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Cell shape development and cytoskeleton

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Cell shape development in plants

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The shape of a plant cell has long been the cornerstone of diverse areas of plant research but it is only recently that molecular-genetic and cell-biological tools have been effectively combined for dissecting plant cell morphogenesis. Increased understanding of the polar growth characteristics of model cell types, the availability of many morphological mutants and significant advances in fluorescent-protein-aided live-cell visualization have provided the major impetus for these analyses. The cytoskeleton and its regulators have emerged as essential components of the scaffold involved in fabricating plant cell shape. In this article, I collate information from recent discoveries to derive a simple cytoskeleton-based operational framework for plant cell morphogenesis.

The fascinating and apparently limitless diversity in the architecture of higher plants results from the three-dimensional assembly of single constituent cells within genetic and environmentally defined developmental frameworks. Initially, however, most plant cells are nearly isodiametric. How does a spherical cell get transformed into just about any other imaginable shape as it matures and occupies its position within the overall developmental pattern of a plant?

The prosaic answer to the above question is that 'differential growth shapes plant cells'. This phrase conveys the fact that, during development, one region of a cell grows differently from another, but it raises the question of how preferences for localized growth can be created within a cell. Furthermore, what molecular hierarchy might operate between cellular components for differential growth to be achieved? Today, different model cell types (Figure 1), a collection of mutants (Table 1), novel cell-biological tools for non-invasive investigations of subcellular events and interactions (Figure 2) [1], and well-established molecular and biochemical techniques allow us to address these questions.

In this review of plant cell morphogenesis, growth is considered as simply a cellular manifestation involving all intracellular resources and processes, and leading to a cumulative and irreversible increase in cell volume. Details of growth-related phenomena such as subcellular targeting and trafficking of vesicles, membrane and cell-wall components, turgor and vacuole biogenesis, as well as the roles of ionic gradients, growth regulators and tropic responses, can be found in other reviews [2–6].

Shape changes result from changing the growth focus of a cell

Differential growth is best studied in easily accessible cell types such as epidermal pavement cells and leaf
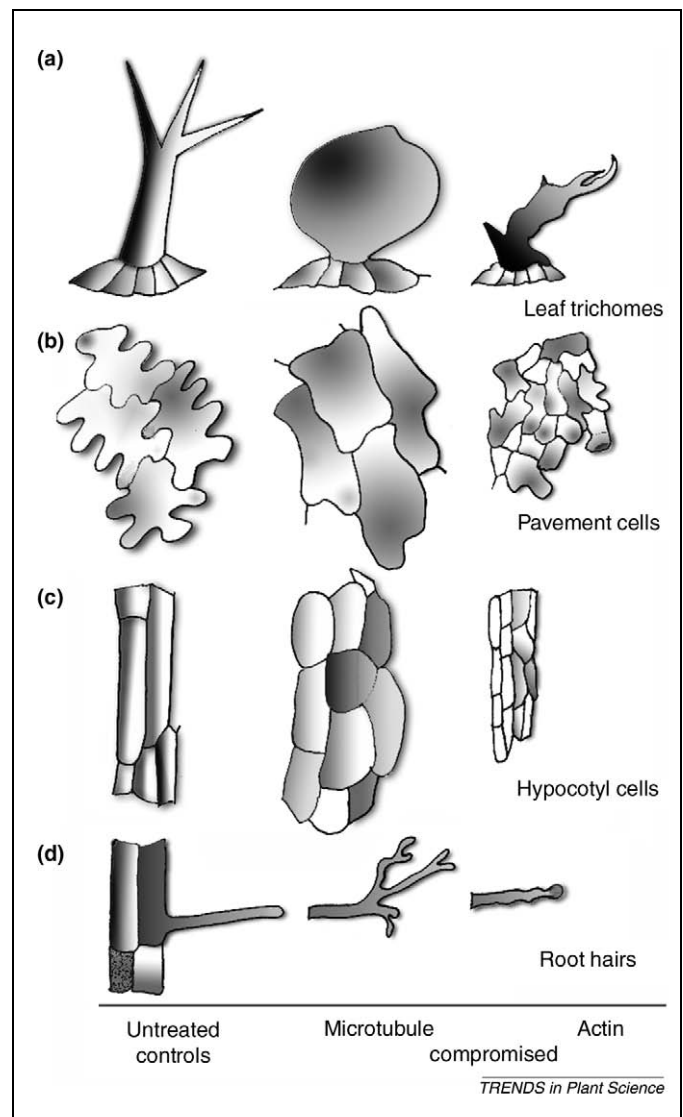
Figure 1. Responses in growth and development of diffusely **(a–c)** and tip-growing **(d)** cells to disturbances in their microtubule (middle column) and actin (right column) cytoskeletons. Pharmaceutical experiments using microtubule-depolymerizing herbicides such as oryzalin and the microtubule-stabilizing drug Taxol suggest that an interference with microtubule cytoskeleton results in loss of polar growth directionality, resulting in more isotropically expanding cells [13–17]. Interference with the dynamics of the actin cytoskeleton using cytochalasins and latrunculins (which interfere with actin polymerization) or phalloidin and jasplakinolide (which stabilize F-actin) limits expansion capability and results in small cells [16–19]. Pollen tubes, which constitute an important model tip-growing cell [11,48,49,73], probably display similar responses to root hairs but are not depicted here because their response to anticytoskeleton drugs under *in vitro* experimental conditions might not precisely reflect their *in vivo* growth responses.

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Table 1. Some molecularly characterized genes and mutants with clear cytoskeleton-linked defects in the morphogenesis of one or more model cell types

Gene	Major mutant phenotype	Gene product	Refs
ANGUSTIFOLIA At1g01510	Narrow leaves, two-branched trichomes	C-terminal binding protein/BARS-like	[40,41]
BRICK1 (maize) putative *Arabidopsis* homologue At2g22640	Defective lobe formation in leaf epidermal cells, stomatal complex, hairs aberrant	Novel actin interacting protein. Homology to HSPC300	[65]
CROOKED At4g01710	Distorted trichomes, unexpanded epidermal cells and short, thick root hairs	Smallest subunit ARPC5 (p16 homologue) of the ARP2/3 complex.	[20,58]
DEFORMED ROOT HAIRS1 At3g18780	Aberrant root-hair initiation site, aberrant tip growth	ACTIN2	[45,46]
DISTORTED1 At1g13180	Distorted trichomes, unexpanded epidermal cells and short, thick root hairs	Actin related protein 3 (ARP3), large subunit of ARP2/3 complex	[57–59]
DISTORTED2 At1g30825	Distorted trichomes, unexpanded epidermal cells and short, thick root hairs	Fourth largest ARPC2 subunit (p35 homologue) of the ARP2/3 complex	[60,61]
ECTOPIC ROOT HAIRS3, FAT ROOT, BOTERO1, FRAGILE FIBER2, LUE1 At2g34560	Ectopic root hairs, swollen root, defective cell wall, fragile stems	Katanin-p60-like, also called AtKSS	[32–35]
FASS/TONNEAU2 At5g18580	Swollen cells, abnormal cortical microtubules, random divisions	Novel higher plant protein, C-terminal similarity to B$'$ regulatory subunits of type 2A protein phosphatases	[36]
GNARLED At2g35110	Distorted trichomes	NAP-125 homologue	[62,63]
KIESEL At2g30410	Weak allele shows swollen trichomes; strong alleles are embryo lethal	Tubulin folding cofactor A	[25,39]
KLUNKER At5g18410[a]	Distorted trichomes	PIR-121 homologue	[64]
LEFTY1/LEFTY2 At1g04820/At4g14960	Shoots, roots twist to the left; right-handed cortical microtubules	α-Tubulin 4 (TUA4) /α-tubulin 6 (TUA6)	[27,28]
MICROTUBULE ORGANIZATION1/GEMINI1 At2g35630	Temperature sensitive, dwarf, short, swollen cells, pollen mother cell defects	TOG-XMAP 215 family; homologue of tobacco TMBP200	[22,26]
PLEIADE At5g51610	Branched root hairs, cytokinesis defects	Microtubule-associated protein AtMAP65-3	[38]
PORCINO At4g39920	weak allele shows swollen trichomes; strong alleles are embryo lethal	Tubulin folding cofactor C	[24,39]
PROPYZAMIDE-HYPERSENSITIVE1 At5g23720	Left-handed helical growth of roots, increased sensitivity to microtubule depolymerizing drugs	Novel mitogen-activated-protein kinase (MAPK) phosphatase-like	[37]
SPIKE1 At4g16340	Aberrant leaf, trichome and cotyledon cell shapes	Adaptor protein CDM family, putative ROP regulator	[43]
SPIRAL1 At2g03680	Right-handed helical growth of hypocotyl and root	Novel microtubule-localized protein	[29,30]
STICHEL At2g02480	Unbranched trichomes on leaves	Novel protein with ATP-binding eubacterial DNA polymerase III γ subunit	[42]
WAVE-DAMPENED2 At5g28646	Suppressed root waving, and rotational polarity and anisotropic expansion altered	KLEEK-domain-containing protein, shows similarity to microtubule destabilizing stathmin proteins	[31]
WURM At3g27000	Distorted trichomes, unexpanded epidermal cells and short, thick root hairs	Actin-related protein 2 (ARP2), homologue of the large subunit of ARP2/3 complex	[57–59]
ZWICHEL At5g65930	Short, swollen, less-branched trichomes	Kinesin-like calmodulin-binding protein (KCBP)	[21,80]

[a]The gene At5g18410 appears as *PIRP* [64], *PIROGI* [81] and *KLUNKER* (R. Saedler *et al.* unpublished). Based on the common properties described, the same *Arabidopsis* database accession number and the precedence of the first given name for a gene it is referred to as *KLUNKER* here.

trichomes [7,8], stomatal guard cells [9], root-hair cells [10], elongating pollen tubes [11] and differentiating tracheids [12], which allow experimental manipulation and can be studied in pertinent morphological mutants. Although the final shapes of these model cells differ greatly, they do share a common growth mechanism [7]. Based on the area over which growth is spread during the development of a cell, the different cell types can be placed into two broad categories. Root hairs and pollen tubes are recognized as tip-growing cells because they display a narrow region of focused growth that creates a cellular tip [10,11]. Alternatively, in most cells of the root, hypocotyl and leaf epidermal trichomes [7,8], growth is spread over a large proportion of the cell surface, producing diffusely growing cells. Development of some cells, such as leaf epidermis pavement cells, might involve a combination of localized tip growth and diffuse growth to produce strangely shaped interlocking cells [7].

Cytoskeletal elements during differential growth

In higher plants, amongst all other cellular components, the two major cytoskeleton elements actin microfilaments and microtubules [11,13] appear to play a decisive role in the shape-determining process. Treatment of plant cells with cytoskeleton-interacting drugs has been particularly helpful in developing this view and has allowed particular changes in cell morphology to be linked to altered activity of specific cytoskeletal proteins [13–18]. The following general observations have emerged (Figure 1). Interference in microtubule dynamics by either microtubule-depolymerizing herbicides (e.g. oryzalin, propyzamide) or microtubule-stabilizing substances such as Taxol results in anisotropically growing cells displaying isotropic growth. This results in large, expanded, spherical forms [13,15,16]. Accordingly, microtubules are implicated in the establishment and maintenance of polar growth directionality [7,14]. By contrast, interference with the
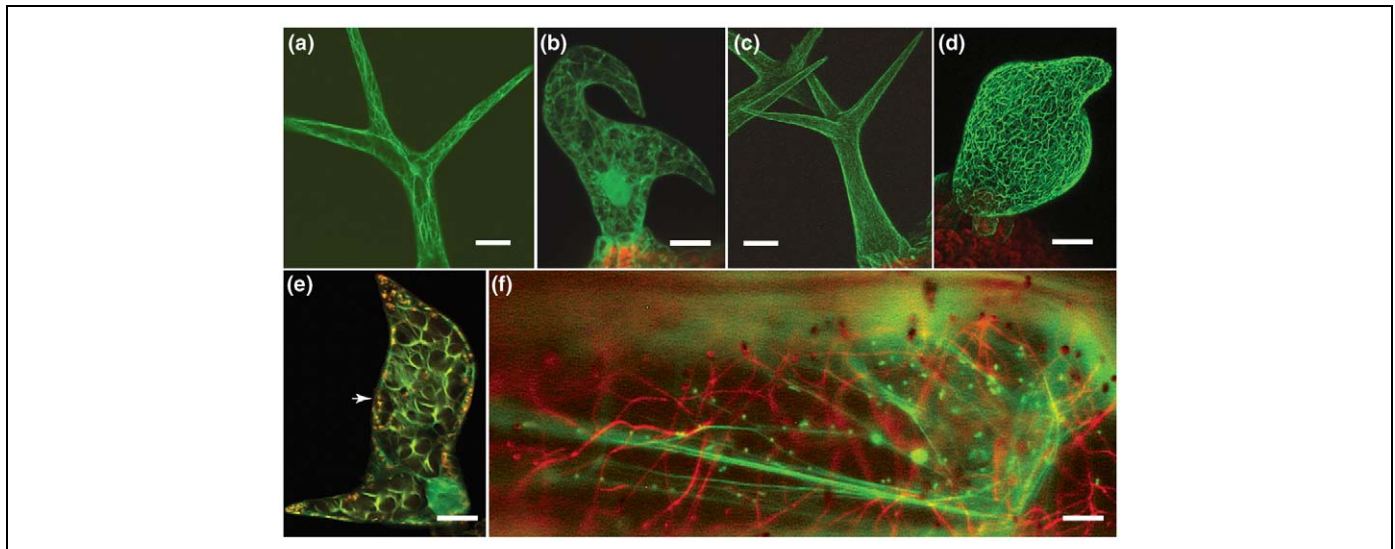
Figure 2. Fluorescent protein-aided labelling of cellular components as a powerful cell-biological tool in plant morphogenesis research. Green fluorescent protein (GFP) labelling allows non-invasive visualization of live cells [1] and is being used extensively to understand the effect of different drugs and proteins on cytoskeleton arrays and subsequently on cell morphology [17–20]. **(a–e)** The effects of different drugs and proteins on cytoskeleton arrays and cell morphology in epidermal trichome cells of *Arabidopsis*. **(a)** Untreated trichome cells possess an extensive network of longitudinally oriented filamentous (F-) actin cables. **(b)** A distorted trichome, as shown here, displayed random, aberrant F-actin organization, which suggested that the actin cytoskeleton could be involved in trichome cell morphogenesis [16,17]. This allowed a candidate gene approach for cloning the *CROOKED* gene [20]. **(c)** Cortical microtubules in wild-type *Arabidopsis* trichomes are organized into dynamic oblique arrays but **(d)** become stabilized and bundled upon treatment with Taxol. Notice the resultant, more isotropic cell shape under microtubule-altered conditions. **(e)** Two GFP variants that were used to label and visualize two separate subcellular structures (GFP-labelled F-actin is depicted in green; yellow fluorescent protein (YFP)-labelled Golgi bodies appear orange–red, white arrowhead) for analysing Golgi-body motility and distribution in the *crooked* mutant [20]. **(f)** A foretaste of multicolour labelling in plants (actin filament label, GFP; microtubule label, red fluorescent protein DsRed; peroxisome label, YFP punctae) that will allow in-depth dissection of subcellular interactions involved in cell shape development. (a–d) Scale bars = 20 μm; (e,f) scale bars = 10 μm.

actin cytoskeleton using drugs such as cytochalasins and latrunculins (actin polymerization inhibitors) or phalloidin and jasplakinolide (which stabilize polymerized actin) produces small, unexpanded cells that retain a semblance of their polar growth characteristics [16–18] but in which organelle and vesicle motility are seriously compromised [19,20]. The actin cytoskeleton is thus implicated in general subcellular motility and vesicle trafficking processes [7,13].

There is a genetic basis for the drug-induced alterations in cell morphology (Figure 1) because mutants with similar morphological defects have been identified in *Arabidopsis* [17,18,20–22]. Furthermore, using immunological [16,23] and live-cell imaging techniques [17,20,24], many of these mutants have been found to possess aberrant actin or microtubule cytoskeletons (Figure 2). Molecular characterization of the respective genes has provided conclusive evidence for a cytoskeletal role in cell morphogenesis (Table 1).

Molecular-genetic link to the microtubule cytoskeleton

The defects seen in mutants in microtubule-associated genes includes an abnormal swelling of diffusely growing cells such as trichomes, root, hypocotyl and pavement cells, whereas tip-growing root hairs usually become sinuous or branched (Figure 1). Mutants in *ZWICHEL* [21], weak mutant alleles for *KIESEL* or *PORCINO* [24,25], and conditional treatments of *MOR1/GEMINI1* mutants [22,26] exhibit these defects particularly well. Additional, spectacular changes attributed to the microtubule cytoskeleton occur in the *lefty* [27,28], *spiral* [29,30] and *wave-dampened* [31] mutants, which exhibit highly skewed left- or right-handed bending of roots and hypocotyl cells (Table 1). Multiple microtubule-associated disorders are observed in *ectopic root hair3* and its allelic mutants [32–35], whereas the severe cellular phenotypes of the *fass/tonneau2* [36], and *propyzamide hypersensitive1* mutants [37] suggest a vital role for protein phosphatases in microtubule cytoskeleton regulation. In addition mutations in different microtubule-associated genes such as *PLEIADE* [38], *CHAMPIGNON/TITAN1*, *HALLIMASCH/TITAN5*, *KIESEL*, *PFIFFERLING* and *PORCINO* display cytokinesis-linked defects [39]. A link to the microtubule cytoskeleton has also been proposed based on the mutant phenotypes of the *ANGUSTIFOLIA* [40,41], *STICHEL* [42] and *SPIKE1* genes [43]. In general, all microtubule-cytoskeleton-associated phenotypes suggest their involvement in cell polarization and maintenance of growth directionality.

Molecular-genetic link to the actin cytoskeleton

Initial observations of mutations in actin-encoding genes in *Arabidopsis* did not reveal morphological defects suggesting a high degree of functional redundancy in these vital genes [44]. Recently, however, many key roles in cell shape determination have been attributed to the actin cytoskeleton. The *actin2/deformed root hairs1* (*act2/der1*) mutants exhibit aberrant root-hair phenotypes, suggesting a crucial role for the actin cytoskeleton in growth-site selection as well as subsequent tip-directed growth [45,46]. The importance of proper regulation of actin dynamics during growth-site selection is also highlighted by the localization of actin-sequestering proteins such as actin depolymerizing factor (ADF) [47,48] and

profilins to the bulged domains in trichoblasts, from which actual tip growth proceeds [49,50]. ADF activity appears to be linked to that of actin interacting protein 1 (AIP1) because an RNA-interference-induced decrease in *Arabidopsis* AIP1 levels lowers F-actin turnover while promoting actin bundling [51]. In both the AIP1 and the ADF1 studies, increased F-actin bundling is associated with inhibition of cell expansion [51,52]. Although several actin-bundling proteins of the fimbrin [53] and villin [54,55] families have been identified in higher plants, mutants attesting to their direct involvement in cell morphogenesis have not been reported so far.

Observations firmly establishing a role for actin in cell morphogenesis have come from the *distorted* class of *Arabidopsis* genes [7,8,16,56]. The *dis* mutants exhibit all the characteristics associated with an aberrant actin cytoskeleton, such as small, abnormally shaped cells and an apparent inability of the cells to respond correctly to conditions requiring rapid cell elongation [20,56–63]. Cell-biological observations in *distorted1*, *distorted2*, *crooked* and *wurm* mutants reveal an aberrant, random distribution of highly bundled, cross-linked cortical F-actin with intervening regions of diffuse F-actin [57–61], as well as impaired organelle motility in regions with dense F-actin [20,59,61]. The corresponding genes encode homologues of different subunits of the ARP2/3 complex [20,57–61], a regulator of actin polymerization in diverse organisms. Two other *dis* mutants *gnarled* and *klunker* display distorted trichome cells that are nearly indistinguishable from those of ARP2/3-complex mutants but in which there is an apparent increase in the cellular area with a diffuse F-actin mesh [62,64]. *GNARLED* and *KLUNKER* encode the *Arabidopsis* homologues of NAP125 (Nck-associated protein 125) [62–64] and PIR121 (p53-inducible mRNA 121), respectively [64] (R. Saedler *et al.*, unpublished). With the *BRK1* gene (HSPC300-hematopoietic stem/progenitor cell clone 300 homologue), which was discovered in maize [65], these two might constitute upstream components of an ARP2/3-complex regulatory machinery [62–65]. In addition, formins, another class of potent actin nucleators have emerged as links between cell membranes and the actin cytoskeleton because overexpression of AtAFH1 results in an abundance of membrane-associated actin cables and leads to growth depolarization of elongating pollen tubes [66].

Mutant characterization and molecular identification of corresponding genes implicates the actin cytoskeleton in cell shape development (Table 1). Cell-biological observations suggest that increased actin polymerization results in the formation of a diffuse, fine mesh of F-actin that correlates with sites of increased cell expansion, whereas non-expanding regions possess dense bundles or aggregates of F-actin. Furthermore, when seen in profile or in two-dimensional compressions of confocal images, the actin organization just behind a region with diffuse F-actin is often relatively dense, comprising motile actin patches [20]. The relevance of these local differences in actin organization becomes evident upon considering the effects of some of the known signalling molecules on plant cell morphogenesis.

Signalling to the cytoskeleton and a twist in the tale

The identification of various actin and microtubule cytoskeleton-associated genes (Table 1) and corresponding mutant phenotypes indicates that a precise orchestration of the activities and functions of both cytoskeletal elements is essential for producing a particular cell shape. In the simple emergent scenario, a direction-defining function is attributed to microtubules, whereas the actin cytoskeleton performs a transport-related function. Presumably, the pathfinder microtubules should be active before the actin-based transportation machinery can get into action. However, how do microtubules find 'their' path? Which intracellular cues do they look for?

A protein high up in the cytoskeleton regulatory hierarchy appears to be the CDM-domain-containing SPIKE1, a potential activator of plant-specific ROPs (Rho-GTPases of plants) [43]. Although the SPIKE1–ROP connection has not been experimentally proved, the more-downstream ROP-mediated regulation of the actin cytoskeleton has been demonstrated [67–69]. Constitutive overexpression of AtROP2 produces cells with an increased area containing a diffuse F-actin mesh [67]. Intriguingly, the final morphology (e.g. swollen hypocotyl and leaf epidermal cells, and branched root hairs) of cells overexpressing AtROP2 [67,68] suggests an impaired microtubule cytoskeleton. Why should an increase in actin polymerization lead to more isotropic growth? More paradoxical observations become apparent.

ARP2/3-complex mutants display short, distorted trichomes and twisted root hairs [20,58,59]. Although the short, unexpanded cellular phenotype is consistent with an impaired actin cytoskeleton, the random cell distortions are not, because they suggest random changes in growth directionality during trichome morphogenesis. However, as described earlier, cellular growth directionality appears to be regulated by the microtubule and not the actin cytoskeleton. Indeed, an analysis of the microtubule organization in the randomly misshapen trichomes of the *distorted2* and *crooked* mutants reveals abnormally clustered endoplasmic microtubules whose distribution pattern closely follows the distribution of dense cortical F-actin patches seen in these mutant cells [61]. What is the relevance of this colocalization of cortical actin patches and microtubules in diffusely growing trichome cells?

Furthermore, aberrant bulge formation in trichoblasts in the *act2/der1*, *crooked* and *wurm* mutants suggests that the selection of a site for polar growth involves actin. Endoplasmic microtubules are usually excluded from these early bulge domains, whereas the later appearance of subapical microtubule bundles in the extending root hair is an indication of polar growth fixation [70]. Indeed, one of the unwavering observations in tip-growing cells has been the presence of a subapical region with sparse but dynamic actin filaments [10,11], with endoplasmic microtubules maintaining a discrete presence behind the diffuse actin zone [10,11,70]. Finally, in the well-studied apolar fucoid zygotes, an actin patch marks the site of polarized growth whereas drug-induced breakdown of actin polymerization results in multiple growth axes [71].

Cortical weakening

Cortical weakening can trigger the growth process

The previous roles attributed to actin and microtubules during cell morphogenesis need to be reassessed so that we can assimilate the observations made above into a coherent operational framework that addresses how growth can be targeted to a region of the cell to make it grow differently from other regions. The observation that all plant cells initially possess a regular shape suggests an even distribution of intracellular resources and the growth machinery at this developmental stage. Presumably, internal forces such as turgor pressure, directed against the layers of filamentous cytoskeletal proteins, plasma membrane and cell wall (Figure 3) that delineate the cell boundaries, must also be well balanced. Embedded in this layered view of the cell is the idea that, for the creation of any shape other than a sphere, a local imbalance needs to be generated so that one region inside the cell becomes radically different from another. How can this be accomplished? Two possibilities are: (1) an outward-directed force (such as that exerted by a minivacuole capable of further expansion) becomes activated internally and impinges on the cell boundary; and (2) that intrusion by an external, inward-directed force (such as the weight exerted by a spore falling on an exposed surface of a cell) creates an effect on the cell periphery. In either case, the bounding layers of the cell might experience a localized tension. Considering that cytoskeletal proteins are capable of exhibiting rapid responses to alterations perceived by the cell-wall and plasma-membrane layers, a cascade of cytoskeletal events might be triggered locally. Signal perception by the bounding layers of the cell thus constitutes the first step in activating the growth machinery (Figure 3).

Cortical weakening can be achieved by loosening the actin mesh

In several cell types, the earliest sign of polar growth initiation is the appearance of a small bulge on the cell surface [10,15,50]. The localization of cell-wall-loosening proteins such as expansins [50], observations of localized ionic changes [4] and the detection of wall-degrading enzymes in exudates of invading organisms [72] suggest that bulge initiation results from internal pressure being exerted against a weakened area of the cell boundary. The cortical cytoskeletal meshwork, consisting of actin filaments and microtubules, also appears to be more labile in the bulged domain [50,69,73]. The increase in local actin dynamics is further supported by observed activation of various actin-polymerization regulators such as ROPs [68,74], profilins [49,50,75], ADFs [47,48] and ARP2/3-complex proteins [69,73] in bulged domains of growing root hairs and characean rhizoids [75]. Experimentally, it has been possible to induce *de novo* bulge formation in root hairs by localized drug-induced loosening of the actin, but not the microtubule, cytoskeleton [76].

In other organisms, experimental evidence links small GTPases to actin cytoskeleton regulation through interactions with actin-nucleating and -polymerizing factors such as formins and the ARP2/3 complex [77]. In higher plants, too, ROP-GTPase-induced enhancement of actin polymerization might be mediated through an activation of the ARP2/3 complex [78] by a regulatory cascade involving putative Scar/WAVE (WASP-family verproline-homologous) proteins [78], an as yet unidentified ABI2 (AB1 interactor 2) homologue, KLK (PIR121 homologue [64]), GRL (NAP125 homologue [62,63]) and BRK1 (HSPC300 homologue [65]) proteins. The molecular capability for increasing cortical actin-mesh lability exists in plant cells. Polarized growth does initiate from bulges containing a fine F-actin mesh [10,50], which is evidence that the growth machinery becomes targeted to these weak cortical domains (Figure 3). Localized actin-mesh loosening and increased actin dynamics could also allow vesicles carrying growth materials to reach the zone of assimilation in an actin-polymerization-driven mechanism similar to that observed for endosomes and small organelles in other organisms [20,73] (Figure 3). The actin mesh can thus act as a barrier when composed of thick F-actin bundles but, when composed of short, dynamic filaments, it can facilitate localized growth by increasing vesicle access to the assimilation zone [20]. The near absence of actin microfilaments from the apex of tip-growing cells and the existence of actin-bundling proteins and dense actin in subtending regions and in regions of low expansion support this view [48,50,75].

Reinforcement of weak cortical sites and fixation of growth directionality requires microtubules

A continuous stream of signals feeding into the actin-mesh-loosening mechanism can potentially extend cortical weakening to the entire cell surface. A general release of growth materials by vesicles in the expanding weak region would ultimately produce an isodiametric cell shape (Figure 3). Because this does not usually happen, the further weakening of the cortex must obviously be curtailed at some stage for growth to become localized. Activation of actin-bundling proteins might counteract the tendency for rapid actin polymerization at a growth site. However, another filamentous protein that is unresponsive to actin regulatory mechanisms would be more suitable for both confining the actin-mesh loosening to a region and simultaneously providing additional reinforcement to the weak site. Such an element should occupy a position juxtaposed to dynamic actin patches.

Endoplasmic microtubules just behind dense actin areas fulfil all these requirements. Additionally, microtubule plus-end binding proteins such as EB1 possess a calponin-homology (CH) domain [79] that can potentially interact with actin microfilaments. In context, an impaired microtubule cytoskeleton invariably leads to loss of growth directionality and isodiametric cell expansion (Figures 1–3) [14,15]. The dispersal of cortical actin patches (as in plants constitutively overproducing AtRop2 [67,68]) would be expected to disperse microtubules, too, and can thus explain why excessive dynamic actin produces cellular phenotypes reminiscent of microtubule defects. Similarly, the random growth directionality of ARP2/3-complex mutant trichomes, with indiscriminately scattered actin patches [20,61], can be explained by the microtubules, which are responsible for fixing polar growth directionality, following cortical cues laid down
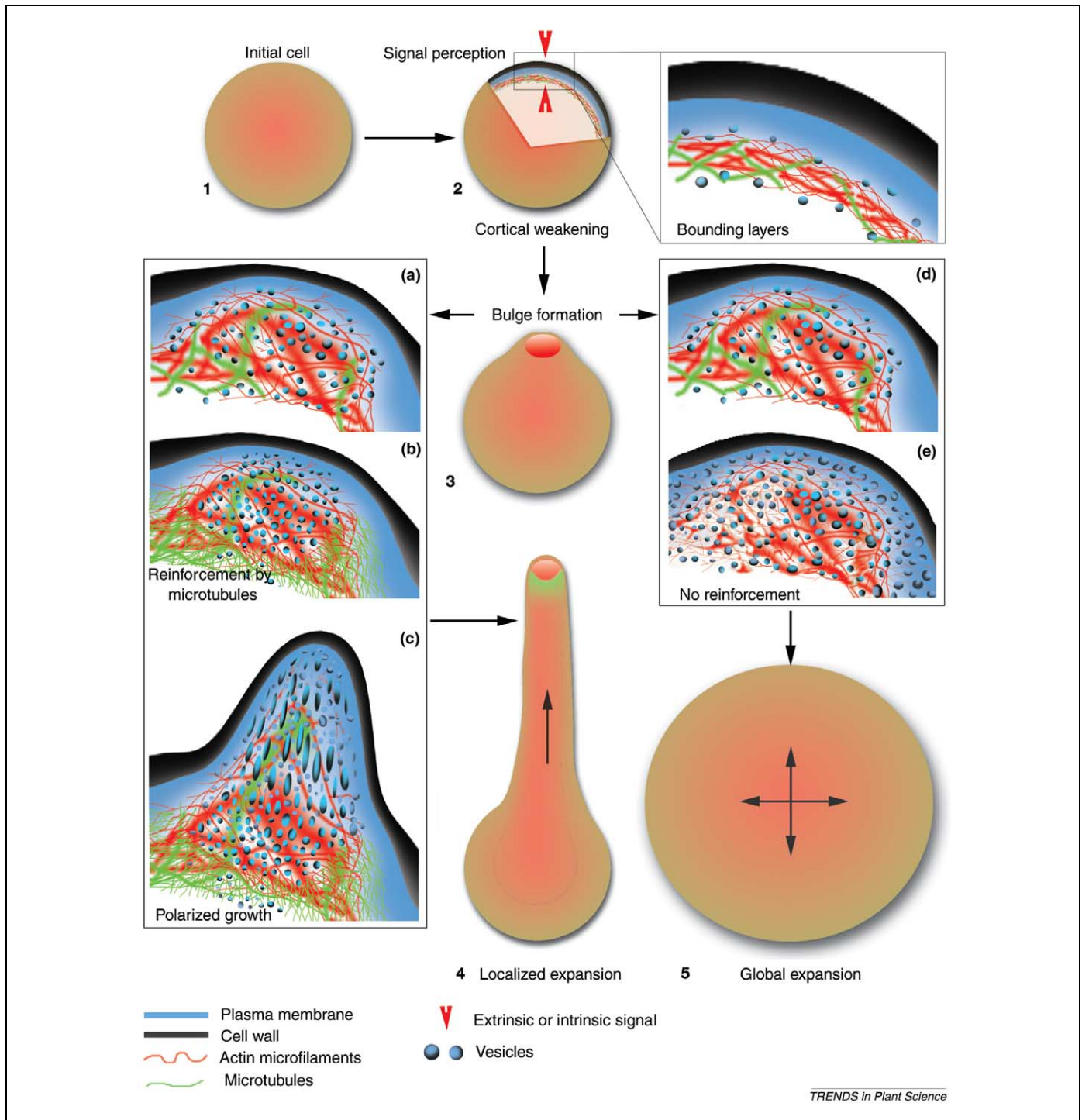
Figure 3. A simple schematic model based on recent studies of cell shape development showing the general sequence of events during plant cell morphogenesis. **(1)** The initial isodiametric shape of a cell reflects a regular distribution of internal resources required for the growth process and an overall balance in the internal forces exerted on the boundaries of the cell. **(2)** The reception and recognition of an extrinsic or intrinsic cue (arrowheads) occurs on the bounding layers of a cell (shown in the magnified transverse section; from the outside, these are the cell wall, the plasma membrane and the cytoskeletal mesh). SPIKE1 [43], ROPs [67,68,74], putative Scar/WAVE-like proteins [78] and members of their regulatory complex [62–65] are probably activated at this early stage. **(3)** The initial responders to environmental cues affect the actin mesh through the activity of membrane- and cytoskeleton-interacting formins [66], the actin polymerization modulating ARP2/3 complex [20,57–61], ADF [47,48,52], AIP1-like proteins [51] and profilins [49,50], which cooperate in the regional reorganization of the actin cytoskeleton by making it more dynamic and flexible. As the actin mesh loosens locally, the cortical site becomes weaker. Internal forces (such as turgor pressure) can now cause a small protuberance (bulge) to emerge on the surface of a cell [10,45,46,50,71,74]. Internally **(a,d)**, the loosening of the actin mesh can greatly facilitate the movement of vesicles carrying growth material closer to the membrane and cell-wall layers for depositing their cargo. **(4)** The cortical weakening caused by the preceding events extends further and depending on how much it is allowed to extend. Two possibilities arise: **(b)** vesicle movement and assimilation of their contents can become targeted to a region; or **(e)** vesicles remain untargeted and deposit their contents anywhere within the cell. Localized vesicle targeting can be achieved in **(b)** if the cortical weakening can become restricted through actin-bundling-protein activity [69,73,75], with additional reinforcement by endoplasmic microtubules regulated by polymerizing factors [24,25,39], microtubule-associated proteins [22,29–35,38,79] and microtubule motors [21,80]. When microtubules are compromised and unable to restrict the cortical weakening from spreading **(e)**, the result is diffuse, untargeted growth **(e)** to produce a globally rounded cell **(5)**. **(4)** Polarity fixation occurs when both cortical actin patches and endoplasmic microtubules display concurrence within a region **(c)**. All cellular growth resources are now recruited to the region for achieving 'directional' growth and the development of a specific cellular form. Although this figure depicts a tip-growing cell, a similar scenario can be envisioned for diffusely growing cells by making the growth focus area larger.

by the actin cytoskeleton [61] and consequently changing the focus of growth randomly to produce distorted trichome cells.

Conclusions and perspectives

Molecular-genetic and cell-biological evidence of cell morphogenesis strongly suggests that localized growth occurs as a cellular response to combat regional weakening of the cortex. The weakening is created by a localized increase in cortical actin dynamics. Polar growth directionality becomes fixed once endoplasmic microtubules approach the weak actin mesh and both reinforce it and establish the concurrence of both cytoskeletal elements at the particular cortical location. All cytoskeleton-dependent, growth-related intracellular traffic now gets diverted to the specified region. Consequently, more growth occurs in the defined region than in other areas of the cell. A spatiotemporal reiteration and readjustment of this 'differential growth' phenomenon in response to specific genetic and environmental patterning cues allows a precise cellular form to emerge.

The identification of more molecular players, their allocation to positions in the molecular hierarchy and cell-biological studies aimed at the simultaneous intracellular visualization of interactions between actin, microtubules and their regulatory proteins should allow further details to be filled in and the global veracity of the framework for cell morphogenesis presented here to be tested.

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