Directional cell expansion - turning toward actin

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Significant recent progress toward understanding directional expansion in diffusely growing plant cells concerns actin. Tools for imaging actin, including both live-cell reporters and fixation protocols, have been improved. Proteins that interact with actin have been identified and their functions probed biochemically and genetically. Specifically, members of the actin-related protein2/3 (ARP2/3) complex and the Wiskott–Aldrich syndrome Verprolin-homologous (WAVE) complex have been identified. These proteins have salient functions in shaping trichomes and leaf pavement cells. Additionally, two targets of a *rho*-of-plants (ROP) G-protein have been discovered that exert opposing regulatory action on actin and microtubules, a pathway that appears to be responsible for establishing the undulating shapes of pavement cells. Finally, several mutants of the *fragile fiber* class have revealed a link between actin organization, cell wall synthesis, and phosphoinositol signaling.

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Introduction

In plants, cell expansion is controlled by turgor pressure, cell wall properties, and the cytoskeleton. Traditionally, microtubules have been implicated in the direction of expansion, probably by orienting newly synthesized cellulose microfibrils [1,2]. By contrast, actin has been implicated in the rate (or overall extent) of expansion [3–6], probably through delivery of Golgi vesicles that contain membrane and wall materials to the site of growth. This traditional distinction is well illustrated by tip-growing cells, such as pollen tubes. Actin plays a primary role in the expansion of these cells whereas the role of microtubules is secondary [7–10]; consistently, the tips of these cells expands approximately isotropically (i.e. at the same rate in all directions) [11].

However, actin has recently emerged as an important contributor to the directionality of expansion in diffusely growing cells, partly through collaboration with microtubules [6,12,13••]. There have been a wealth of papers revealing a role for actin and related proteins in directional cell expansion, papers that are expanding our limited understanding of actin's role in plant growth. This review focuses on the cooperation of actin and microtubules in shaping diffusely growing cells.

Advances in imaging actin

The improvement of imaging techniques in the past few years is a breakthrough in the study of actin. Actin is notoriously problematic to preserve with chemical fixation [12,14]. In animal cells, reference images have been produced by tagging actin, but this approach has yet to be successful for plant actin, whether tagged biochemically (e.g. with rhodamine) or genetically (e.g. with green fluorescent protein [GFP]). Recently, success in plants has been achieved by tagging actin-binding proteins.

Perhaps the first tagged actin-binding protein to be used in plants was mouse talin (GFP–mTalin) [15]. Recently, images of maize leaf cells expressing GFP–mTalin were shown to resemble material that was fixed and stained with phalloidin [16]. However, expression of GFP–mTalin can alter morphology and disrupt actin severely [17,18•,19]. Generally less disturbance to actin and plant growth has been achieved when actin-binding domains (ABDs) from *Arabidopsis* fimbrin are used for tagging. Full-length fimbrin–GFP gives bright fluorescence but reveals few filaments; however, a construct with a part of the protein that includes the binding domains, ABD1/2–GFP, gives dimmer fluorescence but greater detail [19]. Another construct that includes part of the fimbrin protein, GFP–fimbrinABD2, gives striking images of dense actin networks in *Arabidopsis* root, hypocotyl, and leaf epidermal cells, and permits studies of actin dynamics [18•]. Sheahan *et al.* [18•] noted a correspondence between the actin organization seen with this protein construct and that in images of carefully fixed cells. The complexity of the actin cytoskeleton is such that no single actin-binding protein can be expected to stain every bundle and filament, but the congruence of a live-cell reporter and fixation in a given investigation offers assurance that an approximation to the state of the cytoskeleton in the living cell has been reached.

As an alternative approach, Lovy-Wheeler and colleagues [14] improved fixation techniques, specifically for pollen tubes. They took advantage of the fact that cryofixation and freeze substitution provide unmatched structural preservation of single cells and used images from cryofixed pollen tubes as a reference to develop a protocol for

chemical fixation. The advantages of chemical fixation are that it requires no special apparatus, provides higher throughput, and can be applied immediately following live-cell imaging. The resulting protocol for chemical fixation led to images of actin that were all but indistinguishable from the cryofixed images, which, importantly, are somewhat different from images of living pollen tubes expressing fluorescent actin-binding proteins (K Wilsen *et al*., unpublished).

Coordination of microtubules and actin in cells with complex shapes

In general, actin and microtubule arrays in the cell cortex are considered to have a close association [20], and it has been reported frequently that perturbing one can affect the stability of the other [21–24]. Recently, chemical disruption of actin was shown to disrupt severely the phragmoplast in synchronized tobacco tissue-culture cells and to prevent microtubule reorganization at the mitosis–interphase transition [25]. Also, a kinesin that was identified in cotton binds both microtubules and actin filaments and contains an actin-binding calponin-homology domain [26•]. Kinesins that have calponin domains form a small clade that is unique to plants; *Arabidopsis* has seven members, offering scope for interactions between actin and microtubules.

The interlinked status of actin and microtubules has been incisively revealed in pavement cells and trichomes. Pavement cells are characterized by lobed walls that fit together like a jigsaw puzzle across the surface of the leaf; the lobes can be gentle sinusoidal undulations, as in maize, or deeply curved and irregular, as in *Arabidopsis*. Trichomes grow out from the surface of the leaf to form spike-like cells that can be straight or branched. Trichomes in *Arabidopsis* have recently been demonstrated to grow diffusely rather than by tip growth [27]. The morphogenesis of pavement cells and trichomes has been comprehensively reviewed by Smith and Oppenheimer [13••] and that of pavement cells and lobed mesophyll by Panteris and Galatis [28••].

The prevailing theory to explain how the interlocking pattern of pavement cell lobes is formed has been that bands of microtubules cause localized wall thickenings, restricting cell wall expansion in the 'neck' region and forcing the intervening thinner wall regions to stretch under turgor pressure [6,28••]. However, the inability of *Brick1*, a maize mutant in an actin-regulating protein, to form lobes despite the apparently correct formation of microtubule bands highlighted the importance of actin in lobe formation [16]. It is now clear that both actin and microtubules are crucial for the elaboration of lobes.

Likewise, building a trichome appears to require coordinated action from microtubules and actin. Mutants in any of a set of genes that encode actin-regulating proteins have distorted trichomes ([13••] and references below); however, cortical microtubules are aberrant in many of these mutants [27,29]. When these mutations are crossed into *zwichel*, a mutant that has a defect in a microtubule-dependent motor protein, the double mutants have a phenotype that indicates a synthetic interaction [27]. Additionally, endoplasmic microtubules, although less frequently scored than cortical microtubules, are abnormally organized and resistant to chemical depolymerization in at least one of the distorted trichome mutant backgrounds [30•]. Saedler *et al*. [30•] suggest that during trichome formation the cortical actin becomes more dynamic at a site of expansion, allowing vesicle delivery and wall bulging, while microtubules restrict the extent of bulging by hemming in the spread of the increased actin turnover. Intriguingly, localized control of actin dynamics is thought to be the basis of the formation of protrusions in moving animal cells [31,32•].

Modeling and remodeling actin: actin-binding proteins emerge from the genomic shadows

Actin behavior is governed by a suite of actin-associated protein complexes. Recent characterization of components of the actin-regulating ARP2/3 and WAVE complexes, as well as of the ROP-GTPase signaling pathway, has uncovered a complex web of control that potentially allows signal transduction to, and co-coordinated reorganization of, the actin cytoskeleton. These components are reviewed, in the context of cell polarity, by Xu and Scheres, this issue.

ARP2/3 complex

The ARP2/3 complex is pivotal for regulating actin polymerization in animals [32•]. When activated, it enhances the nucleation and polymerization of filamentous actin. It also binds to existing actin filaments, initiating branches and leading to the formation of dendritic actin arrays. Until recently, the ARP2/3 complex was unknown in plants.

Homologs of all seven subunits of the ARP2/3 complex have now been identified in plants on the basis of homology searches and loss-of-function mutants [30•,33–35]. Mutations in the ARP2/3 complex of *Arabidopsis* cause abnormal actin organization and deformation of trichomes, leaf epidermal pavement cells and some hypocotyl epidermal cells, but overall plant morphology is scarcely affected. In these mutants, trichome branches are underdeveloped or twisted, the trichome stalk is unusually thick, and actin organization is compromised [30•,32•,34]. Along with the trichomes, leaf pavement cells and hypocotyl cells are affected: pavement cells are less lobed in the mutants than in wildtype, and some hypocotyl epidermal cells become separated at their end walls under rapid growth conditions [33,35].

Surprisingly, ARP2/3 mutations in *Arabidopsis* affect neither root hairs nor pollen tubes nor the majority of

diffuse growing cells in the plant, suggesting that only select cell types take advantage of the dendritic mode of assembly that is supported by ARP2/3. This is in contrast to animals, in which loss-of-function mutants have extreme, sometimes lethal, phenotypes (summarized by [32•]). Even in the moss *Physcomitrella patens*, loss of one of the ARP2/3 subunits completely blocks the tip-growth of caulonemal cells and thus prevents bud formation and the development of leafy gametophores [36]. Several authors have noted that ARP2/3 mutants have less fine cortical actin and more thick endoplasmic actin cables, and have suggested that fine cortical actin plays a role in cell expansion [16,34,37••]. It will presumably take ultrastructural investigation to reveal how fine actin that is established by ARP2/3 differs from that established by other regulators, such as formin.

WAVE complex

Another complex that is involved in actin regulation is the WAVE complex, members of which have also been recently identified in plants [29,37••]. In animal cells, WAVE proteins regulate ARP2/3 activity and are also known to interact with the G-protein RAC. Hence, the identification of WAVE-like proteins in plants provides a putative link between G-protein-dependent signal transduction and cytoskeletal organization [37••]. Mutations of the members of the plant WAVE complex that have been isolated to date result in phenotypes similar to those of ARP2/3 mutants [29,37••], confirming that they act in the same actin-regulating pathway.

BRICK1 is a WAVE complex member that has been identified in maize and that is homologous to the human WAVE component hematopoietic stem progenitor clone 300 (HSPC300) [16]. BRICK1 revealed the involvement of actin in the formation of pavement cell lobes. Furthermore, lines that are mosaic for *Brick1* showed that wild-type BRICK1 can induce lobe formation in neighboring mutant cells, effective over a distance of two to three cells from the site of transcription. Intercellular interactions are presumably necessary to co-ordinate the interdigitating growth that is typical of pavement cells, and yet BRICK1 is the only cell-shaping protein known to act non cell-autonomously.

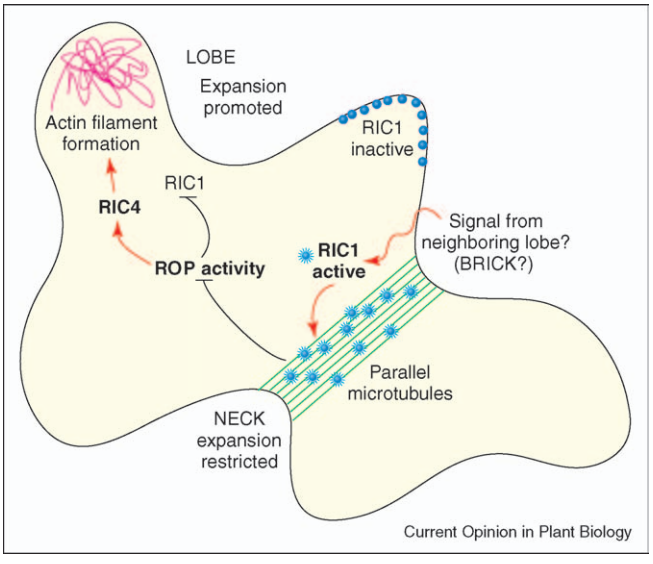
ROP-GTPase signaling pathway in plants

In animal cells, the *rho*-GTPase signaling pathway co-ordinates the cytoskeleton to perform various functions, including cell migration [38]. Previously, it has been established in plants that homologs of *rho*-GTPases, called *rho*-of-plants (ROP), participate in organizing both cortical actin and microtubules ([39,40]; Xu and Scheres, this issue). This year, Fu *et al.* [41••] identified two CRIB-motif-containing ROP target proteins, RIC1 and RIC4, that mediate microtubule and actin organization via counteracting pathways. Furthermore, they proposed a model that accounts for the regulation of both actin and microtubules to generate the convoluted shapes of pavement cells (Figure 1).

According to this model, RIC1 helps to organize microtubules into parallel arrays in the cell 'necks' and thus reins in expansion, whereas RIC4 mediates the polymerization of fine cortical actin filaments in the lobe tips and thus promotes outward growth. What's more, the RIC1 and RIC4 pathways negatively regulate each other to ensure well demarked lobe and neck regions. The discovery of these counterpoised RICs is fascinating because, although numerous microtubule- and actin-associated proteins are known, few cytoskeletal organizers, let alone pathways that coordinate the regulation of microtubules and actin, have been identified.

The interaction between the ROP G-proteins and the downstream RICs presumably depends on a guanine-nucleotide exchange facilitator (GEF) to activate the ROP. In plants, the identification of ROP–GEFs has been hindered by a lack of sequence similarity to known animal GEFs; only this year has their presence in plants been confirmed on the basis of biochemical assays [42••]. ROP–GEFs are represented in *Arabidopsis* by a family of

Figure 1



Cartoon of pavement cell lobe formation that is based on the model by Fu *et al.* [41••]. When ROP GTPase is turned on, it activates RIC4, inducing the formation of fine cortical actin filaments and lobe expansion and concomitantly suppressing RIC1 activity. ROP has limited mobility, so its activity is limited spatially. RIC1 (possibly activated by a signal from the neighboring cell) localizes to microtubules and organizes them into parallel bundles, restricting expansion locally. The polymerization status of microtubules in turn affects local ROP activation. RIC1-organized microtubules sequester and inactivate ROP, preventing its interaction with RIC4; whereas depolymerized microtubules release ROP. This ensures that active RIC4 and RIC1 are spatially separated, allowing the formation of distinct lobes and troughs at the pavement cell margin.

at least 14 highly related proteins, but specifically interacting pairs of ROP and GEF partners have yet to be found. The presence of more than 14 ROP–GEFs in *Arabidopsis* is suggested by the existence of a protein called SPIKE, which was identified from one of the many trichome mutants. SPIKE is also necessary for pavement cell lobe formation and shows characteristics of a *rho*-GEF [43]. In animal cells, GEF-H1 is believed to be activated by microtubule depolymerization [44]; if one of the plant GEFs behaves similarly, this would reinforce the model of Fu *et al.* [41••] by enhancing ROP activity in the lobes, where microtubules are sparse.

Other examples of actin's role in directional cell expansion

In addition to cells that combine overall diffuse growth with localized expansion to create complex cell shapes, cells that grow principally along one axis also need actin. When actin is chemically disrupted, diffuse growth usually becomes less anisotropic [13••]. Because actin and microtubules often have different arrangements at the expanding and non-expanding walls of a cell, Fu *et al.* [41••] suggest that the ROP GTPase signaling pathway coordinates cytoskeletal organization in many, if not all, cell types. Consistently, transgenic constructs that render the ROP constitutively active disrupt actin organization and expansion, causing deformed organs, loss of pavement cell lobes, and roughly isotropic growth in mesophyll cells [45•].

Other examples of actin's involvement in cell wall synthesis and cell elongation come from members of the *fragile fiber* (*fra*) family of mutants [46–48]. Cell walls in the fiber cells of *fra4* (also known as *root hair defective 3*) are abnormally thin, have altered composition and reduced mechanical strength, and have reduced cell elongation in all organs [46]. Actin in *fra4* is clumped into thick cables and has less branching than the actin of wildtype plants. *FRA4* encodes a novel protein that has putative GTP-binding motifs [46]. *FRA3* encodes an inositol polyphosphate phosphatase and *FRA7* encodes a so-called 'suppressor of actin (SAC) domain' phosphatase, which is also active against inositol polyphosphates [47,48]. Both also appear to be necessary for normal actin organization and secondary cell wall formation, with *fra7* mutant plants having reduced expansion in most, if not all, organs [48]. The cell wall defects of these three *fra* mutants are plausibly caused by reduced vesicle delivery to the plasma membrane. In support of this, cytochalasin D, which disrupts actin, also causes reduced wall thickness in fiber cells [46]. Together, the *fra3*, *fra4* and *fra7* mutants link phosphoinositide metabolism, actin organization, and cell wall synthesis. Finally, the link between actin microfilaments, secretion, and directional expansion is underscored by *katamari1*, a mutant that has reduced elongation, and disrupted actin and endoplasmic reticulum. The protein that is defective in this mutant is a Golgi-resident, glycosyl-transferase, previously identified as MUR3 [49].

Conclusions

Less than a decade ago, actin was all but ignored in treatments of the expansion of diffusely growing cells. It has now become clear that actin can promote outgrowth in plant cells, as seen in pavement cells, and that in cooperation with microtubules, it plays a major role in the construction of complex cell shapes and in expansion. We have only just begun, however, to discover the diverse roles of actin and the myriad signaling pathways that govern its organization and function. The recent work discussed here, focusing on signaling molecules and pathways, has opened up a field of study that we are confident will reveal mechanisms that control the plant cytoskeleton and directional cell expansion.

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