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The art of interdigitation: Current views on pavement cell shape acquisition

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Cell shape acquisition is a central feature of morphogenesis, governing tissue organization, organ development, and organismal architecture. In vascular plants, leaf epidermal cells often adopt wavy, interlocking geometries, creating intricate jigsaw puzzle-like patterns. These complex shapes, which develop from simple polyhedral progenitors, provide an excellent model for investigating the mechanisms driving cell shape acquisition. Lobed, interdigitated pavement cells contribute to planar leaf expansion and mechanical stability. Recent advances reveal that the coordination of cell wall remodeling, cytoskeletal organization, and mechanical forces underlies the emergence of lobes (outgrowths) and necks (indentations) that stabilize the tissue and support organ growth. Biomechanical models further demonstrate how spatial modulation of wall stiffness and cytoskeletal dynamics drive interdigitated growth, while phytohormone signaling and communication among neighboring cells fine-tune patterning across the epidermal layer. Here, we bring together current insights into the mechanical, molecular and signaling frameworks that shape pavement cell morphogenesis and highlight key knowledge gaps and future research directions.

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Introduction

Morphogenesis was first defined by Thompson in 1917 as a series of coherent, geometrical transformations of cells that drive organismal development [1]. In plants, this process is particularly complex due to the presence of the cell wall, a polysaccharide-rich network outside the cell membrane. The cell wall must be sufficiently rigid to provide structural support and protection, yet flexible enough to permit cell growth.

During development, cells acquire distinct shapes with remarkable precision and coordination, yet the mechanisms underlying this process remain only partially understood. To achieve their final morphology, cells must increase in volume while modulating surface expansion rates in different regions, giving rise to a wide variety of cell shapes adapted to specific locations and functions [2*,3,4]. This is especially important in the epidermis, the outermost tissue layer, which forms the primary interface with the environment. In both plant and animal epidermis, cells exhibit a broad range of morphologies, such as squamous, cuboidal, columnar, or jigsaw puzzle shape, each associated with specialized functions, including protection, mechanical support, selective permeability, and secretion [3]. In plants, the puzzle-shaped epidermal pavement cells of leaves and cotyledons provide an excellent model for studying complex cell shape acquisition. Their interdigitated growth, characterized by alternating lobes (outgrowths) and necks (indentations), enhances mechanical stability and contributes to organ-level expansion across spatial scales [2*,5,6]. This is even more striking because pavement cells must expand into one another while remaining mechanically constrained by shared rigid cell walls, requiring tightly coordinated growth or local adaptive responses. As a result, cell–cell interactions and tissue-level mechanical coupling play a critical role in determining individual cell geometry and overall leaf morphogenesis.

In this review, we discuss recent advances that reveal how the coordinated regulation of cell wall properties, cytoskeletal dynamics, and mechanical forces drives the

formation of puzzle-shaped pavement cells in plants. We also highlight insights from biomechanical simulations explaining interdigitated growth, as well as the roles of phytohormones and neighboring cell signaling. Finally, we outline current knowledge gaps and propose future directions in this field.

A biomechanical continuum defining pavement cell shape

In plants, cells form a continuous network within a tissue in which their cell walls are physically interconnected. Turgor pressure exerted on the cell walls results in mechanical stress, a reactive force per unit area of the cell wall acting both along (in-plane) and perpendicular (out-of-plane) to the cell wall. The magnitude of this stress along both directions depends on the morphological features of the cell (such as size and shape), meaning that different cells within the same tissue can experience different mechanical conditions [6—9]. These mechanical stresses, together with the intrinsic material properties of the cell wall, shape how a cell grows over time. In turn, the spatiotemporal distribution of these forces at both the tissue and cellular levels plays a central role in determining how cells acquire their characteristic shapes [8,10,11,12**,13,14].

The cell wall is primarily composed of polysaccharides, among which cellulose microfibrils are the main load-bearing element and are interconnected with pectins and hemicelluloses. Together, these components form a strong fibrillar network that helps define cell shape [12**,15]. Cellulose microfibrils are linear chains of β (1,4)-linked D-glucose synthesized by a multimeric CELLULOSE SYNTHASE COMPLEX (CSC) embedded in the plasma membrane [15—17]. CSCs are tethered to cortical microtubules, controlling their deposition in specific orientations [16,18,19]. Localized microtubule arrays are proposed to direct the patterning of cellulose microfibril deposition, producing patches of anisotropic wall expansion that drive symmetry breaking [10,16,20,21]. The dynamic behavior of CSCs during the early stages of pavement cell shape formation has been characterized [16]. During the initial stages of pavement cell development, a population of CSCs moves independently of microtubules. As the cells begin to develop their characteristic shape, microtubules progressively localize to regions of concave curvature (necks), and CSCs increasingly associate with these microtubules, accompanied by rises in both their abundance and velocity. This suggests that CSC distribution, movement, and microtubule association are developmentally regulated. Furthermore, uncoupling microtubule-CSC tethering promotes microtubule organization in the neck regions, thereby imposing heterogenous mechanical stress in a shape-dependent manner. In addition, transient mechanical perturbations such as ablation or compression inhibit CSC movement, leading to reduced CSC density. This reduction allows microtubules to quickly reorganize in the new stress direction. As microtubules realign, they guide the deposition of cellulose microfibrils in a similar orientation, which helps the cell wall adjust to and alleviate the new mechanical stress [16]. Moreover, the orientation of microtubules in response to developmental mechanical stimuli or chemical signals is regulated by the conserved proteins CLIP ASSOCIATED PROTEIN (CLASP) and KATANIN [22]. CLASP specifically regulates microtubule orientation at the subcellular stress level, whereas KATANIN influences the microtubule arrangement in response to supracellular mechanical stress to control pavement cell shape in developing cotyledons. The INCREASED PETAL GROWTH ANISOTROPY 1 (IPGA1) protein was recently shown to coordinate microtubule organization during pavement cell shape development by genetically and physically interacting with ANGUSTIFOLIA (AN) and KATANIN [23].

Pectin polysaccharides and their degree of esterification are key regulators of cell wall mechanical properties. Directional expansion and wall stiffness likely emerge from the interplay between pectin and cellulose organization [8,20,24]. Within pavement cells, lobe and neck formation correlate with localized pectin demethylesterification. Lobe regions show highly methylesterified pectin, whereas neck sites accumulate more demethylesterified pectin [20,25]. Mutants with reduced pectin demethylesterification (*pme3* and *35S:PMEI1*) display wider necks, suggesting that early pectin demethylesterification, along with simultaneous cellulose crystallinity and alignment, play a role in pavement cell morphogenesis [20,25]. Using super-resolution three-dimensional direct stochastic optical reconstruction microscopy (3D-dSTORM), Haas et al. (2020) revealed that homogalacturonan, a type of pectin, aligns and forms crystalline nanofilaments in the anticlinal (side) wall, whose swelling, independent of turgor pressure, drives local expansion and helps shape interdigitated pavement cells [26]. This challenges traditional views of a turgor pressure-dependent role of pectin in cell expansion and suggests a dual function involving both mechanical regulation and potential roles in cellular signaling [26]. Cosgrove and Anderson (2020) emphasize that during lobe formation, the periclinal (top and bottom) and anticlinal walls undergo spatially heterogeneous and anisotropic expansion, coordinated with cytoskeletal reorganization and localized pectin redistribution. Conversely, the oriented deposition of cellulose microfibrils likely imposes mechanical restrictions that limit periclinal wall expansion [27]. Using biochemical and cell biology experiments, it has been shown that FERONIA (FER), a member of the CATHARANTHUS ROSEUS RECEPTOR-LIKE KINASE 1-LIKE (CrRLK1L) family, associates with the demethyesterified pectin in the cell wall and

activates downstream cellular signaling [25]. While these studies implicate pectin demethylesterification in early pavement cell morphogenesis, definitive support for its role will require targeted manipulation of pectin methylation and higher-order mutants. Pectin is also suggested to act as a crucial link between endoreduplication and cell wall remodeling in the regulation of pavement cell size [4], as reduced endoreduplication results in cell wall stiffening and smaller cells [28]. Members of the CINCINNATA-like TEOSINTE BRANCHED 1/CYCLOIDEA/PCF (TCP) clade, which are key regulators of the mitotic cycle, directly activate POLYGALACTURONASE LIKE 1 (PGL1), which decreases pectin polymer abundance. Studies in both wild-type and the *tcpΔ7* mutant demonstrate that lower pectin levels facilitate endocycle progression and promote cell enlargement [4]. Exploring further how cell wall–derived mechanical forces interface with internal cellular processes such as endoreduplication would provide valuable insight into the regulation of pavement cell shape and size.

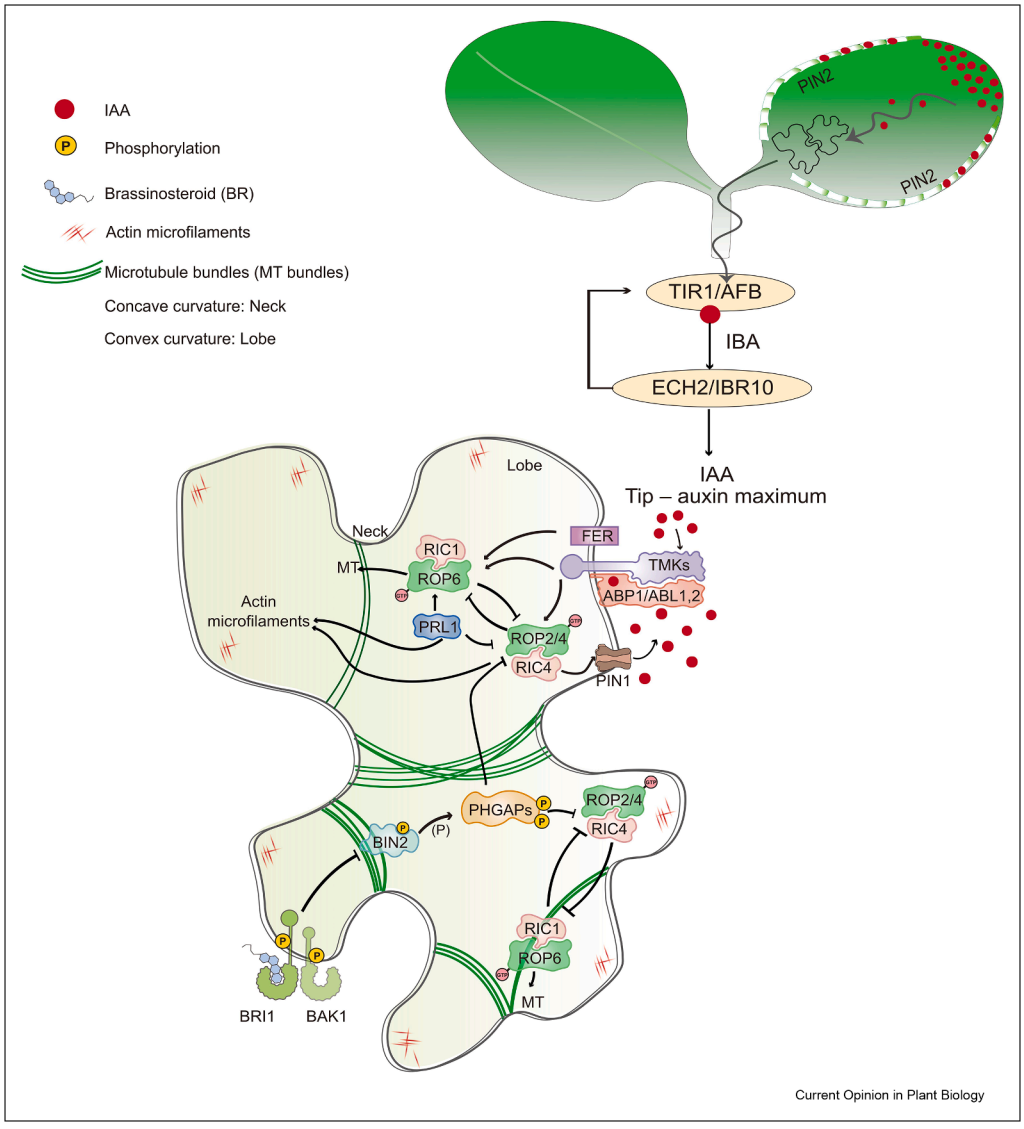
Recently, experimental measurements of cell and tissue elasticity, combined with time-series imaging of cell growth and scanning electron microscopy data on cell wall anatomy, have been used to construct Finite Element (FE) models of pavement cell morphogenesis [12,29,30], providing insights into both the mechanisms and potential functions of pavement cell shape acquisition. FE modeling is used to mathematically predict spatial patterns of mechanical stress in growing cells and tissues [8,10,11,14,26]. It is well established that, for a given turgor pressure, the in-plane mechanical stress patterns experienced by the cell wall depend on the cell's size and shape, as well as the distribution of mechanical properties along the wall [6,8,14,31,32]. Interestingly, this relationship allows predictions that, at both cellular and supra-cellular scales, puzzle-shaped cells generate lower overall tissue mechanical stress (in-plane) compared to simplified, polygonal cells in isotropically expanding tissues [6], offering an initial clue to the evolutionary advantage of this complex cell shape. FE modeling is also used to understand how pavement cells acquire their puzzle shape. Interestingly, the mechano-chemical properties of the anticlinal pavement cell wall are heterogeneous along its length and across its width [8], which has been predicted to create bending under in-plane tensile load by FE simulations [8,14], with mechanically weaker segments adopting convex curvature. The mechanical heterogeneity between the anticlinal cell walls in the lobe and neck regions suggests the involvement of intercellular coordination, with adjacent cells (and their cell walls) playing distinct roles in interdigitating cell shape. Multiple FE model simulations also predict hotspots of in-plane mechanical stress in both anticlinal and periclinal walls in the neck region [10,11,19], which suggests that adjacent periclinal cell walls also experience heterogenous in-plane mechanical stress patterns during lobe formation. Interestingly, microtubule organization, cellulose microfibril organization, and pectin methylesterification patterns [10,11,19,33] reflect this heterogeneity: anticlinal cell walls exhibit more dense, vertically (perpendicular to the plane of the leaf) oriented microtubule bundles at the base of newly forming necks [10] while periclinal walls also show a convergence of microtubules and cellulose microfibrils towards the neck region [10,11,19], along with an apparent reduction in pectin methylesterification levels [11]. These patterns in turn align with the distribution of growth rates in distinct zones along the length of the anticlinal cell wall [11]. Altogether, these results establish a functional link between mechanical stress patterns and cell wall composition and expansion at both anticlinal and periclinal walls, placing mechanical stress patterns as an upstream factor in determining the position and direction of lobe formation. These results also highlight the importance of intercellular coordination in lobe acquisition.

A cellular signaling blueprint behind pavement cell shape

Most of the knowledge about cellular signaling in regulating pavement cell shape acquisition comes from studies of *Arabidopsis thaliana* pavement cells. The phytohormone auxin acts as a signaling molecule coordinating cell morphogenesis by promoting cell wall loosening and microtubule reorientation through activation of RHO OF PLANTS GTPase (ROP) signaling [9,24,34,35*]. The acid growth theory suggests that auxin activates H^+-ATPase pumps, releasing protons into the cell apoplast [36]. This acidifies the apoplast, causing cell wall loosening and thereby cell expansion. In hypocotyl and root tips, auxin has also been shown to regulate methylesterification of homogalacturonan and cell wall-modifying enzymes [37,38]. Specifically, auxin biosynthesis, signaling, and polar transport are known to govern pavement cell shape. Exogenous auxin enhances interdigitation in a dose-dependent manner [34,39], while the auxin biosynthesis quadruple mutant *yuc1,2,4,6* shows reduced interdigitation, and the auxin over-production mutant *yuc1D* exhibits larger cells with more lobes than the wild-type [34,39]. The auxin transporter PINFORMED 1 (PIN1), enriched at lobe tips, promotes localized auxin accumulation (Figure 1), and the PIN auxin transporter mutants *pin1*, *pin2*, and *pin3,4,7* display interdigitation defects, underscoring auxin's role in pavement cell morphogenesis [34,39,40**].

The global and local auxin signaling mediated by the TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALING F-BOX (TIR1/AFB) and TRANSMEMBRANE KINASES (TMKs)/AUXIN BINDING PROTEIN 1 (ABP1)/ABP1-LIKE 1/2 (ABL1/2) modules, respectively, coordinately regulate pavement cell morphogenesis [40**,41,42**,43] (Figure 1). Yu et al. (2023) identified the two germin-family auxin binding

Figure 1



A proposed framework illustrating how cellular pathways coordinate to regulate pavement cell interdigitation. Before interdigitation begins, TRANSPORT INHIBITOR RESPONSE 1/AUXIN-SIGNALING F-BOX (TIR1/AFB1)-dependent signaling promotes indole butyric acid (IBA) conversion into indole acetic acid (IAA) via ENOYL COA HYDRATASE 2 (ECH2) and INDOLE-3-BUTYRIC ACID RESPONSE 10 (IBR10). IAA then promotes PINFORMED 2 (PIN2) accumulation in marginal cells, thereby enhancing auxin transport toward the cotyledon tip, where auxin levels progressively build up (indicated by red dots). The auxin maximum at the cotyledon tip then acts as a global cue, spreading toward other regions of the cotyledon epidermis, likely by diffusion through the apoplast (indicated by wavy black arrow). This localized auxin enrichment (red dots) activates cell surface auxin signaling mediated by TRANSMEMBRANE KINASES (TMKs)/AUXIN BINDING PROTEIN 1 (ABP1)/ABP1-LIKE 1/2 (ABL1/2), which then activates two opposing downstream signaling pathways: the RHO OF PLANTS GTPases 2/4 (ROP2/4) and ROP6 pathways. The ROP2/4–ROP-INTERACTIVE CRIB MOTIF-CONTAINING 4 (RIC4) pathway facilitates the formation of fine actin microfilaments (shown in red), thereby driving lobe expansion. In contrast, the ROP6–RIC1 pathway promotes the alignment of cortical microtubules (MTs, shown in green), leading to localized growth restriction at the neck regions. The accumulated actin microfilaments promote PIN1 polarization at lobe tips. The auxin exported by PIN1 then activates ROP2/4 at lobe regions via the TMK1 auxin-sensing complex in a positive feedback loop. Furthermore, the CrRLK1L receptor kinase FERONIA (FER) binds to demethylesterified cell wall pectin, leading to the activation of the ROP6 signaling pathway. Actin microfilament integrity is also maintained by PLEIOTROPIC REGULATORY LOCUS 1 (PRL1), while simultaneously inhibiting ROP2 and promoting ROP6. Additionally, brassinosteroids (BRs) control pavement cell morphogenesis through two PLECKSTRIN HOMOLOGY GTPase-ACTIVATING proteins (PHGAPs) via BRASSINOSTEROID INSENSITIVE 2 (BIN2), which phosphorylates PHGAPs to maintain their stability and polarized distribution. When BIN2 activity is suppressed by BRs via the BR INSENSITIVE 1 (BRI1)- BRI1 ASSOCIATED RECEPTOR KINASE 1 (BAK1) receptor complex, PHGAPs are degraded, leading to activation of ROP2 GTPase signaling in the lobes. In contrast, where PHGAPs remain present, they inhibit ROP2 activity, thereby restricting signaling in the neck regions.

proteins, ABL1 and 2, that are primarily found in the apoplast at the cell surface, where they bind auxin, and then directly interact with TMKs [42**]. The *abp1,abl1,2* triple mutant and *tmk1,2,3,4* quadruple mutant exhibit defects in pavement cell shape and in PIN1 localization, suggesting an indispensable role of cell surface auxin signaling in shape acquisition [42**]. Furthermore, Perez-Henriquez et al. (2025) showed that pavement cell interdigitation initiates at the apical region of the cotyledon, spreads toward the upper-mid regions, and finally reaches the base of the organ [40**]. This developmental progression depends on coordinated global and local auxin signaling. Specifically, TIR1/AFB-based transcriptional signaling promotes the indole butyric acid (IBA)-dependent auxin biosynthesis pathway by activating ENOYL COA HYDRATASE 2 (ECH2) and INDOLE-3-BUTYRIC ACID RESPONSE 10 (IBR10), leading to auxin accumulation (Figure 1). This promotes accumulation and polarization of the polar auxin transporter PIN2 at marginal cells and contributes to a local auxin maximum at the cotyledon tip [40**,43]. As auxin diffuses from the tip towards the base of the cotyledon, it triggers localized pavement cell interdigitation by activating TMK-dependent cell surface auxin signaling [40**,43] (Figure 1). Interestingly, the auxin maximum at the cotyledon tip also initiates a negative feedback loop. The elevated auxin level activates TRANSPORTER OF IBA 1 (TOB1), which promotes vacuolar internalization of IBA and suppresses PIN2 polar accumulation at marginal cells. This dynamic balance between transient, self-activating and self-terminating polar auxin transport through PIN2 establishes auxin maxima that orchestrate the spatiotemporal regulation of pavement cell interdigitation in the cotyledon [40**,43] (Figure 1). Despite growing evidence implicating auxin dynamics in pavement cell morphogenesis, several questions remain. How auxin gradients are interpreted at the subcellular level to initiate lobe formation is still unclear. Future research should aim to dissect how different auxin transporters coordinate across tissue layers and how they integrate with cytoskeletal elements and mechanical signals of the cell wall to define cell shape.

The local auxin signaling at the cell surface activates the antagonistic ROP GTPases, ROP2/ROP4 and ROP6, which regulate localized actin and microtubule organization via their interactions with ROP-interactive CRIB motif-containing (RIC) proteins to promote lobe (ROP2/ROP4−RIC4) or neck (ROP6−RIC1) formation [9,34,39,40**,41,42**] (Figure 1). Auxin also induces TMK1 nanoclusters at future neck sites, which recruit and activate ROP6, triggering cortical microtubule rearrangement [44,45]. These microtubules, in turn stabilize TMK1 nanoclusters, forming a positive feedback loop that drives neck formation. Meanwhile, the activation of ROP2 at the lobe by auxin inhibits the endocytosis of PIN1, leading to its accumulation at the tip of the lobe. The auxin exported by PIN1 to the cell wall in turn activates ROP2, forming a positive feedback loop during pavement cell interdigitation [46].

Furthermore, the pectin status of the cell wall regulates ROP signaling. FER activates ROP6 in response to the presence of demethylesterified pectin and mechanical cues [25,47]. Loss of FER or impaired pectin demethylesterification disrupts cortical microtubule ordering, widens necks, and reduces pavement cell anisotropy, slightly broadening their circularity [25,47]. It has been found that FER recruits the ROP GUANINE NUCLEOTIDE EXCHANGE FACTOR 14 (RopGEF14) to activate ROP6 signaling, thereby controlling neck formation [25,47]. Additionally, PLEIOTROPIC REGULATORY LOCUS 1 (PRL1), a component of the NINETEEN COMPLEX (NTC) involved in RNA splicing, plays an important role in regulating pavement cell morphogenesis by maintaining actin microfilament integrity. Mutations in *PRL1* lead to a decrease in lobe number and broader necks during pavement cell morphogenesis, primarily due to the disruption and depolymerization of actin microfilaments [35*]. Genetic analyses indicate that PRL1 functions epistatically to ROP2 and ROP6, and differentially modulates their activities, promoting ROP6 activity while inhibiting ROP2 [35*] (Figure 1). This coordinated regulation of opposing ROP GTPase pathways highlights PRL1 as a key upstream factor in establishing proper pavement cell polarity and shape.

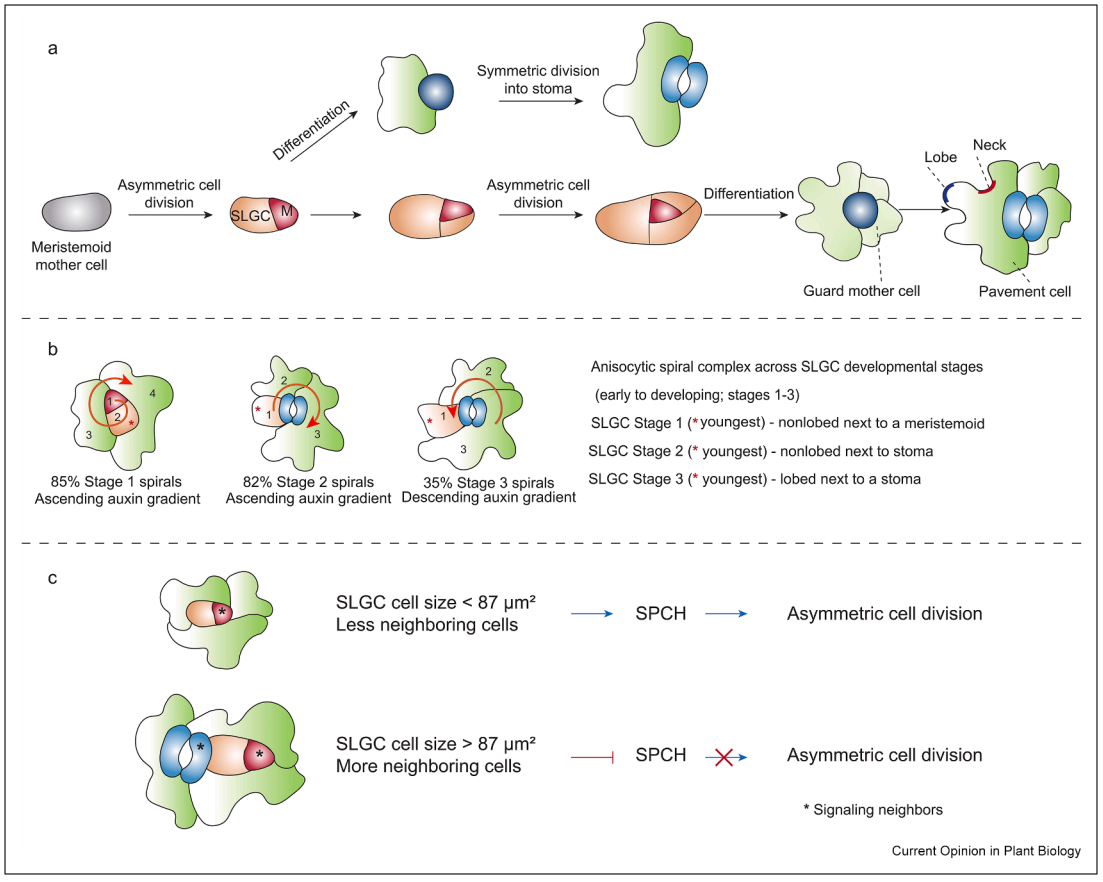
HISTONE DEACETYLASE 6 (HDA6), an epigenetic transcriptional regulator, has been very recently shown to suppress ROP6 expression by activating histone H3K9K14 deacetylation to regulate cell polarity and pavement cell shape [48*]. Mutation in *HDA6* leads to impaired pavement cell interdigitation due to altered ROP6 activity and disorganized microtubule orientation in the necks [48*]. The key regulators PLECKSTRIN HOMOLOGY GTPase ACTIVATING PROTEIN 1/2 (PHGAP1/2), also known as ROP-ENHANCER 2/3 (REN 2/3), have also been shown to control shape formation in pavement cells [49,50]. The *phgap1,2* double mutant exhibits severely altered pavement cell shapes, resembling those seen in mutants with constitutively active ROP2. PHGAP1/2 colocalize with cortical microtubules and accumulate in a microtubule-dependent manner at the neck region along the anticlinal side of the pavement cell. These proteins specifically interact with and inhibit ROP2, but not ROP6, creating a gradient of ROP2 activity in this region, thereby maintaining proper neck formation in pavement cells [49]. Zhang et al. (2022) showed that PHGAP proteins are regulated by brassinosteroids through the GSK3-like kinase BRASSINOSTEROID INSENSITIVE 2 (BIN2), which phosphorylates and stabilizes PHGAPs, promoting their polarization at neck sites [50]. Conversely, brassinosteroid perception by the BR INSENSITIVE 1 (BRI1)-

BRI1 ASSOCIATED RECEPTOR KINASE 1 (BAK1) receptor complex represses BIN2 activity at lobe regions, leading to PHGAP degradation and enabling ROP2 activation to drive lobe formation [50] (Figure 1).

Neighboring cell signaling drives stomatal lineage ground cell (SLGC) fate and shape acquisition

The leaf epidermal layer consists of different cell types such as trichomes, stomata and pavement cells, each with different shapes and sizes. In *Arabidopsis thaliana*, pavement cells arise either directly from protodermal precursors or as secondary products of the stomatal cell lineage, where meristemoid mother cells undergo asymmetric divisions to produce meristemoids, stomatal lineage ground cells (SLGCs) and stomata in anisocytic spiral complexes. Meristemoids subsequently differentiate into guard cells or produce additional SLGCs, while SLGCs can self-renew, generate new meristemoids through spacing divisions, or differentiate into

Figure 2



Intercellular signaling among neighboring cells influences the fate specification and shape development of stomatal lineage ground cells (SLGCs).

(**a**) Pavement cells of *Arabidopsis thaliana* arise either directly from protodermal precursor cells or through the stomatal cell lineage. In this lineage, meristemoid mother cells (gray) undergo asymmetric divisions to generate meristemoids (M, red) and stomatal lineage ground cells (SLGCs, orange). Meristemoids may further differentiate into guard mother cells (dark blue) or undergo additional asymmetric divisions to produce more SLGCs. Guard mother cells divide symmetrically to produce stomatal guard cells (light blue). SLGCs exhibit multiple developmental fates: they can self-renew, generate new meristemoids through spacing divisions, or differentiate into pavement cells (green).

(**b**) Auxin response dynamics in the *Arabidopsis thaliana* stomatal cell lineage during anisocytic spiral complex development. In stage 1 spirals, the meristemoid (cell 1) displays the lowest nuclear auxin response, which increases progressively across older SLGCs, with the oldest cell (cell 4) exhibiting the strongest signal. This pattern persists through stage 2. By stage 3, however, the gradient is inconsistent, with only 46 % of spirals retaining an ascending auxin gradient (from youngest to oldest cells within anisocytic stomatal complexes), while 35 % of the spirals exhibit a descending auxin distribution (from youngest to oldest cells within anisocytic stomatal complexes). These results suggest that low auxin signaling in the youngest SLGC correlates with first-lobe initiation, while auxin responses become increasingly dynamic as morphogenesis proceeds. Red arrow indicates auxin gradient, with the arrowhead representing maximal nuclear auxin response. * Indicates youngest SLGC.

(**c**) SLGC size predicts developmental fate. Smaller cells divide more frequently, whereas larger SLGCs divide less. Geometric analyses show that SLGCs with more signaling neighbors (meristemoids, guard mother cells, and/or stomata) are larger, divide less, and exhibit reduced SPEECHLESS (SPCH) activity. This supports a lateral inhibition model in which stomata and their precursors release mobile peptides that suppress asymmetric divisions in adjacent SLGCs, with meristemoids having the strongest inhibitory effect. * Indicates signaling neighboring cells.

pavement cells (Figure 2A). Grones et al. (2020) provided key insights into how developmental context and auxin signaling influence early pavement cell morphogenesis. Using live imaging, they showed that the first lobe of an SLGC-derived pavement cell consistently forms toward the older neighboring cell and perpendicular to the stoma**,** revealing that lobe initiation is tightly developmentally guided [39]. Their analysis of anisocytic spiral complexes identified three successive developmental stages distinguished by the status of the youngest SLGC: (1) non-lobed and next to a meristemoid, (2) non-lobed and next to a stoma, and (3) lobed and next to a stoma (Figure 2B). Analyses of the nuclear auxin signaling reporter DR5 revealed a robust ascending auxin response gradient (from youngest to oldest cells within anisocytic stomatal complexes) during the early stages of spiral development. The majority of stage 1 complexes (85 %) exhibit the lowest nuclear auxin signal in the meristemoid and progressively higher responses in older SLGCs, an ascending auxin response pattern largely retained in stage 2 spirals (82 %) (Figure 2B). However, this orderly gradient becomes less consistent once lobe formation begins. By stage 3, only 46 % of spirals maintained the ascending gradient, while 35 % of the spirals exhibited a descending auxin gradient (from youngest to oldest cells within anisocytic stomatal complexes; Figure 2B), suggesting that the onset of lobe initiation in the youngest SLGC disrupts the existing auxin response distribution pattern. These findings collectively indicate that a low auxin response state in the youngest SLGC is associated with first-lobe initiation**,** but that auxin signaling becomes highly dynamic as morphogenesis progresses [39].

As discussed above, pavement cell size is closely linked to pectin levels, with reduced pectin promoting endocycle entry. Recent work has further clarified how SLGCs transition into pavement cells. Dubois et al. (2023) showed that the cell-cycle inhibitor SIAMESE-RELATED 1 (SMR1) ensures timely differentiation of SLGCs by terminating their self-renewal capacity, a process dependent on CYCLIN A proteins and CYCLIN DEPENDENT KINASE (CDK) B1 [51*]. Complementing this, Fung et al. (2025) investigated why some SLGCs continue dividing while others differentiate. Using long-term imaging and statistical modeling, they identified cell size as a strong predictor of SLGC fate, with larger SLGCs dividing less frequently than smaller ones [52**]. The geometric and quantitative analyses suggest that SLGCs with more signaling neighbors (meristemoids, guard mother cells, and/or stomata) are larger, divide less, and have lower levels of SPEECHLESS (SPCH), a transcription factor promoting asymmetric division and turnover (Figure 2C). This lateral inhibition model suggests that stomata and their precursors (meristemoids and guard mother cells) may release mobile peptides that suppress asymmetric divisions in adjacent cells, with meristemoids exerting the strongest influence on SLGC division potential [52**]. Together, these studies highlight how cell size and intercellular signaling cooperate to guide SLGC differentiation into pavement cells**.**

Future considerations

A deeper understanding of how pavement cells acquire their jigsaw puzzle shapes will require uncovering how intercellular and intertissue communication integrates mechanical and biochemical cues across the leaf. Although tight coordination between cell types is known to be essential, the precise mechanisms through which neighboring cells, such as mesophyll cells and other epidermal cell types, including trichomes, influence the interdigitation remain largely unresolved. Future research should focus on elucidating how mechanical stress is transmitted between cell types and across tissue layers and how it instructs cytoskeletal organization. Mechanical signals clearly influence cortical microtubule alignment at both cellular and tissue scales; however, the interplay between locally generated cellular stress and globally imposed tissue stress remains poorly defined. Approaches that combine live-cell imaging over time, mechanical perturbations, and computational modeling will be crucial

Glossary

Anisocytic spiral complexes in *Arabidopsis thaliana* comprise three or more unequal subsidiary cells arranged in a spiral around a meristemoid or a pair of stomatal guard cells. Some other plant species display distinct stomatal complex types that differ in the number and orientation of subsidiary cells.

Apoplast is the extracellular matrix of plant cells, comprising the cell wall and the space external to the plasma membrane, and it plays a crucial role in cell growth, signal transduction, and mineral nutrition.

Ascending auxin response gradient describes an increase in auxin concentration from the youngest to the oldest cells within anisocytic stomatal complexes in *Arabidopsis thaliana*.

Descending auxin response gradient describes a decrease in auxin concentration from the youngest to the oldest cells within anisocytic stomatal complexes in *Arabidopsis thaliana*.

Pavement cell anisotropy is defined as the differential cell expansion leading to asymmetric growth (lobe/indentation) of the pavement cell.

Pavement cell polarity is the nanoclustering of sterol lipids and plasma membrane associated signaling proteins leading to polarized domain for the formation of lobes and indentation.

for determining how neighboring cell-derived compressive and/or tensile forces affect anticlinal wall behavior and microtubule dynamics during pavement cell shape acquisition.

Data availability

No original data were employed in this review article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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** of outstanding interest

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