

Control of the Actin Cytoskeleton in Plant Cell Growth

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Abstract

Plant cells grow through increases in volume and cell wall surface area. The mature morphology of a plant cell is a product of the differential rates of expansion between neighboring zones of the cell wall during this process. Filamentous actin arrays are associated with plant cell growth, and the activity of actin-binding proteins is proving to be essential for proper cell morphogenesis. Actin-nucleating proteins participate in cell expansion and cell plate formation whereas the recycling of actin monomers is required to maintain actin dynamics and controlled growth. Coordination of actin-binding protein activity and other aspects of cytoskeletal behavior during cell development maintains cohesive cell expansion. Emerging plant signaling networks are proving to be powerful regulators of morphology-shaping cytoskeletal activity, and in this review we highlight current research in actin network regulation.

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INTRODUCTION

Plant cell growth is a coordinated irreversible increase in plasma membrane and cell wall surface area. The building blocks for cell growth are Golgi-derived vesicles, which consist of cell wall matrix materials surrounded by membrane. These Golgi-derived vesicles are delivered to the cellular location where growth occurs, where they fuse with the plasma membrane and deposit their contents into the cell wall. Due to the inability of cell wall matrix to move within the cell wall, growth is limited to sites of exocytosis (51). In plant cells, different types of cell growth have been defined: isodiametrical growth, anisotropic, intercalary or diffuse growth, and tip growth. Isodiametrical-growing cells expand equally over their whole surface, resulting in a ball-shaped cell. Anisotropic expand-

ing cells restrict growth to large but defined areas, creating elongated and sometimes more complicated cell morphologies. Examples of these cell types are leaf pavement cells that form interlocking lobes and trichomes that exhibit several developmental stages where some parts of the cell expand over a large surface area to form complex branching patterns. In tip-growing cells, expansion occurs over a small area of the cell surface, which results in tubular, elongated cells.

The eukaryote actin cytoskeleton plays a pivotal role in many cellular processes that together regulate cell growth and morphology. Specifically in the case of plant cell growth, filamentous actin (F-actin) coordinates cytoplasmic streaming and guides growth materials to zones of exocytosis (88) although not all actin-dependent vesicle trafficking is coupled to growth (74a). In several plant cell types, such as root hairs and leaf pavement cells, evidence is accumulating for dynamic fine F-actin configurations that localize to cell surface areas undergoing expansion (29, 31, 71). Fine F-actin has been hypothesized to deliver, filter, and retain cell wall matrix containing Golgi-derived vesicles to the plasma membrane area where exocytosis occurs (71) and has been demonstrated in some cell types to determine the cell surface area where growth takes place (50). The formation of F-actin arrays depends on the biochemical interactions of actin monomers and actin-binding proteins (ABPs). Studying the role of actin and ABPs in plant cell growth has consequently provided insights into the biochemistry of the plant actin cytoskeleton. In this review we summarize the experiments that have identified the functions of actin during plant cell growth, and describe the genetic and biochemical evidence for the role of plant ABPs in coordinating F-actin formation to achieve cell expansion. Additionally, the genetic and physical interactions of ABPs with regulatory proteins can be placed into a signaling network to describe the plant morphogenetic pathways that lead to the actin cytoskeleton.

Exocytosis: delivery of vesicles and their contents to the external environment via fusion with the plasma membrane

F-actin: filamentous actin (actin polymer)

Cytoplasmic streaming: the active movement of vesicles and organelles through the cytoplasm

ABP: actin-binding protein

DEFINING THE ROLE OF ACTIN IN PLANT CELL GROWTH

A productive method to investigate the function of the actin cytoskeleton in cellular processes and plant development is to depolymerize the actin cytoskeleton and analyze the consequences. There are two classes of commonly used actin-depolymerizing drugs: the latrunculins and the cytochalasins. Latrunculin forms a high-affinity 1:1 complex with monomeric actin [globular actin (G-actin)], preventing incorporation into filamentous actin (19). The lack of available G-actin for polymerization changes the equilibrium between G-actin and F-actin so that F-actin depolymerizes. Cytochalasins inhibit polymerization by capping free barbed ends, thus preventing the addition of G-actin to actin filaments (13), leading to net-depolymerization. In tip-growing pollen tubes and root hair cells, the consequences of treatment with actin-depolymerizing drugs have been carefully analyzed. In both cell types, growth is inhibited when low concentrations of actin-depolymerizing drugs are applied (31, 47, 50, 71). At still lower concentrations of actin-depolymerizing drugs, the expanding apex of tip-growing cells swells. This root hair swelling is concentration dependent to a concentration of 0.1 μM of cytochalasin D (50). Surprisingly, the amount of available data concerning the consequences of actin depolymerization on intercalary cell growth is limited. Plants grown in medium complemented with a high concentration of latrunculin B (10 μM) for two weeks show little alteration in cell division organization, but cell elongation is reduced dramatically (4). A two-day treatment of *Arabidopsis* roots with cytochalasin B causes root cells to swell radially (6).

Observations made using pharmaceuticals can be compared to the phenotypes of actin mutants. Mutations of the *Arabidopsis* *ACT7* actin gene cause a reduction in the total amount of F-actin in vegetative tissue (32). Consequently, germination is delayed and less efficient, and root growth is retarded and wavy. In plants homozygous for the most se-

vere mutant alleles, root apical cells are not organized in straight files with oblique cell-cell junctions (32). Mutations of *ACT2* (*act2-1*; *deformed root hairs* (*der*) 1-1 to 1-3) cause phenotypes restricted to root hair positioning and growth (69, 79). Although overexpression of *ACT2* under its own promoter does not cause any strong defects, overexpression of *ACT1* under the *ACT2* promoter causes dwarfed plants and morphological changes in most organs, which correlates with a strong increase in actin polymerization and bundling (46). These data show that plant actin isoforms must vary in their biochemical properties, and both the expression levels of actin genes and their developmental context are important during plant development to achieve cell expansion.

In trichomes, the effects of actin depolymerization during different developmental stages of individual cell expansion have been studied in detail. Trichomes are structures that extend from the surface of many aerial organs. *Arabidopsis* leaf trichomes are unicellular, highly polarized, and consist of a stalk with an average of three branches. The relatively consistent size and spacing of branches makes leaf trichomes a powerful morphogenetic model. Treatment with cytochalasin or latrunculin disrupts trichome morphogenesis: The first stages of trichome development (stalk elongation and branch initiation) take place normally (66, 86); however, during later developmental stages, actin depolymerization causes branches to swell, twist, or abort. For distortion to occur, the location of exocytosis must be altered by actin depolymerization, not just inhibited (83). This suggests that during certain types of intercalary growth F-actin guides rather than drives the zones of cell expansion.

ACTIN-BINDING PROTEINS IN PLANTS

In all eukaryote cells, F-actin configuration and actin dynamics are determined by the actions of numerous ABPs. The actions of different classes of ABPs regulate aspects

G-actin: globular actin (actin monomer)

Barbed end: An actin polymer has two ends; the barbed (+) end and the pointed (–) end. The barbed end has a higher affinity for monomers and grows at a greater rate

Bundling: the parallel or antiparallel close alignment of individual actin filaments to form an actin cable

Intercalary growth: relatively unfocused insertion of new cell wall material within a defined area of plant cell wall, resulting in “diffuse” growth, as opposed to the highly focused process of tip growth

Nucleation: the thermodynamically unfavorable stage of actin polymerization in which individual monomers form the “seed” of a new filament

Arp: actin-related protein

of actin biochemistry including nucleation, bundling, filament capping, fragmentation, and monomer availability; other ABPs are involved in transport along actin filaments or use actin as a scaffold. Below, we discuss the functions of ABPs in actin organization and plant development.

Actin Nucleators

Actin nucleation is the formation of a new actin filament from G-actin. It can occur spontaneously when the G-actin concentration is high. However, despite high concentrations of G-actin in living cells, G-actin is prevented from nucleating spontaneously by actin-sequestering proteins such as profilin, and chaotic spontaneous nucleation is unlikely to be responsible for F-actin formation *in vivo*. Recently, specialized actin-nucleating proteins were found to play essential roles in actin-dependent plant growth processes.

The Arp2/3 Complex

In animals, protists, and fungi the activated Arp2/3 complex nucleates actin by promoting barbed-end actin assembly while capping the pointed end. The complex attaches itself to the flanks of existing filaments and initiates a new F-actin branch at an angle of 70° relative to the parent filament. The Arp2/3 complex consists of seven subunits [Arp2, Arp3, ArpC1/p41, ArpC2/p31, ArpC3/p21, ArpC4/p20, and ArpC5/p16 (39)]. Homologs of all Arp2/3 complex subunits are present in plants (64), but to date there is no *in vitro* biochemical evidence that a plant Arp2/3 complex nucleates actin filaments in a similar fashion to other eukaryote Arp2/3 complexes. However, components of the *Arabidopsis* Arp2/3 complex can complement mutations of yeast homologs, and vice versa, inferring the existence of a plant complex (25, 59). In addition, mutations in the plant Arp2/3 complex can be complemented with mammalian subunits (65).

Three “distorted” class mutants have been shown to encode subunits of the Arp2/3 com-

plex: *wurm* is a mutant of the Arp2 subunit, *distorted1* is a mutant of the Arp3 subunit, and *crooked* represents the ArpC5/p16 subunit (59, 61, 64, 65). The most dramatic phenotype can be observed in trichomes, which develop a distorted morphology highly similar to that caused by actin-depolymerizing drugs, indicating that these mutant phenotypes are generated by defects in the actin cytoskeleton (66, 86). Besides defects in trichome development, root hair growth under certain conditions is disturbed in Arp2/3 mutants (64, 65). The root hair phenotype varies from wavy growth to a widened diameter to root hairs with multiple tips. Also, less pronounced interlocking of lobes between leaf pavement cells and the curling of hypocotyl epidermal cells during periods of rapid elongation in Arp2/3 mutants have been reported (64, 65).

The changes in actin organization, caused by mutations in Arp2/3 complex subunits, have best been studied in trichomes. Using the GFP-mTalin probe, Mathur et al. (64, 65) show severe, localized aggregations of actin. Using immunolocalization and phalloidin staining, the Szymanski group (25, 59) concludes that the actin filaments failed to localize as coherent populations that are aligned with the long axis of the cell. In addition they show that the amount of actin in the core of trichome branches, compared to the total amount of actin (core and cortical actin together), is reduced in Arp2/3 complex mutants. From these descriptions it can be concluded that the actin organization in Arp2/3 mutant trichomes differs somewhat when alternative actin visualization techniques are used.

A functioning Arp2/3 complex is essential in yeast where deletion of several subunits is lethal (93). The Arp2/3 complex is also essential in *C. elegans* (82) and *Drosophila* (43, 100). In mammalian cells, RNAi inhibition of the Arp2/3 complex inhibits cell growth (37). In contrast, plants only develop several subtle, tissue-specific, developmental defects, as discussed above. Also, the loss of total F-actin content appears to be far less severe in

plants compared to animal and protist cells. This points to a less pronounced role of the Arp2/3 complex in the biochemistry of plant actin nucleation than in other eukaryotic organisms.

Formins

Formins represent a second major group of actin nucleators that stimulate de novo actin nucleation and extension from the barbed end. Paradoxically, fungal and animal formins partially cap the growing barbed end (56, 76, 102), yet in some cases can accelerate monomer incorporation at the barbed end beyond the limits of free diffusion (80).

Unlike the putative plant Arp2/3 complex, plant formins have been studied to some extent in vitro. Four plant formins (AtFH1, AtFH4, AtFH5, and AtFH8) have been shown to nucleate purified actin, and allow extension from the barbed end of filaments (44, 70, 96, 20a). Like other formins, the plant formins appear to bind to the barbed end of F-actin, inhibit actin depolymerization from the barbed end, and partially protect the barbed end from other proteins that otherwise would terminate barbed-end growth. The study of the biochemistry of plant formins is complicated by several factors including the division of plant formins into two large and distinct clades, the absence of recognizable autoinhibition domains that are found in animal and yeast formins, and the direct tethering of group I formins to lipid membranes. The 21 plant formins are divided by sequence similarity and domain organization into groups I and II (20, 21), but only group I formins have been studied in vitro. The divergence between groups I and II extends to the residues predicted to make contact with actin monomers, suggesting that group II formins may have a very distinct biochemistry. Also, few in vitro studies have included longer fragments containing transmembrane domains and putative control regions that might influence the interactions between plant formins and actin.

When overexpressed in tobacco pollen tubes, the actin-nucleating domains of group I formin AtFH1 increase the number of actin cables (18), indicating that group I plant formins can induce actin polymerization in vivo. Pollen tubes transformed with less than 1 µg of full-length *AtFH1* transgene show an initial increase in growth rate followed by subsequent growth inhibition as F-actin cables begin to accumulate (18). In root hairs, overexpression of full-length AtFH8 can induce the accumulation of fine F-actin and the disruption of tip growth (96). Expression of the N terminus of AtFH4 without the actin-nucleating C terminus also disrupts root hair growth (20a). The overexpression of AtFH1, AtFH4, and AtFH8 shows that formins have the potential to affect growth through F-actin formation, but the actual function of most plant formins remains unknown. So far only one isoform, AtFH5, has been reported to have any null phenotype (44). Interestingly, this is a reduction in the rate of cell wall formation, supporting the hypothesis that plant formins within a natural context participate in growth processes.

Gelsolin

A gelsolin-like protein that can nucleate actin polymerization from monomers has been identified in poppy pollen tubes (40). Gelsolin can tightly cap barbed ends in vitro, and only allows extension from the pointed end of filaments (40). The pointed end has distinct biochemical properties to the barbed end, and the action of proteins such as profilin are likely to inhibit pointed-end growth within a plant cell. Gelsolin also severs actin filaments and blocks the assembly of profilin-actin complex onto actin filament ends and enhances profilin-mediated actin depolymerization. The localization and function of gelsolin have not been investigated in vivo.

Heterodimeric Capping Protein

Heterodimeric capping protein binds tightly to the barbed end of actin filaments. Like

AtFH: *Arabidopsis thaliana* formin homolog

Sequestering activity: the ability of some actin-binding proteins to bind G-actin and temporarily remove the monomer from the cytoplasmic pool of “free” actin available for polymerization

ADF: actin depolymerization factor

gelsolin, the barbed-end binding affinity of plant capping protein allows it to act as a nucleator that facilitates pointed-end elongation (41). The elongation rate of filaments in vitro is significantly slowed by a combination of capping protein and profilin, as capping protein blocks barbed-end growth and profilin actin is unable to associate with the pointed end (41). In animals, capping protein dramatically alters F-actin arrays generated by Arp2/3 complex activity (10), and it remains to be seen whether plant capping protein function is required for plant Arp2/3 complex-dependent growth processes.

To date, the number of varieties of actin-nucleating proteins in plants is unknown, and other classes of actin nucleators may yet be identified. F-actin severing proteins can conceivably produce a significant contribution of free F-actin ends for plant actin polymerization. Future work will reveal additional insight into the regulation of free barbed and pointed-end generation in plants and how these processes contribute to plant development.

Other ABPs that Affect Cell Growth: Profilin

Profilin specifically binds G-actin. When bound to profilin, G-actin cannot incorporate at the pointed ends of actin filaments or nucleate, whereas incorporation at the barbed ends continues at the normal rate (75). The sequestering action of proteins like profilin allows cells to maintain high levels of actin monomers without risking spontaneous nucleation or filament extension. In some plant cells actin exists at a 1:1 molar concentration with profilin (31). Because pollen grains are packed with such a high concentration, profilin is a major antigen responsible for pollen allergies. Formins are designed to exploit actin monomers bound to profilin, and the presence of profilin greatly influences formin biochemistry (56, 80). Five isoforms of profilin have been identified in the *Arabidopsis* genome, and the biochemical proper-

ties of profilin vary from isoform to isoform in Maize (54). In pollen tubes and *Tradescantia* stamen hair cells, profilin is evenly distributed throughout the cytoplasm, although some of the profilin accumulates in the nucleus of *Tradescantia* stamen hair cells for unknown reasons (38, 89, 90). Transgenic plants overexpressing PFN-1 have longer roots and root hairs that are twice as long as wild-type hairs (78). An increase in the amount of growth, but no change in cell shape, suggests that profilin does not play a role in spatially restricting actin turnover. It is not clear whether the growth rate or the duration of the growth period is increased in these lines. When expression of profilin was inhibited by antisense RNA expression, a variety of developmental changes were found, including an overall dwarf phenotype with short hypocotyls and early flowering. The dwarf phenotype is caused by the development of shorter and more isodiametrically shaped cells (78). This indicates that a minimum amount of profilin-bound monomeric actin has to be available for proper cell expansion.

ADF/cofilin

ADF/cofilin binds both to G- and F-actin and enhances actin dynamics by severing actin filaments and increasing the depolymerization from the pointed end (14, 35). The activity of plant ADF is influenced by several factors. Phosphorylation of Ser-6 decreases the activity of plant ADF (85) and undoes the localization of overexpressed GFP-ADF to actin filaments (16). In contrast to animals, phosphorylation of plant ADF is regulated by a calmodulin-like domain protein kinase (CDPK) (2, 85). The activity of ADF is also inhibited by phosphatidylinositol 4,5-bisphosphate (PIP₂) or phosphatidylinositol 4-monophosphate (PIP) binding (35). Finally, the activity of ADF is pH dependent. At high pH (8.0), ADF severs actin filaments, whereas it binds F-actin at a lower pH (6.0) (36). Dong et al. (23) tested the consequences of overexpression and inhibition of the ADF1 gene

in *Arabidopsis*. Overexpression of ADF causes irregular cellular and tissue morphogenesis and reduces the growth of cells and organs (23). In contrast, ADF inhibition results in a delay in flowering and stimulated cell expansion, as well as organ growth (23). The actin cytoskeleton was visualized with the live cell actin probe GFP-mTalin. When ADF1 was overexpressed, this revealed the disappearance of thick actin cables in different cell types. ADF1 inhibition caused an induction of actin cable formation. Although it should be kept in mind that GFP-mTalin has been shown to compete for binding places on F-actin with ADF and thus inhibits the activity of the latter (49), it is likely that an increase in actin polymerization when ADF is inhibited and a decrease in actin polymerization when ADF is overexpressed occur.

In tip-growing cells, ADF plays an important role in regulating actin dynamics. In root hairs, ADF overexpression leads to a highly irregular F-actin organization and the disappearance of thick bundles of actin, resulting in an increase in the radial root hair diameter, whereas underexpression inhibits root hair growth (23). In pollen tubes, a different response is observed: Overexpression of ADF inhibits pollen tube growth in a dose-dependent manner (16). Differences between the biochemical properties of pollen and vegetative ADFs have been found, which may cause these contrasting responses (1).

AIP1

Plant Actin Interacting Protein 1 (AIP1), a protein also conserved in yeast and animals, enhances the activity of the lily pollen-specific ADF1 in vitro in a synergistic manner (1). The activity of this ADF isoform in the absence of AIP1 is remarkably low, and it is not phosphorylated, even though the conserved Ser-6 is present (1). When at an equimolar concentration, AIP1 enhances the activity of LiADF1 in vitro by nearly three times (1). Upon the expression of AIP1 RNAi species and the subsequent reduction of endogenous

AIP1 protein levels, *Arabidopsis* leaves, roots, shoots, and root hairs fail to expand normally (48). These cell expansion defects are fatal in lines where AIP1 expression is inhibited strongly. The actin organization in intercalary growing cells and root hairs is severely disrupted. Thick bundles of actin appear in the cytoplasm of intercalary growing cells, and (unlike control root hairs) F-actin cables are observed in the root hair tip (48). If AIP1 solely enhances the activity of ADF, a similar phenotype would be expected in ADF-inhibited plants (see previous section), but this is not the case. Even though actin bundling has been reported in both situations (23, 48), ADF inhibition stimulates cell expansion and organ growth whereas AIP1 underexpression inhibits these processes. Further characterization of the ADF-AIP1 biochemical relationship is required to understand these phenotypic contrasts. The possibility exists that the functions of plant AIP1 may extend beyond the stimulation of ADF activity.

CAP

In yeast, Cyclase Associated Protein (CAP) is a subunit of the cAMP-generating adenylyl cyclase complex. CAP interacts with the actin cytoskeleton in many eukaryotic species and inhibits actin polymerization in vitro by sequestering monomeric actin (27, 33). In *Arabidopsis*, a CAP homolog was successfully used to pull down actin and vice versa from cytoplasmic extracts (5), which might indicate a direct interaction with plant actin. Overexpression of the *Arabidopsis* CAP homolog resulted in a lower level of fluorescence in Bright Yellow 2 (BY-2) Tobacco tissue culture cells stained with fluorescent phalloidin, from which the authors conclude that CAP induces actin depolymerization. BY-2 cells overexpressing AtCAP are inhibited from entering mitosis. Overexpression of CAP in *Arabidopsis* induces growth defects such as size reduction of leaves and petioles caused by decreased cell size and cell number. *Arabidopsis* plants overexpressing profilin isoform PFN1 do not show

AIP1: actin interacting protein 1

CAP: cyclase associated protein

ROP: Rho of Plants

Endocytosis: the internalization and recycling of vesicles from the plasma membrane

growth inhibition, but instead some cell types show excessive expansion (78). Therefore, the actin-sequestering role of AtCAP cannot be entirely equivalent to that of profilin. Recent work has shown that the *S. cerevisiae* homolog of CAP prevents actin monomer addition to the barbed end of F-actin whereas profilin prevents monomer addition to the pointed end (67). *S. cerevisiae* CAP also appears to enhance actin turnover mediated by ADF and profilin (3). Plant CAP is therefore likely to play a unique biochemical role in cytoskeletal organization and plant development.

Actin-Bundling Proteins

Plants possess at least three classes of actin-bundling proteins: villins (42, 91, 99), fimbrins (55, 57, 68), and elongation factor-1 α (36). Villin bundles actin filaments in a unipolar fashion (97) and localizes to actin cables in pollen tubes (91, 98) and root hairs (87). In root hairs, microinjection of an antibody raised against villin causes unbundling of F-actin (52, 87) and migration of the nucleus toward the apex of growing root hairs (52). Although villin-mediated actin bundling reinforces F-actin against depolymerization by ADF, plant villins do not appear to possess other activities of proteins from the villin/gelsolin family (these include actin nucleation, capping, depolymerization, and filament severing) (42). The activity of plant villin isoforms can either be sensitive (99) or insensitive (42) to the concentration of calcium ions.

Fimbrins are actin filament cross-linkers that are calcium concentration independent (57), localize to actin filaments (55), and protect actin filaments against profilin-induced depolymerization (57). Fimbrin has two actin-binding domains. The second domain, fused to GFP, is used for in vivo visualization of the actin cytoskeleton in plant cells (48, 84, 92).

Elongation Factor-1 α (EF-1 α) is a protein with a dual function. It binds aminoacyl-tRNA to the ribosome, but it also binds to actin and bundles it, while inhibiting incorpo-

ration of monomeric actin at low pH (36). The activity of EF-1 α is enhanced by ADF (36). Lopez-Valenzuela et al. (63a) show that during maize endosperm development, the actin-bundling properties of EF-1 α differ.

Actin-bundling activity is shared by several different families of plant ABPs, but much remains to be discovered concerning the functions of actin-bundling proteins in vivo and their importance during plant development.

SIGNALING TO ACTIN

A striking example of the coordinated power of ABPs comes from the in vitro reconstitution of actin-based motility. This requires the unpolarized biochemical activity of profilin and ADF to maintain a pool of free G-actin, and the polar stimulation of the Arp2/3 complex to produce localized F-actin (74). In a plant cell the formation of F-actin arrays must require the coordinated activation and/or repression of a variety of ABPs in time and space. Recent developments have begun to identify signaling systems that orchestrate this activity in developing plant cells (see **Figure 1**). The function of small GTPases in cytoskeletal control is currently receiving wide attention.

ROPs

In tip-growing cells and leaf epidermal pavement cells, Rho of Plants (ROP) GTPases are involved in regulating cell expansion and localize to sites of tip growth and intercalary growth (15, 17, 29, 45, 72). In addition, ROPs localize to developing cell plates and cross walls (72) and to Golgi bodies (17), as well as participate in endocytosis (11). The animal, protist, and fungal homologs of ROPs (the RHOs, RACs, and CDC42) are major regulators of the actin cytoskeleton.

Overexpression of constitutively active ROP (CA-rop) leads to the production of root hairs with multiple tips and isodiametric swelling. Dominant negative forms of ROP (DN-rop) cause inhibition of cell growth (45, 72). In both cases, changes in the actin

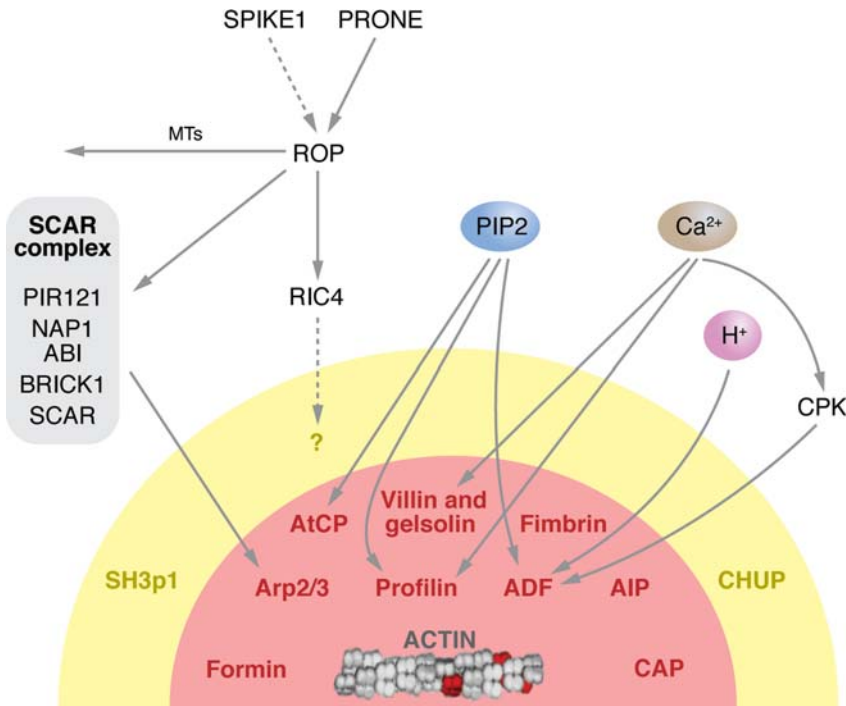


Figure 1

Major known control pathways to the plant actin cytoskeleton. Proteins within the red zone have been proven to alter actin biochemistry directly whereas ABPs within the yellow zone (CHUP and SH3p1) bind actin but with unknown effects (58, 73). SPIKE1 (77) and the PRONE family (9) are ROP GTPase exchange factors with the potential to stimulate ROP activity.

configuration correlate with defects in cell growth. Root hairs expressing DN-rop2 have a reduced amount of fine F-actin in comparison to wild-type hairs, whereas in hairs expressing CA-rop2 a dense network of fine F-actin is present (45). Molendijk et al. (72) show that the polar ROP signaling occurs before root hair growth initiates, indicating that ROPs may serve as a polarity marker. Once root hair growth is initiated, ROPs localize to the expanding root hair tip. Changes in the actin cytoskeleton and cell expansion have also been reported in pollen tubes when ROPs are overexpressed (17, 30). Excessive fine F-actin accumulates in the apical region and a thick band of transverse F-actin is formed in the subapex, which correlates with a switch from polar growth to isodiametrical swelling (30). Overexpression of GTPase-activating protein (RopGAP1) (30), and injection of antibodies against Rop1 (63), caused a similar decrease in the amount of apical fine F-actin and growth inhibition. During the early expansion phase of leaf pavement cells, the location of fine F-

actin formation and cell expansion are determined by ROP proteins. Expression of CA-rop2 leads to expansion over the whole cell surface, which correlates with the formation of cortical fine F-actin over the whole cell surface. In contrast, expression of DN-rop2 leads to inhibition of growth and a decrease in the amount of cortical fine F-actin (29). These observations resemble observations made in root hairs and pollen tubes: At the membrane surface where expansion takes place, ROPs are activated and fine F-actin is formed.

RICs: Effectors of ROP

RICs are CRIB motif-containing proteins that interact with the active (GTP-bound) form of ROP isoforms (94). Different classes of RICs have been identified that activate antagonizing pathways in pollen tube and leaf pavement cell growth. In pollen tubes, Rop1 interacts directly with Ric3 and Ric4 (34, 94). Both Ric3 and Ric4 cause growth depolarization when overexpressed (34, 94). Ric4

RIC: ROP interacting CRIB motif protein

CRIB: Cdc42/Rac interactive binding protein

stimulates the formation of F-actin in the pollen tube apex (34). Ric3 overexpression leads to an increased cytoplasmic calcium concentration in the apex of growing pollen tubes that disassembles fine F-actin (34). Combined overexpression of both Ric3 and Ric4 does not cause changes in pollen tube growth, indicating that the balance between Ric3 and Ric4 activity is critical (34).

In leaf epidermal cells, a similar mechanism that requires balanced levels of two counteracting Rics is essential for the intercalation of adjoining cells (28). Leaf epidermal cells adhere together by forming a series of interlocking lobes. GTP-bound Rop2 and Rop4 activate Ric4, which in turn induces local assembly of cortical F-actin in zones of growth that become lobes (28). Simultaneously, Rop2 and Rop4 inhibit Ric1 (28). Ric1 promotes the formation of organized cortical microtubules and in turn inhibits the formation of a growing lobe and Rop2/4 activation. This interrelationship between actin, microtubules, and the cell wall during plant cell expansion has been recently reviewed by Smith and Oppenheimer (85a). Through the local changes in Rop2/4 activity, indentations and outgrowths are formed by the two counteracting pathways of Ric1 and Ric4 (28).

The components of the ROP pathway downstream of Ric4 that effect changes to the actin cytoskeleton remain to be identified, but this pathway appears to be a major component in the regulation of localized growth.

The SCAR Complex

The SCAR complex is an effector of Rac cytoskeletal reorganization in animals and protists, and recent work has identified homologs of SCAR complex components in plants (7, 8, 12, 22, 24, 62, 81, 101, 103). Yeast-2-hybrid assays and in vitro pull-down experiments have demonstrated binary interactions between these plant protein homologs that are equivalent to those characterized in the mammalian complex (7, 8, 24, 26, 101). One member of the putative plant complex, PIR121,

binds the active form of ROP2 (7) whereas plant SCAR, another component, can activate the Arp2/3 complex in vitro and bind G-actin (8, 22, 26). Null mutants of components PIR121 and NAP1 phenocopy the knockouts of the Arp2/3 complex, showing almost an identical distortion of trichomes and other epidermal cell types. This indicates that the SCAR complex is required to activate the Arp2/3 complex. To date, mutant alleles of only one isoform of *Arabidopsis* SCAR (SCAR2) have been found to exhibit a phenotype (8, 101). This again resembles that of other Arp2/3 pathway knockouts, but is less severe, indicating that other SCAR isoforms or other classes of Arp2/3 regulators may perform similar functions to SCAR2 via the SCAR complex.

CONCLUDING REMARKS

Accumulating evidence points toward the importance of a subpopulation of dynamic F-actin in growth processes. The morphogenesis of different cell types appears reliant on the activities of different subsets of ABPs: Pollen growth is insensitive to Arp2/3 mutations but is sensitive to manipulation of profilin or ADF, whereas trichome growth is so dependent on Arp2/3 activity that Arp2/3 null mutants resemble the effects of total actin depolymerization. Many plant ABPs have been studied in biochemical assays in vitro, but their precise role in plant growth is uncharacterized. Very fundamental questions concerning how actin influences growth remain to be answered: How do actin filaments guide vesicles? Do polymerizing actin filaments exert forces against membranes, and if so, which ones? How much of the influence of actin filaments on growth is through the organization of other systems such as the microtubule cytoskeleton?

Some of these questions might be answered in part by discovering how and where ABPs are manipulated within a plant cell. The recent developments in the understanding of ROP effectors has added support to

the role of ROPs in signaling to the plant cytoskeleton. Activated ROP localizes to zones of growth and F-actin dynamics. One intact pathway from GTPase signaling to F-actin formation is beginning to emerge from the plant SCAR complex, although the biological significance of the ROP2-PIR121 interaction remains to be proven. Still to be considered are the actions of other signal-

ing systems such as phospholipids and calcium ions. Both affect the activities of multiple ABPs, and it has been suggested that both systems are downstream of ROP signaling (53, 60). However, lessons from the study of animal signaling pathways to the cytoskeleton show that ABP control is often dependent upon multiple pathways with extensive cross talk and self-regulation.

SUMMARY POINTS

1. In plant cells, growth is limited to sites of exocytosis and coincides with local fine F-actin arrays.
2. Actin and the Arp2/3 complex govern the locations of cell growth in *Arabidopsis* trichome cells, but unlike other eukaryotes, the plant Arp2/3 complex is not essential for life.
3. Plant formins nucleate F-actin that grows from the barbed end in vitro and are involved in plant growth processes in vivo.
4. Capping protein and gelsolin also have the potential to nucleate filaments, but these grow from the pointed end only.
5. Profilin prevents spontaneous nucleation and chaotic polymerization by sequestering G-actin, whereas capping protein potentially controls the availability of growing F-actin barbed ends.
6. Recycling actin monomers through the actions of ADF and AIP1 maintains actin dynamics and has complex effects on plant growth.
7. ROP GTPases promise to be the focus of a signaling network that controls multiple cytoskeletal processes, including F-actin formation and cell morphogenesis.
8. RIC proteins are ROP effectors that act differentially to regulate actin and microtubules during cell morphogenesis.
9. The plant SCAR complex regulates the Arp2/3 complex and is possibly a ROP effector.

LITERATURE CITED

1. Allwood EG, Anthony RG, Smertenko AP, Reichelt S, Drobak BK, et al. 2002. Regulation of the pollen-specific actin-depolymerizing factor LIADF1. *Plant Cell* 14:2915–27
2. Allwood EG, Smertenko AP, Hussey PJ. 2001. Phosphorylation of plant actin-depolymerising factor by calmodulin-like domain protein kinase. *FEBS Lett.* 499:97–100
3. Balcer HI, Goodman AL, Rodal AA, Smith E, Kugler J, et al. 2003. Coordinated regulation of actin filament turnover by a high-molecular-weight Srv2/CAP complex, cofilin, profilin, and Aip1. *Curr. Biol.* 13:2159–69
4. Baluska F, Jasik J, Edelmann HG, Salajová T, Volkmann D. 2001. Latrunculin B-induced plant dwarfism: Plant cell elongation is F-actin-dependent. *Dev. Biol.* 231:113–24
5. Barrero RA, Umeda M, Yamamura S, Uchimiya H. 2002. Arabidopsis CAP regulates the actin cytoskeleton necessary for plant cell elongation and division. *Plant Cell* 14:149–63

6. Baskin TI, Bivens NJ. 1995. Stimulation of radial expansion in Arabidopsis roots by inhibitors of actomyosin and vesicle secretion but not by various inhibitors of metabolism. *Planta* 197:514–21
7. Basu D, El-Assal Sel D, Le J, Mallery EL, Szymanski DB. 2004. Interchangeable functions of Arabidopsis PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development. *Development* 131:4345–55
8. Basu D, Le J, El-Essal Sel D, Huang S, Zhang C, et al. 2005. DISTORTED3/SCAR2 is a putative Arabidopsis WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis. *Plant Cell* 17:502–24
9. Berken A, Thomas C, Wittinghofer A. 2005. A new family of RhoGEFs activates the Rop molecular switch in plants. *Nature* 436:1176–80
10. Blanchoin L, Amann KJ, Higgs HN, Marchand JB, Kaiser DA, Pollard TD. 2000. Direct observation of dendritic actin filament networks nucleated by Arp2/3 complex and WASP/Scar proteins. *Nature* 404:1007–11
11. Bloch D, Lavy M, Efrat Y, Efroni I, Bracha-Drori K, et al. 2005. Ectopic expression of an activated RAC in Arabidopsis disrupts membrane cycling. *Mol. Biol. Cell* 16:1913–27
12. Brembu T, Winge P, Seem M, Bones AM. 2004. NAPP and PIRP encode subunits of a putative wave regulatory protein complex involved in plant cell morphogenesis. *Plant Cell* 16:2335–49
13. Brown SS, Spudich JA. 1979. Cytochalasin inhibits the rate of elongation of actin filament fragments. *J. Cell Biol.* 83:657–62
14. Carlier MF, Laurent V, Santolini J, Melki R, Didry D, et al. 1997. Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: implication in actin-based motility. *J. Cell Biol.* 136:1307–22
15. Chen CY, Cheung AY, Wu HM. 2003. Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth. *Plant Cell* 15:237–49
16. Chen CY, Wong EI, Vidali L, Estavillo A, Hepler PK, et al. 2002. The regulation of actin organization by actin-depolymerizing factor in elongating pollen tubes. *Plant Cell* 14:2175–90
17. Cheung AY, Chen CY, Tao LZ, Andreyeva T, Twell D, Wu HM. 2003. Regulation of pollen tube growth by Rac-like GTPases. *J. Exp. Bot.* 54:73–81
18. Cheung AY, Wu HM. 2004. Overexpression of an Arabidopsis formin stimulates supernumerary actin cable formation from pollen tube cell membrane. *Plant Cell* 16:257–69
19. Coué M, Brenner SL, Spector I, Korn ED. 1987. Inhibition of actin polymerization by latrunculin A. *FEBS Lett.* 213:316–18
20. Cvrckova F, Novotny M, Pickova D, Zarsky V. 2004. Formin homology 2 domains occur in multiple contexts in angiosperms. *BMC Genomics* 5:44
- 20a. Deeks MJ, Cvrckova F, Machesky LM, Mikitova V, Ketelaar T, et al. 2005. Arabidopsis group Ie formins localize to specific cell membrane domains, interact with actin-binding proteins and cause defects in cell expansion upon aberrant expression. *New Phytol.* 168:529–40
21. Deeks MJ, Hussey PJ, Davies B. 2002. Formins: intermediates in signal-transduction cascades that affect cytoskeletal reorganization. *Trends Plant Sci.* 7:492–98
22. Deeks MJ, Kaloriti D, Davies B, Malho R, Hussey PJ. 2004. Arabidopsis NAP1 is essential for Arp2/3-dependent trichome morphogenesis. *Curr. Biol.* 14:1410–14
23. Dong CH, Xia GX, Hong Y, Ramachandran S, Kost B, Chua NH. 2001. ADF proteins are involved in the control of flowering and regulate F-actin organization, cell expansion, and organ growth in Arabidopsis. *Plant Cell* 13:1333–46

24. El-Assal Sel D, Le J, Basu D, Mallery EL, Szymanski DB. 2004. Arabidopsis GNARLED encodes a NAP125 homolog that positively regulates ARP2/3. *Curr. Biol.* 14:1405–9
25. El-Din El-Assal S, Le J, Basu D, Mallery EL, Szymanski DB. 2004. DISTORTED2 encodes an ARPC2 subunit of the putative Arabidopsis ARP2/3 complex. *Plant J.* 38:526–38
26. Frank M, Egile C, Dyachok J, Djakovic S, Nolasco M, et al. 2004. Activation of Arp2/3 complex-dependent actin polymerization by plant proteins distantly related to Scar/WAVE. *Proc. Natl. Acad. Sci. USA* 101:16379–84
27. Freeman NL, Chen Z, Horenstein J, Weber A, Field J. 1995. An actin monomer binding activity localizes to the carboxyl-terminal half of the *Saccharomyces cerevisiae* cyclase-associated protein. *J. Biol. Chem.* 270:5680–85
28. Fu Y, Gu Y, Zheng Z, Wasteneys G, Yang Z. 2005. Arabidopsis interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis. *Cell* 120:687–700
29. Fu Y, Li H, Yang Z. 2002. The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during Arabidopsis organogenesis. *Plant Cell* 14:777–94
30. Fu Y, Wu G, Yang Z. 2001. Rop GTPase-dependent dynamics of tip-localized F-actin controls tip growth in pollen tubes. *J. Cell Biol.* 152:1019–32
31. Gibbon BC, Kovar DR, Staiger CJ. 1999. Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell* 11:2349–63
32. Gilliland LU, Pawloski LC, Kandasamy MK, Meagher RB. 2003. Arabidopsis actin gene ACT7 plays an essential role in germination and root growth. *Plant J.* 33:319–28
33. Gottwald U, Brokamp R, Karakesisoglou I, Schleicher M, Noegel AA. 1996. Identification of a cyclase-associated protein (CAP) homologue in *Dictyostelium discoideum* and characterization of its interaction with actin. *Mol. Biol. Cell* 7:261–72
34. Gu Y, Fu Y, Dowd P, Li S, Vernoud V, et al. 2005. A Rho family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes. *J. Cell Biol.* 169:127–38
35. Gungabissoon RA, Jiang CJ, Drobak BK. 1998. Interaction of maize actin-depolymerising factor with actin and phosphoinositides and its inhibition of plant phospholipase C. *Plant J.* 16:689–96
36. Gungabissoon RA, Khan S, Hussey PJ. 2001. Interaction of elongation factor 1 alpha from *Zea mays* (ZmEF-1 alpha) with F-actin and interplay with the maize actin severing protein, ZmADF3. *Cell Motil. Cytoskel.* 49:104–11
37. Harborth J, Elbashir SM, Bechert K, Tuschl T, Weber K. 2001. Identification of essential genes in cultured mammalian cells using small interfering RNAs. *J. Cell Sci.* 114:4557–65
38. Hepler PK, Vidali L, Cheung AY. 2001. Polarized cell growth in higher plants. *Annu. Rev. Cell Dev. Biol.* 17:159–87
39. Higgs HN, Pollard TD. 2001. Regulation of actin filament network formation through ARP2/3 complex: activation by a diverse array of proteins. *Annu. Rev. Biochem.* 70:649–76
40. Huang S, Blanchoin L, Chaudhry F, Franklin-Tong VE, Staiger CJ. 2004. A gelsolin-like protein from *Papaver rhoeas* pollen (PrABP80) stimulates calcium-regulated severing and depolymerization of actin filaments. *J. Biol. Chem.* 279:23364–75
41. Huang S, Blanchoin L, Kovar DR, Staiger CJ. 2003. Arabidopsis capping protein (AtCP) is a heterodimer that regulates assembly at the barbed ends of actin filaments. *J. Biol. Chem.* 278:44832–42

42. Huang S, Robinson RC, Gao LY, Matsumoto T, Brunet A, et al. 2005. Arabidopsis VILLIN1 generates actin filament cables that are resistant to depolymerization. *Plant Cell* 17:486–501
43. Hudson AM, Cooley L. 2002. A subset of dynamic actin rearrangements in *Drosophila* requires the Arp2/3 complex. *J. Cell Biol* 156:677–87
44. Ingouff M, Fitz Gerald JN, Guerin C, Robert H, Sorensen MB, et al. 2005. Plant formin AtFH5 is an evolutionarily conserved actin nucleator involved in cytokinesis. *Nat. Cell Biol.* 7:374–80
45. Jones MA, Shen JJ, Fu Y, Li H, Yang Z, Grierson CS. 2002. The Arabidopsis Rop2 GTPase is a positive regulator of both root hair initiation and tip growth. *Plant Cell* 14:763–76
46. Kandasamy MK, McKinney EC, Meagher RB. 2002. Functional nonequivalency of actin isovariants in Arabidopsis. *Mol. Biol. Cell* 13:251–61
47. Ketelaar T. 2002. *Spatial Organisation of Cell Expansion by the Cytoskeleton*. Wageningen: Wageningen Univ.
48. Ketelaar T, Allwood EG, Anthony R, Voigt B, Menzel D, Hussey PJ. 2004. The actin-interacting protein AIP1 is essential for actin organization and plant development. *Curr. Biol.* 14:145–49
49. Ketelaar T, Anthony RG, Hussey PJ. 2004. Green fluorescent protein-mTalin causes defects in actin organization and cell expansion in Arabidopsis and inhibits actin depolymerizing factor's actin depolymerizing activity in vitro. *Plant Physiol.* 136:3990–98
50. Ketelaar T, de Ruijter NC, Emons AM. 2003. Unstable F-actin specifies the area and microtubule direction of cell expansion in Arabidopsis root hairs. *Plant Cell* 15:285–92
51. Ketelaar T, Emons AM. 2001. The cytoskeleton in plant cell growth: lessons from root hairs. *New Phytol.* 152:409–18
52. Ketelaar T, Faivre-Moskalenko C, Esseling JJ, de Ruijter NC, Grierson CS, et al. 2002. Positioning of nuclei in Arabidopsis root hairs: an actin-regulated process of tip growth. *Plant Cell* 14:2941–55
53. Kost B, Lemichez E, Spielhofer P, Hong Y, Tolia K, et al. 1999. Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J. Cell Biol.* 145:317–30
54. Kovar DR, Drobak BK, Staiger CJ. 2000. Maize profilin isoforms are functionally distinct. *Plant Cell* 12:583–98
55. Kovar DR, Gibbon BC, McCurdy DW, Staiger CJ. 2001. Fluorescently-labeled fimbrin decorates a dynamic actin filament network in live plant cells. *Planta* 213:390–95
56. Kovar DR, Kuhn JR, Tichy AL, Pollard TD. 2003. The fission yeast cytokinesis formin Cdc12p is a barbed end actin filament capping protein gated by profilin. *J. Cell Biol.* 161:875–87
57. Kovar DR, Staiger CJ, Weaver EA, McCurdy DW. 2000. AtFim1 is an actin filament crosslinking protein from Arabidopsis thaliana. *Plant J.* 24:625–36
58. Lam BC, Sage TL, Bianchi F, Blumwald E. 2001. Role of SH3 domain-containing proteins in clathrin-mediated vesicle trafficking in Arabidopsis. *Plant Cell* 13:2499–512
59. Le J, El-Assal Sel D, Basu D, Saad ME, Szymanski DB. 2003. Requirements for Arabidopsis ATARP2 and ATARP3 during epidermal development. *Curr. Biol.* 13:1341–47
60. Li H, Lin Y, Heath RM, Zhu MX, Yang Z. 1999. Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell* 11:1731–42
61. Li S, Blanchoin L, Yang Z, Lord EM. 2003. The putative Arabidopsis arp2/3 complex controls leaf cell morphogenesis. *Plant Physiol.* 132:2034–44

62. Li Y, Sorefan K, Hemmann G, Bevan MW. 2004. Arabidopsis NAP and PIR regulate actin-based cell morphogenesis and multiple developmental processes. *Plant Physiol.* 136:3616–27
63. Lin Y, Yang Z. 1997. Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth. *Plant Cell* 9:1647–59
- 63a. Lopez-Valenzuela JA, Gibbon BC, Hughes PA, Dreher TW, Larkins BA. 2003. eEF1A isoforms change in abundance and actin-binding activity during maize endosperm development. *Plant Physiol.* 133:1285–95
64. Mathur J, Mathur N, Kernebeck B, Hulskamp M. 2003. Mutations in actin-related proteins 2 and 3 affect cell shape development in Arabidopsis. *Plant Cell* 15:1632–45
65. Mathur J, Mathur N, Kirik V, Kernebeck B, Srinivas BP, Hulskamp M. 2003. Arabidopsis CROOKED encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation. *Development* 130:3137–46
66. Mathur J, Spielhofer P, Kost B, Chua N. 1999. The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in *Arabidopsis thaliana*. *Development* 126:5559–68
67. Mattila PK, Quintero-Monzon O, Kugler J, Moseley JB, Almo SC, et al. 2004. A high-affinity interaction with ADP-actin monomers underlies the mechanism and in vivo function of Srv2/cyclase-associated protein. *Mol. Biol. Cell* 15:5158–71
68. McCurdy DW, Kim M. 1998. Molecular cloning of a novel fimbrin-like cDNA from *Arabidopsis thaliana*. *Plant Mol. Biol.* 36:23–31
69. McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, et al. 1995. Sequence-based identification of T-DNA insertion mutations in Arabidopsis: actin mutants act2-1 and act4-1. *Plant J.* 8:613–22
70. Michelot A, Guerin C, Huang S, Ingouff M, Richard S, et al. 2005. The formin homology 1 domain modulates the actin nucleation and bundling activity of Arabidopsis FORMIN1. *Plant Cell* 17:2296–13
71. Miller DD, de Ruijter NCA, Bisseling T, Emons AMC. 1999. The role of actin in root hair morphogenesis: studies with lipochito-oligosaccharide as a growth stimulator and cytochalasin as an actin perturbing drug. *Plant J.* 17:141–54
72. Molendijk AJ, Bischoff F, Rajendrakumar CS, Friml J, Braun M, et al. 2001. *Arabidopsis thaliana* Rop GTPases are localized to tips of root hairs and control polar growth. *EMBO J.* 20:2779–88
73. Oikawa K, Kasahara M, Kiyosue T, Kagawa T, Suetsugu N, et al. 2003. Chloroplast unusual positioning1 is essential for proper chloroplast positioning. *Plant Cell* 15:2805–15
74. Pantaloni D, Le Clainche C, Carlier MF. 2001. Mechanism of actin-based motility. *Science* 292:1502–6
- 74a. Parton RM, Fischer-Parton S, Trewavas AJ, Watahiki MK. 2003. Pollen tubes exhibit regular periodic membrane trafficking events in the absence of apical extension. *J. Cell Sci.* 116:2707–19
75. Pollard TD, Blanchoin L, Mullins RD. 2000. Molecular mechanisms controlling actin filament dynamics in nonmuscle cells. *Annu. Rev. Biophys. Biomol. Struct.* 29:545–76
76. Pruyne D, Evangelista M, Yang C, Bi E, Zigmund S, et al. 2002. Role of formins in actin assembly: nucleation and barbed-end association. *Science* 297:612–15
77. Qiu JL, Jilk R, Marks MD, Szymanski D. 2002. The *Arabidopsis* SPIKE1 gene is required for normal cell shape control and tissue development. *Plant Cell* 14:101–18

78. Ramachandran S, Christensen HE, Ishimaru Y, Dong CH, Chao-Ming W, et al. 2000. Profilin plays a role in cell elongation, cell shape maintenance, and flowering in *Arabidopsis*. *Plant Physiol.* 124:1637–47
79. Ringli C, Baumberger N, Diet A, Frey B, Keller B. 2002. ACTIN2 is essential for bulge site selection and tip growth during root hair development of *Arabidopsis*. *Plant Physiol.* 129:1464–72
80. Romero S, Le Clainche C, Didry D, Egile C, Pantaloni D, Carlier MF. 2004. Formin is a processive motor that requires profilin to accelerate actin assembly and associated ATP hydrolysis. *Cell* 119:419–29
81. Saedler R, Zimmermann I, Mutondo M, Hulskamp M. 2004. The *Arabidopsis* *KLUNKER* gene controls cell shape changes and encodes the AtSRA1 homolog. *Plant Mol. Biol.* 56:775–82
82. Sawa M, Suetsugu S, Sugimoto A, Miki H, Yamamoto M, Takenawa T. 2003. Essential role of the *C. elegans* Arp2/3 complex in cell migration during ventral enclosure. *J. Cell Sci.* 116:1505–18
83. Schwab B, Mathur J, Saedler R, Schwarz H, Frey B, et al. 2003. Regulation of cell expansion by the *DISTORTED* genes in *Arabidopsis thaliana*: actin controls the spatial organization of microtubules. *Mol. Genet. Genomics* 269:350–60
84. Sheahan MB, Staiger CJ, Rose RJ, McCurdy DW. 2004. A green fluorescent protein fusion to actin-binding domain 2 of *Arabidopsis* fimbrin highlights new features of a dynamic actin cytoskeleton in live plant cells. *Plant Physiol.* 136:3968–78
85. Smertenko AP, Jiang CJ, Simmons NJ, Weeds AG, Davies DR, Hussey PJ. 1998. Ser6 in the maize actin-depolymerizing factor, ZmADF3, is phosphorylated by a calcium-stimulated protein kinase and is essential for the control of functional activity. *Plant J.* 14:187–93
- 85a. Smith LG, Oppenheimer DG. 2005. Spatial control of cell expansion by the plant cytoskeleton. *Annu. Rev. Cell Dev. Biol.* 21:271–95
86. Szymanski DB, Marks MD, Wick SM. 1999. Organized F-actin is essential for normal trichome morphogenesis in *Arabidopsis*. *Plant Cell* 11:2331–47
87. Tominaga M, Yokota E, Vidali L, Sonobe S, Hepler PK, Shimmen T. 2000. The role of plant villin in the organization of the actin cytoskeleton, cytoplasmic streaming and the architecture of the transvacuolar strand in root hair cells of *Hydrocharis*. *Planta* 210:836–43
88. Valster AH, Pierson ES, Valenta R, Hepler PK, Emons AMC. 1997. Probing the plant actin cytoskeleton during cytokinesis and interphase by profilin microinjection. *Plant Cell* 9:1815–24
89. Valster AH, Vidali L, Hepler PK. 2003. Nuclear localization of profilin during the cell cycle in *Tradescantia virginiana* stamen hair cells. *Protoplasma* 222:85–95
90. Vidali L, Hepler PK. 1997. Characterization and localization of profilin in pollen grains and tubes of *Lilium longiflorum*. *Cell Motil. Cytoskeleton* 36:323–38
91. Vidali L, Yokota E, Cheung AY, Shimmen T, Hepler PK. 1999. The 135kDa actin-bundling protein from *Lilium longiflorum* pollen is the plant homologue of villin. *Protoplasma* 209:283–91
92. Wang YS, Motes CM, Mohamalawari DR, Blancaflor EB. 2004. Green fluorescent protein fusions to *Arabidopsis* fimbrin 1 for spatio-temporal imaging of F-actin dynamics in roots. *Cell Motil. Cytoskeleton* 59:79–93
93. Winter DC, Choe EY, Li R. 1999. Genetic dissection of the budding yeast Arp2/3 complex: a comparison of the in vivo and structural roles of individual subunits. *Proc. Natl. Acad. Sci. USA* 96:7288–93

94. Wu G, Gu Y, Li S, Yang Z. 2001. A genome-wide analysis of Arabidopsis Rop-interactive CRIB motif-containing proteins that act as Rop GTPase targets. *Plant Cell* 13:2841–56
 95. Yamaguchi H, Lorenz M, Kempiak S, Sarmiento C, Coniglio S, et al. 2005. Molecular mechanisms of invadopodium formation: the role of the N-WASP-Arp2/3 complex pathway and cofilin. *J. Cell Biol.* 168:441–52
 96. Yi K, Guo C, Chen D, Zhao B, Yang B, Ren H. 2005. Cloning and functional characterization of a formin-like protein (AtFH8) from Arabidopsis. *Plant Physiol.* 138:1071–82
 97. Yokota E, Shimmen T. 1999. The 135-kDa actin-bundling protein from lily pollen tubes arranges F-actin into bundles with uniform polarity. *Planta* 209:264–66
 98. Yokota E, Takahara K, Shimmen T. 1998. Actin-bundling protein isolated from pollen tubes of lily. Biochemical and immunocytochemical characterization. *Plant Physiol.* 116:1421–29
 99. Yokota E, Vidali L, Tominaga M, Tahara H, Orii H, et al. 2003. Plant 115-kDa actin-filament bundling protein, P-115-ABP, is a homologue of plant villin and is widely distributed in cells. *Plant Cell Physiol.* 44:1088–99
 100. Zallen JA, Cohen Y, Hudson AM, Cooley L, Wieschaus E, Schejter ED. 2002. SCAR is a primary regulator of Arp2/3-dependent morphological events in *Drosophila*. *J. Cell Biol.* 156:689–701
 101. Zhang X, Dyachok J, Krishnakumar S, Smith LG, Oppenheimer DG. 2005. IRREGULAR TRICHOME BRANCH1 in Arabidopsis encodes a plant homolog of the actin-related protein2/3 complex activator scar/WAVE that regulates actin and microtubule organization. *Plant Cell* 17:2314–26
 102. Zigmund SH, Evangelista M, Boone C, Yang C, Dar AC, et al. 2003. Formin leaky cap allows elongation in the presence of tight capping proteins. *Curr. Biol.* 13:1820–23
 103. Zimmermann I, Saedler R, Mutondo M, Hulskamp M. 2004. The *Arabidopsis* GNARLED gene encodes the NAP125 homolog and controls several actin-based cell shape changes. *Mol. Genet. Genomics* 272:290–96
-

RELATED RESOURCES

- Staiger CJ. 2000. Signaling to the actin cytoskeleton in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 51:257–88
- Wasteneys GO, Galway ME. 2003. Remodeling the cytoskeleton for growth and form: an overview with some new views. *Annu. Rev. Plant Biol.* 54:691–722
- Welch MD, Mullins RD. 2002. Cellular control of actin nucleation. *Annu. Rev. Cell Dev. Biol.* 18:247–88