanodomain-delimited ABA signaling pathway regulates the anion channel SLAH3. *Proc. Natl Acad. Sci. USA* **110**, 8296–8301 (2013).
59. Bücherl, C. A. et al. Plant immune and growth receptors share common signalling components but localise to distinct plasma membrane nanodomains. *eLife* **6**, e25114 (2017).
60. Martiniere, A. et al. Osmotic stress activates two reactive oxygen species pathways with distinct effects on protein nanodomains and diffusion. *Plant Physiol.* **179**, 1581–1593 (2019).
61. Platre, M. P. et al. Developmental control of plant Rho GTPase nano-organization by the lipid phosphatidylserine. *Science* **364**, 57–62 (2019).
62. Jaillais, Y. & Ott, T. The nanoscale organization of the plasma membrane and its importance in signaling: a proteolipid perspective. *Plant Physiol.* **182**, 1682–1696 (2020).
63. Groves, J. T. & Kuriyan, J. Molecular mechanisms in signal transduction at the membrane. *Nat. Struct. Mol. Biol.* **17**, 659–665 (2010).
64. Sadegh, S., Higgins, J. L., Mannion, P. C., Tamkun, M. M. & Krapf, D. Plasma membrane is compartmentalized by a self-similar cortical actin meshwork. *Phys. Rev.* **7**, 011031 (2017).
65. Szymanski, W. G. et al. Cytoskeletal components define protein location to membrane microdomains. *Mol. Cell. Proteom.* **14**, 2493–2509 (2015).
66. Liang, P. et al. Symbiotic root infections in *Medicago truncatula* require remorin-mediated receptor stabilization in membrane nanodomains. *Proc. Natl Acad. Sci. USA* **115**, 5289–5294 (2018).
67. Yanagisawa, M., Alonso, J. M. & Szymanski, D. B. Microtubule-dependent confinement of a cell signaling and actin polymerization control module regulates polarized cell growth. *Curr. Biol.* **28**, 2459–2466.e2454 (2018).
68. Martinière, A. et al. Cell wall constrains lateral diffusion of plant plasma-membrane proteins. *Proc. Natl Acad. Sci. USA* **109**, 12805–12810 (2012).
69. McKenna, J. F. et al. The cell wall regulates dynamics and size of plasma-membrane nanodomains in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **116**, 12857–12862 (2019).
70. Daněk, M. et al. Cell wall contributes to the stability of plasma membrane nanodomain organization of *Arabidopsis thaliana* FLOTILLIN2 and HYPERSENSITIVE INDUCED REACTION1 proteins. *Plant J.* **101**, 619–636 (2020).
71. Pan, X. et al. Auxin-induced signaling protein nanoclustering contributes to cell polarity formation. *Nat. Commun.* **11**, 3914 (2020).
72. Sampathkumar, A. Mechanical feedback-loop regulation of morphogenesis in plants. *Development* **147**, dev177964 (2020).
73. Hamant, O. & Haswell, E. S. Life behind the wall: sensing mechanical cues in plants. *BMC Biol.* **15**, 59 (2017).
74. Paradez, A., Wright, A. & Ehrhardt, D. W. Microtubule cortical array organization and plant cell morphogenesis. *Curr. Opin. Plant Biol.* **9**, 571–578 (2006).
75. Hamant, O., Inoue, D., Bouchez, D., Dumais, J. & Mjolsness, E. Are microtubules tension sensors? *Nat. Commun.* **10**, 2360 (2019).
76. Buschmann, H. & Lloyd, C. W. *Arabidopsis* mutants and the network of microtubule-associated functions. *Mol. Plant* **1**, 888–898 (2008).
77. Mirabet, V. et al. The self-organization of plant microtubules inside the cell volume yields their cortical localization, stable alignment, and sensitivity to external cues. *PLoS Comput. Biol.* **14**, e1006011 (2018).
78. Durand-Smet, P., Spelman, T. A., Meyerowitz, E. M. & Jönsson, H. Cytoskeletal organization in isolated plant cells under geometry control. *Proc. Natl Acad. Sci. USA* **117**, 17399–17408 (2020).
79. Li, S., Lei, L., Somerville, C. R. & Gu, Y. Cellulose synthase interactive protein 1 (CSI1) links microtubules and cellulose synthase complexes. *Proc. Natl Acad. Sci. USA* **109**, 185–190 (2012).
80. Bringmann, M. et al. POM-POM2/cellulose synthase interacting1 is essential for the functional association of cellulose synthase and microtubules in *Arabidopsis*. *Plant Cell* **24**, 163–177 (2012).
81. Gardiner, J. C. et al. A 90-kD phospholipase D from tobacco binds to microtubules and the plasma membrane. *Plant Cell* **13**, 2143–2158 (2001).
82. Oda, Y. Emerging roles of cortical microtubule–membrane interactions. *J. Plant Res.* **131**, 5–14 (2018).
83. Nakamura, M., Ehrhardt, D. W. & Hashimoto, T. Microtubule and katanin-dependent dynamics of microtubule nucleation complexes in the acentrosomal *Arabidopsis* cortical array. *Nat. Cell Biol.* **12**, 1064–1070 (2010).
84. Heisler, M., Hamant, O., Krupinski, P., Uyttewaal, M. & Ohno, C. Alignment between PIN1 polarity and microtubule orientation in the shoot apical meristem reveals a tight coupling between morphogenesis and auxin transport. *PLoS Biol.* **8**, e1000516 (2010).
85. Eren, E. C., Dixit, R. & Gautam, N. A three-dimensional computer simulation model reveals the mechanisms for self-organization of plant cortical microtubules into oblique arrays. *Mol. Biol. Cell* **21**, 2674–2684 (2010).
86. Ambrose, J. C., Shoji, T., Kotzer, A. M., Pighin, J. A. & Wasteneys, G. O. The *Arabidopsis* CLASP gene encodes a microtubule-associated protein involved in cell expansion and division. *Plant Cell* **19**, 2763–2775 (2007).
87. Verger, S., Long, Y., Boudaoud, A. & Hamant, O. A tension–adhesion feedback loop in plant epidermis. *eLife* **7**, e34460 (2018).
88. Routier-Kierzkowska, A.-L. et al. Cellular force microscopy for in vivo measurements of plant tissue mechanics. *Plant Physiol.* **158**, 1514–1522 (2012).
89. Chan, J. Microtubule and cellulose microfibril orientation during plant cell and organ growth. *J. Microsc.* **247**, 23–32 (2012).
90. Crowell, E. F. et al. Differential regulation of cellulose orientation at the inner and outer face of epidermal cells in the *Arabidopsis* hypocotyl. *Plant Cell* **23**, 2592–2605 (2011).
91. Robinson, S. & Kuhlemeier, C. Global compression reorients cortical microtubules in *Arabidopsis* hypocotyl epidermis and promotes growth. *Curr. Biol.* **28**, 1794–1802.e1792 (2018).
92. Geitmann, A., Hush, J. & Overall, R. Inhibition of ethylene biosynthesis does not block microtubule re-orientation in wounded pea roots. *Protoplasma* **198**, 135–142 (1997).
93. Hejnowicz, Z., Rusin, A. & Rusin, T. Tensile tissue stress affects the orientation of cortical microtubules in the epidermis of sunflower hypocotyl. *J. Plant Growth Regul.* **19**, 31–44 (2000).
94. Jacques, E., Verbelen, J.-P. & Vissenberg, K. Mechanical stress in *Arabidopsis* leaves orients microtubules in a 'continuous' supracellular pattern. *BMC Plant Biol.* **13**, 163 (2013).
95. Colin, L. et al. Cortical tension overrides geometrical cues to orient microtubules in confined protoplasts. *Proc. Natl Acad. Sci. USA* **117**, 32731–32738 (2020).
96. Yu, M. et al. mDia1 senses both force and torque during F-actin filament polymerization. *Nat. Commun.* **8**, 1650 (2017).
97. Risca, V. I. et al. Actin filament curvature biases branching direction. *Proc. Natl Acad. Sci. USA* **109**, 2913–2918 (2012).
98. Chugh, P. et al. Actin cortex architecture regulates cell surface tension. *Nat. Cell Biol.* **19**, 689–697 (2017).
99. Simon, C., Caorsi, V., Campillo, C. & Sykes, C. Interplay between membrane tension and the actin cytoskeleton determines shape changes. *Phys. Biol.* **15**, 065004 (2018).
100. Zhang, L., Xing, J. & Lin, J. At the intersection of exocytosis and endocytosis in plants. *N. Phytol.* **224**, 1479–1489 (2019).
101. Houk, A. R. et al. Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* **148**, 175–188 (2012).
102. Thottacherry, J. J. et al. Mechanochemical feedback control of dynamin independent endocytosis modulates membrane tension in adherent cells. *Nat. Commun.* **9**, 4217 (2018).
103. Wen, P. J. et al. Actin dynamics provides membrane tension to merge fusing vesicles into the plasma membrane. *Nat. Commun.* **7**, 12604 (2016).
104. Stewart, M. P. et al. Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* **469**, 226–230 (2011).
105. Basu, R. et al. Cytotoxic T cells use mechanical force to potentiate target cell killing. *Cell* **165**, 100–110 (2016).
106. Ranade, S. S., Syeda, R. & Patapoutian, A. Mechanically activated ion channels. *Neuron* **87**, 1162–1179 (2015).
107. Singer, S. J. & Nicolson, G. L. The fluid mosaic model of the structure of cell membranes. *Science* **175**, 720–731 (1972).
108. Diz-Muñoz, A., Fletcher, D. A. & Weiner, O. D. Use the force: membrane tension as an organizer of cell shape and motility. *Trends Cell Biol.* **23**, 47–53 (2013).
109. Shi, Z. & Baumgart, T. Membrane tension and peripheral protein density mediate membrane shape transitions. *Nat. Commun.* **6**, 5974 (2015).
110. Kusumi, A. et al. Paradigm shift of the plasma membrane concept from the two-dimensional continuum fluid to the partitioned fluid: high-speed single-molecule tracking of membrane molecules. *Annu. Rev. Biophys. Biomol. Struct.* **34**, 351–378 (2005).
111. Fogelson, B. & Mogilner, A. Computational estimates of membrane flow and tension gradient in motile cells. *PLoS ONE* **9**, e84524 (2014).
112. Lieber, A. D., Schweitzer, Y., Kozlov, M. M. & Keren, K. Front-to-rear membrane tension gradient in rapidly moving cells. *Biophys. J.* **108**, 1599–1603 (2015).
113. Schweitzer, Y., Lieber, A. D., Keren, K. & Kozlov, M. M. Theoretical analysis of membrane tension in moving cells. *Biophys. J.* **106**, 84–92 (2014).
114. Shi, Z., Graber, Z. T., Baumgart, T., Stone, H. A. & Cohen, A. E. Cell membranes resist flow. *Cell* **175**, 1769–1779.e1713 (2018).
115. García-Sáez, A. J., Chiantia, S. & Schwille, P. Effect of line tension on the lateral organization of lipid membranes. *J. Biol. Chem.* **282**, 33537–33544 (2007).
116. Oglęcka, K., Rangamani, P., Liedberg, B., Kraut, R. S. & Parikh, A. N. Oscillatory phase separation in giant lipid vesicles induced by transmembrane osmotic differentials. *eLife* **3**, e03695 (2014).

117. Ho, J. C., Rangamani, P., Liedberg, B. & Parikh, A. N. Mixing water, transducing energy, and shaping membranes: autonomously self-regulating giant vesicles. *Langmuir* **32**, 2151–2163 (2016).
118. Oglecka, K., Sanborn, J., Parikh, A. N. & Kraut, R. S. Osmotic gradients induce bio-reminiscent morphological transformations in giant unilamellar vesicles. *Front. Physiol.* **3**, 120 (2012).
119. Colom, A. et al. A fluorescent membrane tension probe. *Nat. Chem.* **1**, 1118–1125 (2018).
120. Riggi, M. et al. Decrease in plasma membrane tension triggers PtdIns (4, 5) P 2 phase separation to inactivate TORC2. *Nat. Cell Biol.* **20**, 1043–1051 (2018).
121. Michels, L. et al. Complete microviscosity maps of living plants cells and tissues with a toolbox of targeting mechanoprobes. *Proc. Natl Acad. Sci. USA* **117**, 18110–18118 (2020).
122. Homann, U. Fusion and fission of plasma-membrane material accommodates for osmotically induced changes in the surface area of guard-cell protoplasts. *Planta* **206**, 329–333 (1998).
123. Fricke, W., Jarvis, M. C. & Brett, C. T. Turgor pressure, membrane tension and the control of exocytosis in higher plants. *Plant Cell Environ.* **23**, 999–1003 (2000).
124. Wolfe, J. & Steponkus, P. L. Mechanical properties of the plasma membrane of isolated plant protoplasts: mechanism of hyperosmotic and extracellular freezing injury. *Plant Physiol.* **71**, 276–285 (1983).
125. Apodaca, G. Modulation of membrane traffic by mechanical stimuli. *Am. J. Physiol. Renal Physiol.* **282**, F179–F190 (2002).
126. Asnacios, A. & Hamant, O. The mechanics behind cell polarity. *Trends Cell Biol.* **22**, 584–591 (2012).
127. Sorre, B. et al. Curvature-driven lipid sorting needs proximity to a demixing point and is aided by proteins. *Proc. Natl Acad. Sci. USA* **106**, 5622–5626 (2009).
128. Subramaniam, A. B., Lecuyer, S., Ramamurthi, K. S., Losick, R. & Stone, H. A. Particle/fluid interface replication as a means of producing topographically patterned polydimethylsiloxane surfaces for deposition of lipid bilayers. *Adv. Mater.* **22**, 2142–2147 (2010).
129. Feriani, L., Cristofolini, L. & Cicuta, P. Soft pinning of liquid domains on topographical hemispherical caps. *Chem. Phys. Lipids* **185**, 78–87 (2015).
130. Rinaldin, M., Fonda, P., Giomi, L. & Kraft, D. J. Geometric pinning and antimixing in scaffolded lipid vesicles. *Nat. Commun.* **11**, 4314 (2020).
131. Ren, C. et al. Leukocyte cytoskeleton polarization is initiated by plasma membrane curvature from cell attachment. *Dev. Cell* **49**, 206–219.e207 (2019).
132. Chan, J., Mansfield, C., Clouet, F., Dorussen, D. & Coen, E. Intrinsic cell polarity coupled to growth axis formation in tobacco BY-2 cells. *Curr. Biol.* **30**, 4999–5006 (2020).
133. Zhao, H. et al. Membrane-sculpting BAR domains generate stable lipid microdomains. *Cell Rep.* **4**, 1213–1223 (2013).
134. Simunovic, M. et al. Friction mediates scission of tubular membranes scaffolded by BAR proteins. *Cell* **170**, 172–184.e111 (2017).
135. Altartouri, B. et al. Pectin chemistry and cellulose crystallinity govern pavement cell morphogenesis in a multi-step mechanism. *Plant Physiol.* **181**, 127–141 (2019).
136. Bidhendi, A. J., Altartouri, B., Gosselin, F. P. & Geitmann, A. Mechanical stress initiates and sustains the morphogenesis of wavy leaf epidermal cells. *Cell Rep.* **28**, 1237–1250.e1236 (2019).
137. Sassi, M. et al. An auxin-mediated shift toward growth isotropy promotes organ formation at the shoot meristem in *Arabidopsis*. *Curr. Biol.* **24**, 2335–2342 (2014).

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Competing interests

The authors declare no competing interests.

Additional information

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