

# Microtubules and Microfilaments in Cell Morphogenesis in Higher Plants Review

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**Microtubules and microfilaments play important roles in cell morphogenesis. The picture emerging from drug studies and molecular-genetic analyses of mutant higher plants defective in cell morphogenesis shows that the roles played by them remain the same in both tip-growing and diffuse-growing cells. Microtubules are important for establishing and maintaining growth polarity whereas actin microfilaments deliver the materials required for growth to specified sites. The recent cloning of several cell morphogenesis genes has revealed that conserved mechanisms as well as novel signal transduction pathways spatially organize the plant cytoskeleton.**

## Introduction

Polarized growth of higher plant cells is considered to follow either of two mechanisms: tip-growth, during which growth is limited to a small region of the cell and diffuse; or intercalary growth, where cell expansion takes place at all surfaces. Tip growth is believed to be limited mainly to root hairs and pollen tubes, and most other cell types are thought to expand by diffuse growth. Tip-growing cell growth was shown to depend on the actin cytoskeleton which has led to the misconception that during tip-growth microtubules have little or no role [1]. Conversely, the correlation of rearrangements of microtubule arrays with the directionality of turgor-driven growth in various cell types has led to the suggestion that such growth involves microtubules mainly and that actin plays only a minor role. Over time, tip growth and diffuse growth have begun to be viewed as separate processes [1].

A comparison of the respective roles of microfilaments and microtubules in various cell types of higher plants has led to a reassessment of this view. It now appears that all cells, irrespective of their growth focus, use microtubules for polar-axis determination and microfilaments for the targeted delivery of components necessary for growth. In this review we discuss the general role of microtubules and microfilaments in cell morphogenesis as revealed by drug-based studies, and their regulation as uncovered by the molecular characterization of cell morphogenesis mutants.

## Cell Responses to Microtubular and Microfilament Disrupting Drugs

One basic difference between animal and plant cells is that plant cells are encased by a rigid cell wall. During cell expansion an inner osmotic pressure — cell turgor — exerts a force that impinges on the restraining cell

wall. In this situation a cell will grow only where the cell wall is remodeled. The role of microtubules and microfilaments during cell growth has been dissected by using specific drugs that disrupt cytoskeletal function, and is summarized in Figure 1. As detailed below, the cellular phenotypes elicited suggest that microtubules and microfilaments have distinct functions in cell morphogenesis.

## Role of Microtubules during Cell Morphogenesis

Microtubules have frequently been found aligned with wall microfibrils that are arranged like ‘hoops around a barrel’ in an orientation transverse to the direction of cell expansion. Microtubule orientation is thus believed to determine the direction of growth though deviations in microtubule–microfibril coalignment have been reported in certain cell types [2,3]. The coalignment of microfibrils and microtubules suggests that the microtubule cytoskeleton is responsible for targeting membrane and wall material required for building a cell and thereby determining growth direction.

Microtubule drug treatments in diffuse growing cells appear to support this. Cells of the leaf epidermis, hypocotyl, and root with impaired microtubules swell isotropically (Figure 1), suggesting that growth takes place but its directionality is lost [4,5]. Similarly, branched, anisotropically growing leaf trichome cells lose their branching capability and become globular following similar treatment (Figure 1) [6].

In tip-growing root hair cells, under normal conditions a single trichoblast gives rise to one root hair [7]. Trichoblasts on *Arabidopsis* seedlings treated with microtubule drugs often have more than one root-hair initiation site and thus multiple root hairs [4,8]. This implies that the selection of a site for focusing tip growth may depend upon microtubules. Further, microtubule drug treatment of elongating root hairs also results in their becoming branched through the formation of multiple tips (Figure 1), suggesting that microtubules are important for maintaining one growth axis [8].

This view is supported by the finding that in microtubule drug-treated root hair cells, but not in untreated root hairs, new tips can be initiated by locally applying  $\text{Ca}^{2+}$ . Normally, root hairs show a tip-focused  $\text{Ca}^{2+}$  gradient which appears to be critical for tip focused growth because dissipating this gradient with specific buffers or channel blockers arrests tip growth. Manipulations of  $\text{Ca}^{2+}$  concentration in the tip region may result in a new growth directionality but elicit no effect in more distal regions of the root hair cell [8]. Therefore, the finding that  $\text{Ca}^{2+}$  can induce new growth centers only in the absence of microtubules shows that microtubules are important for maintaining growth polarity.

In further support of a role of microtubules in determination of growth directionality, it was shown that transient microtubule stabilization could trigger

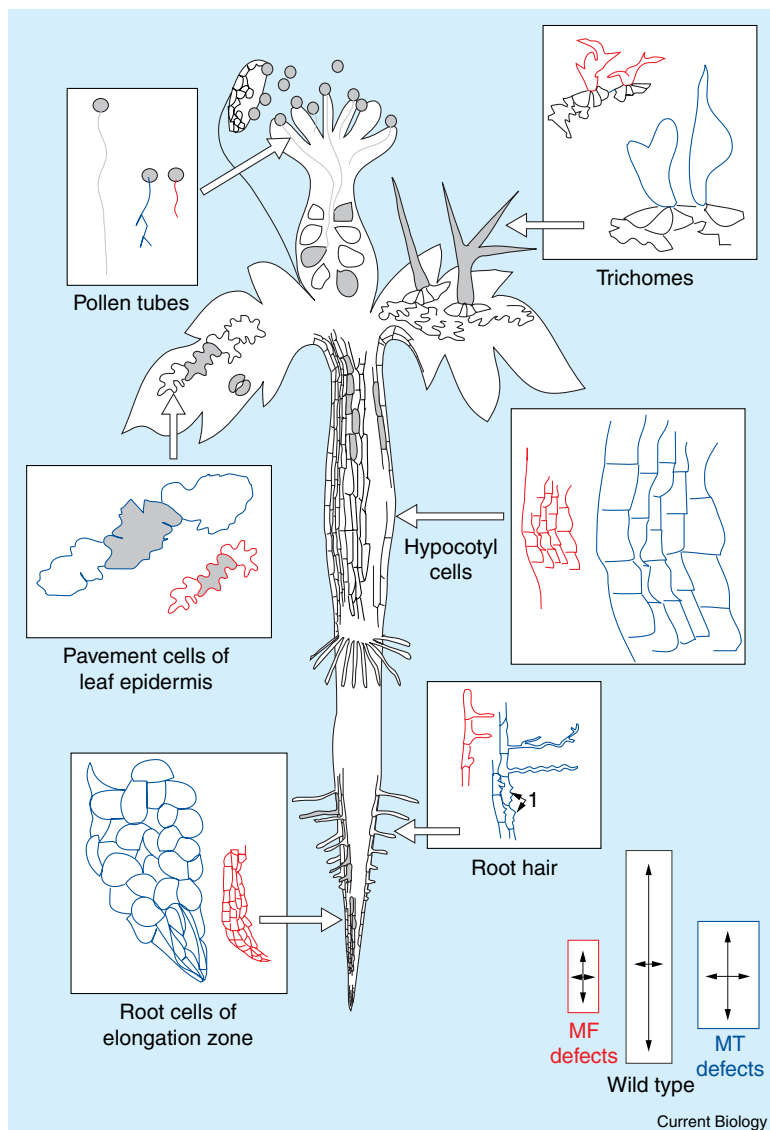


Figure 1. Schematic representation of common phenotypes of model cell types resulting from defects in actin (red) and microtubule (blue) cytoskeleton.

The three rectangles at the bottom of the figure compare cytoskeleton-linked changes in cell size with the wild-type cell (middle). In general actin cytoskeleton defects result in reduced cell elongation/expansion. Microtubule defects lead to loss of growth focus, increased expansion and a general rounding up of the cell shape. During root hair morphogenesis, specifically, both the selection of a site for tip growth (1) as well as the maintenance of tip growth in an elongating hair are influenced by the microtubule cytoskeleton.

morphogenetic events in unicellular *Arabidopsis* trichomes. Transient stabilization of microtubules in elongating trichomes of the unbranched *stichel* mutant and less-branched *zwichel* mutant using taxol was sufficient to initiate branch-like outgrowths [6].

Taken together, the phenotypes obtained by microtubule drug treatments suggest that microtubules are important for initiating changes in growth directionality, as well as maintaining growth directionality in both diffuse and tip-growing cells. As growth itself remains unaffected in such treated cells, microtubules do not appear to be involved in the transport of cell membrane and wall material required for growth.

#### Role of Actin Microfilaments during Cell Morphogenesis

Cell expansion is drastically reduced in all cell types after microfilament specific drug treatments. Treatment of *Arabidopsis* seedlings with the actin polymerization inhibitor latrunculin results in small 'bonsai' plants containing unexpanded cells [9]. Cells of the

leaf-epidermis, root and the hypocotyl (Figure 1) remain relatively small and unexpanded; and trichomes initiate branches that often remain short or expand randomly leading to a distorted phenotype (Figure 1). In most cases trichome distortion can be traced back to aberrant actin organization [10,11]. Similarly, root hairs and pollen tubes (Figure 1) do not elongate properly and remain short and stubby [9,12]. Both actin stabilizing drugs as well as those that interfere with actin polymerization result in the same phenotype. How can this be explained?

The apex of tip-growing cells is a region of dynamic actin organization where numerous actin-interacting proteins such as profilin, actin-depolymerizing factors (ADFs) and villin accumulate [9,12,13]. Based primarily on visualization of the actin cytoskeleton in growing root hairs and pollen tubes it has been proposed that Golgi-derived vesicles carrying cell-building material are liberated from the ends of very fine microfilament bundles into a vesicle incorporation zone at the cell apex [13,14] (Figure 2A). Video-enhanced microscopic

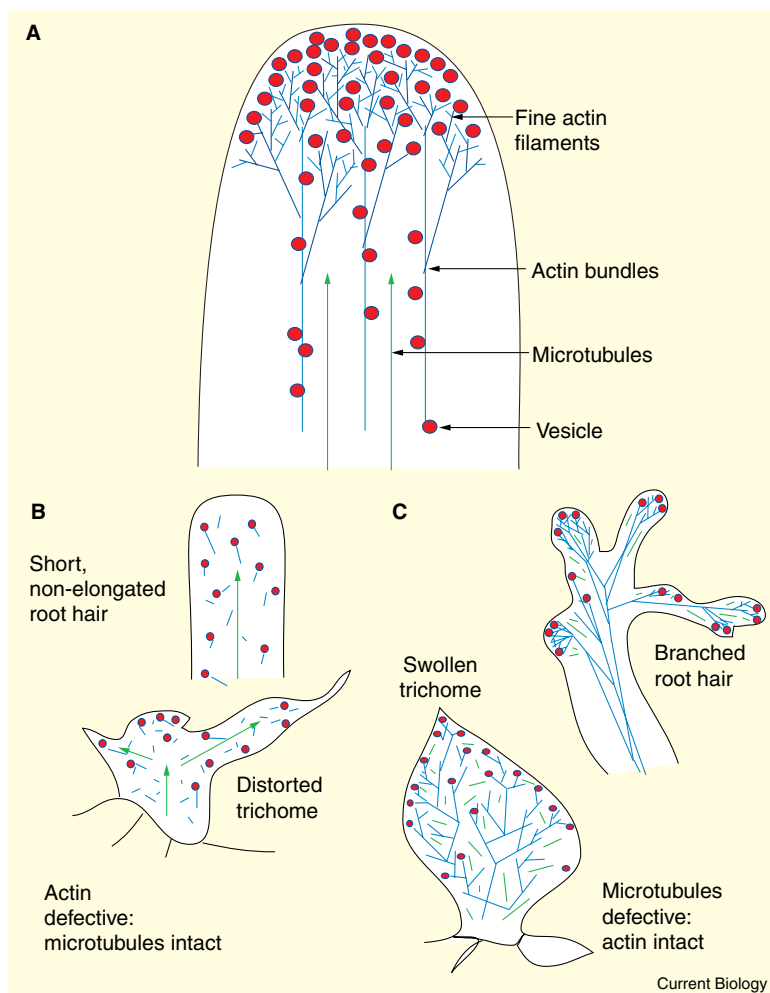


Figure 2. Schematic representation depicting the hypothesized roles played by microtubules (shown in green) and actin microfilaments (shown in blue) in all expanding cells.

(A) Fine actin filament bundles are concentrated near the apex in a tip-growing cell. These branch out from thicker actin bundles and probably are the final tracks from which vesicles (shown in red) carrying cell-building materials are eased off into the vesicle incorporation zone resulting in cell extension. Microtubules do not appear to pervade the zone occupied by fine microfilaments. In diffuse growing cells the vesicle incorporation zone, signifying the region of cell expansion would be much larger compared to that of a tip-growing cell, as in (A). However, vesicles carrying cell membrane/cell wall material probably still get released from the ends of fine actin filaments while microtubules maintain the growth directionality. (B) Perturbations in the actin cytoskeleton in a tip-growing cell, such as a root hair cell (upper part), or a diffuse growing cell such as a trichome (lower part), possibly result in random movement of vesicles and aberrant deposition of cell-building material. Though microtubule-aided growth axially as well as turgor force is still maintained this results in reduced tip extension and a short cell (root hair), or cell distortion as in trichome cells. (C) Disturbance in the microtubular cytoskeleton results in a loss of focused growth. However, movement and delivery of vesicles carrying cell-building material continues along the actin cytoskeleton. In the absence of a clear focus vesicular contents are possibly released at numerous aberrant positions. In tip-growing cells (upper part of figure) this frequently results in the formation of multiple growth apices while diffuse growing cells like trichomes (lower part of figure) swell up tremendously.

analysis of vesicle dynamics and exocytosis at wound sites in *Chara* internodal cells has linked vesicle delivery to rearrangements of fine actin microfilaments [15].

More recently, the movement of Golgi vesicles and membrane-bound organelles such as peroxisomes and mitochondria has been shown to occur along actin microfilaments using dual labeling strategies in living cells ([16] and references therein). This movement ceases upon a drug-induced interference with the actin cytoskeleton [16–18]. It is therefore conceivable that a disturbed actin cytoskeleton also causes reduced and/or misdirected delivery of different vesicles to the cell cortex and ultimately results in growth reduction or cellular distortion (Figure 2B).

As actin drug treated cells show no initial defects in growth directionality the actin cytoskeleton does not appear to be involved directly in establishment or maintenance of growth directionality. On the contrary microtubule defects disturb growth axially without perturbing the actin cytoskeleton-based movement and delivery of vesicles, and result in a different cellular phenotype (Figure 2C).

In summary, the relative roles of microtubules and microfilaments are similar to those in other eukaryotes except that a large percentage of subcellular trafficking in higher plants is actin rather than microtubule-dependent. This suggests that the transport machinery in plants may differ considerably from that in animal cells. Indeed dynein motors and associated proteins appear to be absent in higher plants [19]. There is increasing evidence, however, that the choice of a particular cytoskeletal track for intracellular motility may be subject to conditions of growth and development and that, in both animal and plants, some organelles may switch from one cytoskeletal track to another [20,21].

#### Genetic and Molecular Analysis of the Cytoskeletal Function in Cell Morphogenesis

Compared to animals and yeast, the respective cytoskeletal gene families are much larger in plants allowing perhaps a more complex regulation [22–24]. Whereas the drug-based approach has revealed the general role of microtubules and microfilaments during cell morphogenesis, it does not show how their

spatial arrangement or their function is regulated. Approaches to dissect these processes include the analysis of cell morphogenesis mutants and the biochemical analysis of interacting proteins known from other systems to affect cytoskeletal assembly, dynamics and structural behavior. The recent characterization of many mutants with defects indicative of cytoskeletal malfunction and the cloning of relevant genes has begun to reveal some of the molecular mechanisms involved in shaping plant cells.

### Genetic and Molecular Analysis of the Microtubule Cytoskeleton

Studies in animals and yeast cells have shown that microtubule function is controlled by many proteins at different levels that influence microtubule biogenesis, their dynamics, their association with particular sub-cellular structures and microtubule-specific transport processes. It is the combination of these steps that establishes microtubule spatial organization and specifies cellular responses.

Several genes involved in different aspects of microtubule cytoskeleton regulation have been cloned recently. Microtubule biogenesis involves several proteins that after translation capture  $\alpha$  or  $\beta$  tubulin monomers and facilitate their proper folding and the formation of assembly competent  $\alpha$ - $\beta$  heterodimers [25]. Biochemical and genetic analyses in animals and yeast have isolated several tubulin-folding cofactors (TFCs) and chaperonins involved in microtubule biogenesis [26].

Genes involved in the biogenesis of microtubules in plants have been identified based on a group of embryo lethal mutants that are collectively called *pilz* mutants [27]. Mutations in any of the *PILZ* genes result in one or a few-celled giant embryos [27]. The recent cloning of these genes has shown that they encode for TFC-A (*KIESEL*), TFC-C (*PORCINO*), TFC-D (*CHAMPIGNON*) TFC-E (*PIFFERLING*), and a small GTP-binding protein *ARL2* (*HALLIMASCH/TITAN5*) [28,29] (Table 1).

Weak alleles of *KIS* and *POR* produce viable embryos that develop into short plants with both cell division and expansion defects [30,31]. Microtubule density does not appear to be affected in such plants and it is likely that the phenotypes are due to a disturbed balance of monomers or dimers. These data indicate that the factors involved in the biogenesis of microtubules are conserved between animals and plants, and that their absence affects cell division as well as cell morphogenesis.

The importance of a proper tubulin subunit balance is also evident in experiments where normal levels of one  $\alpha$ -tubulin (*AtTUA6*) are reduced through an antisense approach. *AtTUA6* antisense lines show dramatically swollen root cells and branched root hairs [32]. Mutations affecting overall growth directionality have also been identified. Thus *spiral1* and *spiral2* have a biased right-handed twisting [33] whereas *lefty1* and *lefty2* [34] constitute two of their suppressor mutants. *lefty1* and *lefty2* mutations specifically alter the inter-dimer interface of  $\alpha$ -tubulins *AtTUA6* and *AtTUA4*, respectively, and alter growth directionality

of various cell types [34]. Cortical microtubules in the *lefty* mutants have increased sensitivity to microtubule-disrupting drugs suggesting that reduced microtubule stability can lead to left-handed helical growth in plants [34,35].

Net extension of a given microtubule is regulated by controlling the polymerization and depolymerization rates at its ends [36]. In animals and yeast, the microtubule minus end remains either embedded or in the vicinity of a microtubule organizing center (MTOC) and is thus protected from destabilization. Higher plants lack well-defined MTOCs and the rapid reorganization and reorientation of microtubules in their absence still remains one of the most intriguing areas of plant cytoskeletal research [37].

One way by which new ends, each capable of fresh polymerization and depolymerization, can be generated is by cutting an existing microtubule into smaller segments. This can be achieved by the action of microtubule-severing proteins such as katanins. Katanins are conserved, heterodimeric proteins comprising a p60 subunit that severs microtubules and a p80 subunit required for targeting the protein complex to its intracellular site of action [38]. Mutations in katanin p60 have recently been isolated in *Arabidopsis* [39–41]. Microtubule organization is altered in such mutants – *botero1*, *fat root*, *fragile fibre2*, *ectopic root hair 3* – and leads to multiple cellular defects (Table 1).

Microtubule dynamics and associated processes can also be controlled through selective stabilization of microtubules. A putative stabilizer of microtubules has recently been identified with the cloning of the *MICROTUBULE ORGANIZATION 1* (*MOR1*) gene in *Arabidopsis*. *MOR1* encodes a homologue of the TOGp-XMAP215 class of microtubule-associated proteins that are conserved through the different kingdoms [42]. The *mor1* mutant phenotype is characterized by disorganized cortical microtubules at elevated temperatures suggesting that *MOR1* is important for the stabilization of microtubule arrays [42,43].

Among the large number of kinesins and kinesin-like motor proteins that have been discovered in higher plants only one, *KCBP/ZWICHEL*, has so far been shown to be involved in the control of cell morphogenesis [44–46]. *KCBP/ZWICHEL* is a kinesin bearing a calmodulin-binding domain and acts as a minus-end-directed motor protein in *in vitro* assays [45]. *zwichel* mutants have short, swollen and less branched trichomes, slightly reminiscent of microtubule drug-treated trichome phenotypes [46].

It is still unclear whether *ZWI* is involved in cargo movement or, as suggested by the isolation of a suppressor of *zwichel* (*SUZ2*), whether *ZWI* may play a role in stabilizing microtubule subpopulations required for reorienting microtubules [47]. Motor proteins are known to stabilize and nucleate microtubules [48], and in the absence of bona-fide MTOCs in higher plants the formation of transient, stable microtubular foci may well serve the purpose of reorienting growth directionality.

Microtubule organization is also altered in the *an* (*angustifolia*) mutant [49]. *ANGUSTIFOLIA* is a novel carboxy-terminal binding protein/Brefeldin A



Table 1. Some mutants in higher plants with demonstrated cytoskeleton-linked cell-morphology defects

Mutant (group)	Plant	Major phenotype	Cytoskeletal defect	Gene product	References
<i>angustifolia</i>	A*	Narrow leaf, 2-branched trichomes	CMT arrangement abnormal	CtBP/BARS-like	[49,50]
<i>brick1</i>	Z*	Lobes not formed in epidermal cells stomatal complex, hairs aberrant	MF do not localize to correct position	Novel protein	[52]
<b>distorted</b> group ( <i>ali</i> , <i>crk</i> , <i>dis1</i> , <i>dis2</i> , <i>gr1</i> , <i>klk</i> , <i>spi/sin</i> , <i>wrm</i> )	A	Malformed, fat supine trichomes	MF organization defective	Unknown	[10,11]
<i>ectopic root hairs 3/ botero1/fat root/ fragile fiber2</i>	A	Ectopic root hairs/swollen root/ defective cell wall	Cortical/perinuclear MT organization aberrant	Katanin-p60 like	[39–41]
<i>fass/tonneau2</i>	A	Swollen cells, overall plant morphology	CMT arrangement disturbed Lack pre-prophase band	Novel PP2A regulatory subunit	[65,66]
<i>lefty1/lefty2</i>	A	Shoots, roots twist left	Cortical MT defect	$\alpha$ -tubulin4/ $\alpha$ -tub6	[34]
<i>lilliputian</i>	Z	Miniature seedlings, aberrant root elongation	Cortical MF assembly defective	Unknown	[68]
<i>microtubule organization 1</i>	A	Temperature sensitive dwarf short, swollen cells	CMT organization defective at restrictive temperature	Homolog of TOG-XMAP215 family	[42]
<b>pilz</b> group ( <i>titan1</i> , <i>titan5</i> )	A	Strong alleles, mushroom-shaped embryos	Lack organized MT arrays	Tubulin folding cofactors	[27,29]
<i>champignon/titan1 hallimasch/titan5 pfifferling porcino</i>		Weak allele shows trichome phenotype	Cortical MT arrays defective	TFC-D Arl2 TFC-E TFC-C	[28,29] [28,29] [28] [28,31]
<i>kiesel</i>		Weak allele shows trichome phenotype	Cortical MT arrays defective	TFC-A	[28,30]
<i>spike 1</i>	A	Aberrant leaf, trichome, cotyledon morphology	MT cytoskeleton misregulated	Adapter protein CDM family	[56]
<i>spiral1/spiral2</i>	A	Right-handed helical growth of root, hypocotyl, petiole epidermal cells	Cortical MT defects	Unknown	[33]
<i>yin-yang</i>	O*	Altered epidermal cell length, enhanced sensitivity to actin drugs	Aberrant MF response to auxin	Unknown	[69]
<i>zwichel</i>	A	Short, less branched trichomes	Defects in CMT rearrangement?	Kinesin-like calmodulin binding protein (KCBP)	[44,46]

\*A, *Arabidopsis thaliana*; O, *Oryza sativa*; Z, *Zea mays*. MT, microtubules; CMT, cortical microtubules; MF, microfilaments.

ADP-ribosylated substrate (CtBP/BARS)-related protein that interacts with ZWI in a yeast two-hybrid screen suggesting that the two proteins may act together to organize microtubules [50].

### Molecular Analyses of the Actin Cytoskeleton

Actin filaments (F-actin) are polymers composed of monomeric G-actin. The formation and stability of actin filaments is controlled by factors that affect actin nucleation, actin monomer sequestration, actin polymerization and depolymerization kinetics and in the creation and tightening of actin filament bundles [24,51]. Similar proteins have been found in higher plants though functional studies have not been carried out in most cases [51].

The only putative actin cytoskeleton regulatory gene that has been identified is the *BRICK1* in maize. In *brick1* mutants, the formation of lobes in epidermal cells does not occur [52]. In wild-type maize leaves, lobe formation correlates with an accumulation of actin at the growth site which is also absent in *brick1*

mutants. It is therefore assumed that the novel 8 kDa protein encoded by *BRICK1* is involved in the regulation of actin organization. As *BRICK1* is highly conserved in animals and yeast its further characterization may lead to the understanding of new actin-dependent aspects of cell polarization [52].

Much of our current knowledge of the role of actin-associated proteins in cell morphogenesis is based on over-expression and antisense studies of proteins of the ADF/cofilin and profilin families [53,54]. Transgenic *Arabidopsis* over-expressing ACTIN DEPOLYMERIZATION FACTOR 1 (*AtADF1*) have reduced plant size, fewer actin cables, and aberrant hypocotyl and root hair cell growth. Transgenic plants with reduced levels of *AtADF1* have an increased size with a notable increase in the average length of root hairs [53].

Opposite effects on plant growth are seen with over-expression of *PROFILIN1*; plants have longer roots and root hairs. Reduction of *PROFILIN1* levels to 50% of wild type gives dwarf plants with short roots, nearly isodiametric hypocotyl cells and a rough epidermis

[54]. A similar reduction in PROFILIN1 expression levels was observed in a T-DNA insertion line [55]. However, in contrast to the phenotype displayed by *PROFILIN1*-antisense plants [54], mutants have longer hypocotyls and produce a greater number of longer root hairs [55].

### Signaling to the Cytoskeleton

Little is known about the signal transduction pathways controlling cytoskeletal organization in higher plants. The recent cloning of *SPIKE1* suggests that in plants a conserved mechanism recruits microtubules to intracellular regions through specific adaptor proteins [56]. *SPIKE1* is a 207 kDa protein sharing amino acid similarity with the CDM family of adapter proteins present in many organisms (*Caenorhabditis elegans* CED-5; human DOCK180; *Drosophila* Myoblast city). CDM proteins are believed to integrate extracellular signals and cytoskeleton reorganization [57]. *spike1* mutants have reduced trichome branching and stalk elongation, and seedling viability and polarized growth in cotyledon and leaf epidermal cells is defective. The localized lateral microtubule clustering that often precedes lobe formation in developing epidermal cells does not occur in mutant plants [56].

A second conserved regulatory mechanism is the control of actin cytoskeleton by small GTPases of the Rho family that act as molecular switches in yeast and animals [58]. Rho-like proteins in plants form a distinct phylogenetic group and are termed ROPs (Rho-like proteins of plants). Although ROPs have several unique characteristics they appear to be involved in recruiting actin to active growth regions [58–60]. Cells constitutively expressing the *Arabidopsis* *ROP2* gene have increased levels of fine actin microfilaments [60]. Conversely, the expression of a dominant negative version of the *Arabidopsis* *Rop1* in tobacco pollen tubes and a dominant negative version of *Rop2* in root hairs and in epidermal pavement cells disrupts the accumulation of short actin filaments suggesting that ROPs control the actin cytoskeleton organization [60,61].

It is noteworthy that cellular phenotypes induced by constitutive over-expression of *ROP2* resemble those obtained upon interference with the microtubule cytoskeleton. This may indicate that similar to animals and yeast, Rho-like proteins in plants also interact with microtubules. Possible candidates for this regulation are mDia-like formin-homology proteins [62]. Indeed formin-like proteins are present in *Arabidopsis* though their role in regulating the plant cytoskeleton is not well understood [63,64].

The analysis of *FASS/TONNEAU2* has revealed a novel pathway regulating microtubule organization and cell polarity. Plants mutant for *FASS/TON2* lack the preprophase band, a plant specific, microtubule and actin-containing ring that marks the future cell division plane [65]. Cell divisions that take place in such mutants are random, suggesting a role for *FASS/TON2* in the establishment of cell polarity.

*FASS/TON2* encodes a putative novel protein phosphatase 2A regulatory subunit and is probably involved in the control of the phosphorylation status of proteins [66]. A role for protein phosphorylation

in regulating microtubule cytoskeleton dynamics has been shown using phosphatase inhibitors such as calyculin A and okadaic acid [67]. The specificity of the *fass/ton2* phenotype shows that this gene specifically controls a hitherto undefined process involved in cell polarity establishment via the control of phosphorylation.

### Perspective

The picture emerging from drug studies and molecular and genetic studies in higher plants is that the roles of microtubules and microfilaments are similar in both tip and diffuse growing cells. Microtubules are important for establishing and maintaining growth directionality and focus, whereas microfilaments are required for delivering material to the actual growth sites. Beyond these generalities it remains to be determined how microtubules and the actin cytoskeleton act in concert in higher plants. For example, is it actin that marks a new growth site and recruits microtubules or is it microtubules that respond first to a cue and guide actin microfilaments?

That novel mechanisms are expected to be discovered is evident from various plant-specific phenomena such as the existence of a preprophase band that accurately marks the position of the division plane, the lack of microtubule-organizing centers, the apparent absence of minus end directed dynein motor molecules and the finding that intracellular movement of cell organelles is predominantly actin rather than microtubule-based.

The molecular characterization of morphogenesis mutants such as the *distorted* mutants in *Arabidopsis* [10,11], *lilliputian* in maize [68], and *yin-yang* mutant in rice [69], and the development of new marker lines enabling us to monitor intracellular processes *in vivo* may provide the means to unravel how the plant cytoskeleton controls cell morphogenesis.

### Note Added in Proof

Recent reports provide information on the involvement of microtubules [70,71], and actin cytoskeleton [72] in cellular morphogenesis. Using field emission scanning electron microscopy Burk and Ye [70] present new observations on altered orientation of cellulose microfibril deposition in the katanin mutant *fra2*. They conclude that cortical microtubules play a direct role in determining the direction of cell elongation. Observations on *radially swollen 4* (*rsw4*) and *rsw7* mutants of *Arabidopsis*, however, show that in swollen roots of both mutants, cortical microtubules are neither depleted nor disoriented. As microtubule-microfibril orientation is insufficient for limiting radial expansion, other factors, supplied in part by *RSW4* and *RSW7*, may be necessary for maintaining growth anisotropy [71].

The direct effects of missense mutations in an *Arabidopsis* actin gene (*AtACTIN2*) have been seen in *deformed root hairs1* (*der1*) mutants [72]. Observations of *der1* suggest that *ACTIN2* is involved in selection of the correct site for bulge initiation during early root hair development as well as in later tip growth.

## Acknowledgements

We are grateful to Frantisek Baluska, Anne Mie Emons, Michael Melkonian, Burkhard Becker, Arp Schnittger and Siegfried Roth for their critical comments on the manuscript.

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