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Local interactions shape plant cells

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Plant cell expansion is usually attributed to the considerable osmotic pressure that develops within and impinges upon the cell boundary. Whereas turgor containment within expandable walls explains global expansion, the scalar nature of turgor does not directly suggest a mechanism for achieving the localized, differential growth that is responsible for the diversity of plant-cell forms. The key to achieving local growth in plant cells appears to lie not in harnessing turgor but in using it to identify weak regions in the cell boundary and thus creating discrete intracellular domains for targeting the growth machinery. Membrane-interacting phospholipases, Rho-like proteins and their interactors, an actin-modulating ARP2/3 complex with its upstream regulators, and actin–microtubule interactions play important roles in the intracellular cooperation to shape plant cells.

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Introduction

The walled plant cell develops a considerable internal osmotic or turgor pressure that impinges upon its boundary and causes it to expand. However, in contrast to the phenomenal increases in turgor observed in specialized penetrating structures such as fungal appressoria [1], turgor does not appear to change locally within expanding plant cells. Furthermore, it is a scalar quantity, meaning that turgor effects on the cell boundary should be equally distributed in all directions. Turgor-mediated cell expansion should therefore lead to globular cells. Instead, plants display an amazing diversity of cell forms (Figure 1). How does a plant cell achieve, in the words of Frank Harold [2], ''local compliance with the global force of turgor''?

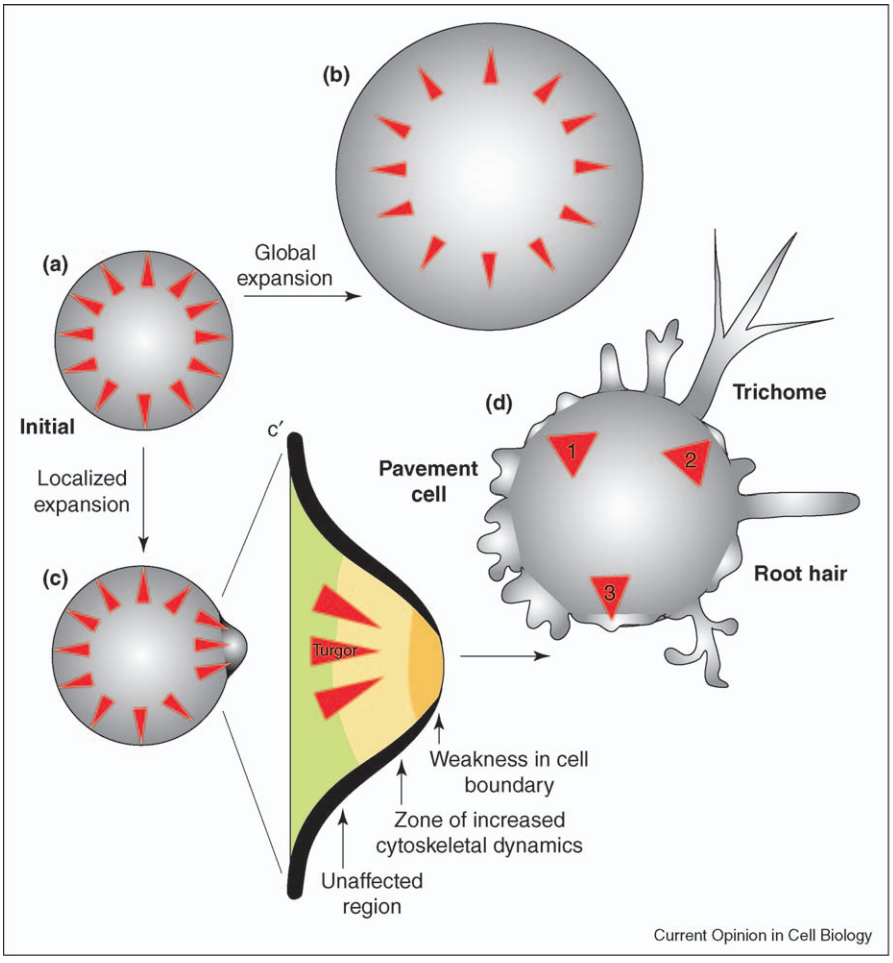
Alteration of the regional characteristics of the cell boundary (which comprises the cell wall, the plasma membrane and underlying cytoskeletal mesh) can be an alternative way of achieving localized cell protrusion. Indeed, numerous observations on expanding plant cell walls [3], plasma membrane and cytoskeletal elements [4,5] suggest that regional alterations in the cell boundary precede localized growth. Because of internal turgor, changes that produce a local weakening of the cell boundary are discernible as a small bulge on the cell periphery (Figure 1). Observation of the early developmental stages of different model cell types traces each of them back to the bulge stage (Figure 1d). Since further elaboration of shape to achieve the final mature cell form can be visualized as a continuum of boundary-loosening events followed by wall rigidification, it seems obvious that the key to understanding the launching of shape diversification in plants lies within the nondescript bulged domain on the cell initial (an initial being defined here as that early developmental stage when a cell has entered a specific pathway of differentiation but when its shape does not suggest its final polarized form).

Recent studies have been exciting in this respect as plant biologists have started to identify, with an amazing rapidity, the molecular basis for shape modulation in plant cells. The viewpoint being presented here is from inside the plant cell and focuses on our recent understanding of molecular players that have a role in localized reorganization of the cell boundary. Though exocytosis events involving targeted secretion and the laying down of the primary wall are clearly affected by internal reorganization in the expanding cell, I do not discuss them here but instead direct the reader to excellent recent reviews dealing with the subject [6,7].

Creation of a bulge

Bulge initiation in a nearly spherical plant cell results from a local breakdown of the balance existing between internal forces and the cell boundary and represents a weakening in the cell boundary. Wall loosening involving the activity of wall-modifying proteins such as xyloglucan endotransglucosylase/hydrolase [8] and expansins [9] clearly takes place, as these proteins have been found to be enriched at bulge initiation sites. Membrane modifications involving a lipid-based machinery have also been highlighted through the recent characterization of the CAN OF WORMS 1 (COW1) gene, which encodes a phosphatidylinositol transfer protein (PITP) [10], and the TIP GROWTH DEFECTIVE1 (TIP1) gene, which confers membrane-modifying S-acyl transferase activity [11]. Further, overexpression of AtPLDζ1, a phospholipase-D, results in ectopic bulge initiation in atrichoblasts (non-root-hair-forming cells) in *Arabidopsis* [12], whereas phosphatidic acid, a product of PLD activity, stimulates

Figure 1



A diagrammatic depiction of ''local compliance with the global force of turgor'' [2], as suggested by observations on the morphogenesis of turgor-containing plant cells. **(a)** A non-vectorial turgor force stretches the cell boundary equally in all directions to produce a spherical initial. **(b)** Global expansion relying upon turgor only would lead to a larger spherical cell. **(c)** When encountering a localized weakening in the cell periphery, the scalar nature of turgor is manifested in the form of a small bulge. This weak, bulged domain polarizes the cell as it becomes the focus of sub-cellular activities aimed at reinforcing the weak region. The enlarged cross-sectional view of the bulge shown here reveals that the weak region is followed by a zone of increased subcellular dynamics including rapid interactions between cytoskeletal elements, their regulators, organelles and vesicles. The base of the bulge represents a relatively unaffected zone where, perhaps through cytoskeletal reinforcement, the boundary-weakening has been prevented from spreading further. **(d)** Observing the stages in morphogenesis for three model plant cell types (trichome, root hair and pavement cell) reveals that polar growth in each of these cells initiates from a small bulge (arrow heads: 1, trichome; 2, root hair; 3, pavement cell) on the cell periphery. Subsequent elaboration of polar growth requires coordination between actin and microtubule cytoskeletal systems, and possibly again relies upon turgor to identify local weakness for targeting growth. (Note that an aberrant, branched root hair has also been shown. Such root hairs generally arise from a broad bulge and in most cases can be clearly associated with cytoskeletal malfunctioning.)

AGC2-1, a protein kinase that localizes to rapidly growing root hairs in a AtPDK1 (3′phosphoinositide-dependent kinase-1)-dependent manner [13]. Interestingly, PLD activation in plant cells results in cortical microtubule rearrangement [14], and a tobacco PLD localizes to microtubules [15]. Following the break-up of earlier cortical attachments that tether the cytoskeleton to the plasma membrane, the weak bulge domain presents a locale for focusing sub-cellular events aimed at combating the weakness (see enlargement in Figure 1c). The formerly balanced cell is now polarized and embarks upon polar or directional growth.

Beyond the bulge: establishment of polar growth

Whereas reduced microtubule dynamics promote further expansion of the bulged domain, the initiation of polarized growth from the domain seems to be intimately linked to the appearance of an actin patch. This has been best described during rhizoid initiation from spherical

fucoid zygotes [16], but regional actin accumulation has also been observed in germinating pollen grains and root hairs embarking on tip growth [9,17], in leaf hair (trichome) initials [18] and in lobe-forming regions of leaf epidermal pavement cells [19]. Accordingly an inhibitor-induced interference with actin dynamics leads to patch dispersal and aberrant rhizoid formation in fucus zygotes [20], inhibition of tube formation in germinating pollen grains [21], and an inability to initiate a tip region for focusing growth in *Arabidopsis* trichoblasts [9]. Mutations in the *Arabidopsis ACTIN2* gene lead to larger bulges and aberrant root-hair initiation sites [22]. In the absence of ultrastructural detail on the actin patch in plant cells, it is unclear whether, as in yeast [23], it represents an aggregation of fine-branched networks, which could suggest a region of focused actin nucleation and polymerization activities. Nevertheless, an ARP2 (actin-related protein 2) homolog localizes to the bulge domain in polarized fucus zygotes [24••], and ARP3 is enriched in bulged domains in trichoblasts [25]. Both ARP2 and ARP3 are major subunits of a highly conserved actin-polymerization-modulating ARP2/3 complex [26]. In other organisms this complex has been implicated in membrane-protrusive and organelle-propulsive activities aided by actin polymerisation [26,27]. The complex is regulated by Rho-GTPases and several plant Rho-like proteins (ROPs) have been shown to accumulate very early at sites of bulge initiation [28–30]. Molecular activity that would lead to an increase in actin dynamics is clearly evident within the bulged domain even before any signs of polar growth are apparent. Inhibition of actin polymerization in pollen tubes [21] and RNAi experiments using the ARPC1 subunit of the ARP2/3 complex in *Physcomitrella patens* [31••] suggest that actin polymerization could contribute directly to membrane protrusion in plants as well as in animal cell lamellipodia. However, as shown for the ARP2/3-complex-aided rocketing motility of certain microbes and subcellular structures [27], mediation of actin polymerization could equally be required for the propulsion of membrane-bound vesicles to the cell boundary. Polarized vesicle trafficking secretion at this stage requires a substantial involvement of Rab-GTPases [32•] and ADP-ribosylation factors (ARFs) and the reader is directed to recent reviews on these topics [33,34].

Elaboration of polar growth: recent case studies

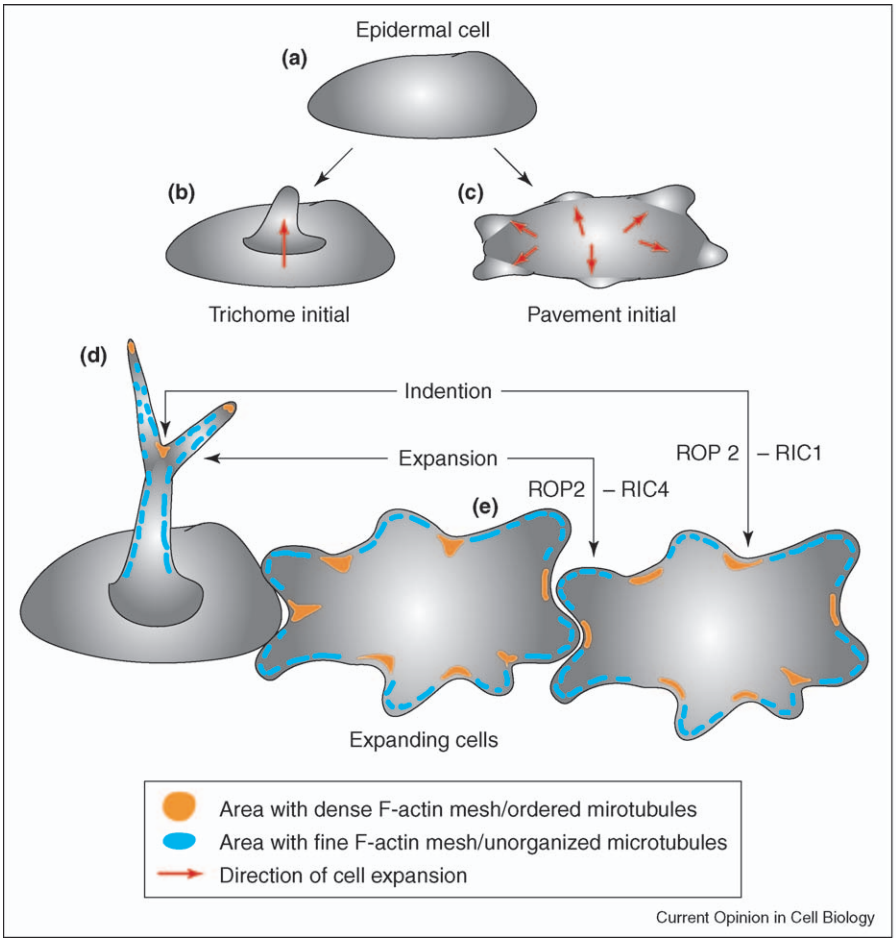
The polar growth of cells like pollen tubes and root hairs, which is limited to a small region that forms the cell tip, has been the subject of many reviews [4,35,36]. The more recent elucidation of the molecular mechanisms underlying polarized growth in plants is focused upon here and has come from observations on diffuse growing leaf epidermal trichomes and pavement cells. Morphologically, the early stages in the differentiation of a trichome cell or a lobed pavement cell from a relatively flat epidermal cell differ only in the directionality of growth (Figure 2a,b). A trichome initial projects almost perpendicular to the epidermal plane, whereas a pavement cell extends horizontally and develops multiple lobes.

Polar growth of trichomes

The unicellular *Arabidopsis* trichome, whether branched or unbranched, displays a precise, symmetrical form where the cell tapers to a narrow tip. Alterations in trichome shape can thus be easily screened for and several genes sharing a mutant phenotype of randomly misshapen trichomes have been grouped into a class called *distorted* (*dis*) [37]. Molecular characterization of these genes has identified an ARP2/3 complex and its upstream regulators, which belong to a ROP-regulated pathway involving SCAR/WAVE, HSPC300-like, NAP125-like and PIR121-like proteins [38•,39•,40–42]. ARP2/3 complex activation enhances actin polymerization and results in the formation of a fine dendritic F-actin mesh. However, observations on F-actin organization revealed that *dis* mutant cells often have random pockets of fine and dense F-actin instead of the regular fine cortical F-actin meshwork characteristic of expanding wild-type cells. Cellular areas with dense F-actin aggregation do not expand as easily as regions with fine F-actin mesh, and mutant cells thus become randomly misshapen. In two studies the regional accumulation of actin correlated with increased aggregation and decreased motility of small organelles like Golgi bodies, peroxisomes and mitochondria [43,44]. These observations suggested that F-actin might act as an intracellular barrier. In accordance with this 'actin barrier' concept, a fine F-actin meshwork resulting from increased actin dynamics mediated by ARP2/3 complex [38•] and formins [45••] would allow and perhaps even aid the rapid motility of organelles and exocytotic vesicles [43]. This would promote local growth. In contrast, a dense web comprising F-actin aggregates and bundles in an intracellular locality would hinder organelle motility and vesicular trafficking and thereby restrict growth. Cytoplasmic aggregation and growth inhibition occurs in a small sub-apical region in tubular stage-2 trichomes and leads to the bifurcation of the tip [43] into two divergently expanding branches (Figure 2c). The observation of reduced growth upon local induction of F-actin bundling [46] together with the large number of actin nucleators, polymerization promoters, F-actin bundling proteins and actin-monomer-binding proteins identified in plants [47] supports the actin mesh hypothesis.

Interestingly, the locality of actin aggregates in trichomes coincides with the regional clustering of cytoplasmic microtubules [44]. This observation points to an intricate regional cooperation between actin and microtubule cytoskeletons and suggests that microtubules, with their own set of interactors and regulators, might act to confine the actin-based expansion to a locality [5].

Figure 2



Observations on morphogenesis of single celled trichomes [43] and pavement cells [48••] reveal the link between cytoskeletal organization and regional growth. **(a)** A polygonal epidermal cell that acts as the initial for both trichomes and pavement cells (an initial being defined here as that early developmental stage when a cell has entered a specific pathway of differentiation but when its shape does not suggest its final polarized form). **(b)** A trichome initial creates a bulge that projects outwards (directional arrow) from the horizontal epidermal plane. **(c)** The pavement cell initial creates multiple horizontally aligned bulges (arrows) along its periphery. **(d)** Local dense (orange patches) and fine (blue lines) F-actin meshworks coincide with non-expanding and expanding regions, respectively [43]. Accordingly, the dense actin mesh is suggested to act as a barrier for vesicle trafficking and thus restricts regional growth. Fine F-actin meshworks resulting from increased actin nucleating and polymerizing allow vesicles access to the cell boundary and promote regional expansion. **(e)** Dissection of the molecular mechanisms underlying shaping of single pavement cells and interdigitating growth of neighbouring pavement cells reveals that the creation of a fine F- actin mesh and a dense cytoskeletal region depends upon ROP2–RIC4 and ROP2–RIC1 interactivity, respectively [48••]. Expanding regions with dynamic actin usually display unorganized microtubules whereas regions with well-aligned microtubules usually display dense F-actin. The lobes of one pavement cell fit into the indentions of its neighbouring cells.

The idea of regional cytoskeletal cooperation during differential growth and its upstream molecular regulation has been convincingly dissected in a seminal study involving pavement cell morphogenesis in *Arabidopsis* [48••].

Interdigitating growth of pavement cells

The final 'jigsaw puzzle' shape of epidermal pavement cells arises from multiple local projections (lobes) of a polygonal initial (Figure 2a,b). During expansion, the lobes of one pavement cell fit into the indentions of its neighbours to produce an epidermal surface with an interdigitating pattern. Fu *et al.* [48••] reveal a ROP-GTPase signalling network underlying the creation of these lobes and indentions by pavement cells. Local activation of a ROP (AtROP2) activates RIC4 (ROP-interactive CRIB-motif-containing protein 4) to enhance actin dynamics and promote localized growth. However, ROP2 activity leads to the inactivation of another target RIC1 that localizes to cortical microtubules and promotes their ordering into parallel arrays. RIC1-dependent microtubule organization not only inhibits cell outgrowth locally but also suppresses ROP2 activation in the indention zone. Thus, while the ROP2–RIC4 interaction promotes cell outgrowth, ROP2–RIC1 restrains outgrowth

(Figure 2c). Such coordinated activity in epidermal cells creates the interdigitations between adjacent pavement cells. It is interesting that the regions of indention in a pavement cell with aligned microtubules coincide with the regions of increased actin aggregation observed earlier [43]. As suggested before [5], a loose or dense F-actin meshwork with a strong dependence on microtubule elements is essential for regulating localized growth to achieve a particular cell form.

Who is in charge? A tripartite coalition?

Recent findings on plant cell morphogenesis identify three major players: ROPs, microtubules and actin microfilaments, each with its own retinue of effectors and regulators. At first glance ROPs appear to be the master molecules that regulate both microtubule and microfilament activities. However, as shown for animal cells [49], the local activation of ROPs by pertinent GEFs (guanine nucleotide exchange factors) might require the active participation of microtubule plus ends. The recent identification of at least 14 proteins with potent GEF activity [50••] and the presence of multiple ROPs, RICs [51] and microtubule-TIP proteins [52] in *Arabidopsis* promises to reveal the plant cell cortex as a veritable maze of ROP–microtubule interactions. Again, while cortical actin appears to be a major target for ROPs, the effect of altered actin organization on microtubule localization and dynamics is still a relatively uncharted territory in plant cells. Finally, despite the obvious importance of the cytoskeleton in setting up and promoting polarized growth of plant cells, turgor might still be a major factor aiding the movement of polysaccharides into growing cell walls [53] and thus cannot be totally discounted.

Conclusions and perspectives

As presented above, the walled cell achieves ''local compliance with the global force of turgor'' not by succumbing to its non-vectorial nature but by using this very property to identify a weakness in the cell boundary, where a sub-cellular domain is created for focusing resources. The execution of cytoskeleton-aided polar growth by a cell also perhaps requires turgor force to continue identifying weak regions for reinforcement. While we are gaining some understanding of the mechanisms underlying the seemingly simple operation of converting a spherical plant cell into any other shape, the challenge now is to unravel the core sensing machinery whereby a plant cell, driven by genetic mechanisms, is able to create a weakness in a specific locale and produce a precise polar shape again and again.

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