Signaling to the Actin Cytoskeleton During Cell Morphogenesis and Patterning

Alison Sinclair, Mike Schenkel, and Jaideep Mathur

Abstract The plant is a supracellular organism whose cells are locked in position through shared walls but maintain apoplastic and symplastic connectivity. Their fixed position places each cell into a unique niche within the organism. Consequently, every environmental cue is perceived slightly differently by each cell. The response of each plant cell varies accordingly. Thus, plant growth and development reflect a progression of accommodative arrangements reached between constituent cells. In recent years the actin cytoskeleton, through its direct involvement in subcellular compartmentation, organelle and vesicle trafficking, and structural reinforcement, has emerged as a key player during accommodative growth and development. Here, using salient actin-cytoskeleton-associated cellular phenotypes, we elaborate upon the molecular-cell biological machinery involved in organizing the actin cytoskeleton during cell shape development in plants.

1 Introduction

A series of division, expansion, differentiation, and cell death events are involved in the fabrication of a multicellular plant. These events occur within a genetically defined framework in response to environmental cues and result in the precise patterns that provide each plant species with a unique, recognizable identity. However, if the millions of cells that make up a plant are considered individually, it becomes apparent that each wall-encased plant cell occupies a unique niche with

A. Sinclair
Laboratory of Plant Development & Interactions, Department of Molecular and Cellular Biology, CBS, University of Guelph, 50 Stone Road, Guelph, ON N1G 2W1, Canada

M. Schenkel
Laboratory of Plant Development & Interactions, Department of Molecular & Cellular Biology, CBS, University of Guelph, 50 Stone Road, Guelph, ON N1G 2W1, Canada

J. Mathur (✉)
Department of Molecular and Cellular Biology, 4463 Science Complex, University of Guelph, 488 Gordon Street, Guelph, ON N1G 2W1, Canada
e-mail: jmathur@uoguelph.ca

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respect to the environment. Owing to its fixed location within the plant, each cell perceives and responds to environmental cues in a manner slightly different from that of its neighbors. Further, each cell has only limited response options for contributing to the genetically determined architectural master plan: it can divide to add more adjustable units (cells); it can expand in a polar fashion to fill out spaces; or it can die and thus vacate space to help reorganize the overall shape. In addition, as components of a supracellular organism, individual cells can differentiate into specialized forms for fulfilling specific functions. The creation of a precise form by numerous plant cells thus reflects an innate ability to accommodate cell structure and function to that of neighboring cells. How is this cooperation and coordination of developmental activities achieved by wall-locked plant cells?

A number of signaling cascades, including Ca^{2+}/H^{+}, reactive oxygen species (ROS), heterotrimeric G-proteins, phospholipases, phosphoinositides, mitogen-activated protein kinases, cyclic AMP, and GTPases, are recognized in plants (Hirt 2000; Hong-Bo et al. 2008; Sumimoto 2008; Shao et al. 2008; Chen 2008;Cheung and Wu 2008; Kost 2008). In recent years the actin cytoskeleton has emerged as a central responder to many of these signaling channels (Brenner et al. 2006; Nibau et al. 2006; Kost 2008). The actin cytoskeleton also constitutes the signaling hub through which numerous subcellular activities are regulated. These include subcellular compartmentation, organelle and vesicle motility, as well as structural reinforcement in co-operation with microtubules. With each new study it becomes increasingly apparent that almost every actin-associated activity has an impact on cell growth and morphogenesis. Interestingly, seemingly different cues appear to produce similar responses from the actin cytoskeleton. Moreover, as we follow molecular cascades elicited in response to diverse signals, we become aware of the considerable gaps in our ability to directly link environmental cues to the far-downstream behavior of the actin cytoskeleton and the subsequent elicitation of a developmental response. While one can easily envision a kind of inverted pyramid of subcellular events whereby diverse cues get parsed into increasingly narrower response categories, the molecular-cell biological mechanisms behind this thinking applied to the actin cytoskeleton are only beginning to be understood.

As detailed and excellent reviews on different aspects of signaling to the actin cytoskeleton continue being published, here we have adopted a broad definition for the term "signaling." The actin cytoskeleton has been placed at a hub: on one hand we explore the putative molecular-cell biological events that organize actin within the plant cell, and on the other we link information on actin organization to the morphogenesis and development of model cell types in different organisms and assorted mutants of *Arabidopsis thaliana*.

2 Actin Organization in Plant Cells

Our understanding of signaling to the actin cytoskeleton first requires a characterization of the typical actin cytoskeleton organization in plants. The actin gene family in plants is diverse and built up of multiple isoforms with unique patterns of regulation (Meagher et al. 2000). The actin cytoskeleton, comprising actin monomers (G-actin)

and polymerized filamentous actin (F-actin) can change rapidly through cue-mediated depolymerization and repolymerization cycles. In living cells, F-actin strands twist around each other to produce helical arrangements of microfilaments possessing an approximate diameter of 7 nm. Immunocytochemical methods have informed us a lot about the actin organization in different cell types in plants (Vitha et al. 2000; Staiger and Schliwa 1987). More recent advances in live imaging of F-actin arrays have greatly shaped our present views on the dynamic actin cytoskeleton, notably, the introduction of fluorescent phalloidin into plant cells through micro injection (Schmit and Lambert 1990), microabrasion (Ramachandran et al. 2000), or after enzymatic digestion and freeze shattering (Wasteneys et al. 1997) has been pursued successfully. Synthetic fluorescent fusion proteins created using the filamentous actin binding domain of the Talin gene (Kost et al. 1998) and the actin binding domain 2 of the Fimbrin 1 gene (Sheahan et al. 2004; Wang et al. 2008) are the most used live probes for visualizing F-actin arrays in plants. G-actin pools in plant cells appear predominantly cytosolic and show up as diffuse fluorescence. Three morphologically distinguishable, but readily interchangeable states characterize the polymerized actin. These are seen as very fine meshworks comprising actin filaments, thick F-actin bundles, and loose F-actin strands (Staiger et al. 2000; Mathur et al. 1999; Wang et al. 2008). Since at the light-microscopy level it is not possible to resolve individual filaments of actin, the states probably reflect different levels of actin bundling. Actin patches as described for yeasts (Young et al. 2004) have not been observed in plant cells; however, brightly fluorescent regional aggregates of F-actin can be clearly observed in a variety of plant cells. Whether these represent true actin patches comprising interwoven actin filaments or reflect subcellular compartmentation is still an open question. Actin cages around large organelles have been described (Kandasamy and Meagher 1999). In addition to the above, short-lived arrays of actin filaments that contribute to the preprophase band and to stages of phragmoplast formation have been observed in dividing cells (Kakimoto and Shibaoka 1987; Sano et al. 2005).

Occasionally, 3–5-μm-long, brightly fluorescent bundles of F-actin might be encountered in cells that are experiencing subcellular stress or have been physically injured. All arrays are readily convertible to each other and reflect the dynamic nature of the actin cytoskeleton.

2.1 Different Actin Regulators Organize the Actin Cytoskeleton

A large number of actin genes and actin-interacting proteins have been identified and cloned from plants (Staiger et al. 2000; Meagher and Fechheimer 2003; Ketelaar et al. 2004; Staiger and Blanchoin 2006; Grunt et al. 2008). Since many actin interactors are conserved between eukaryotes, in vitro studies have been very instructive in ascribing roles for different proteins involved in actin cytoskeleton organization (Ayscough 1998). The direct visualization of actin arrays in plant cells through fluorescent imaging (Miller et al. 1996; Kost et al. 1998; Mathur et al. 1999; Blancaflor, 2000; Kovar et al. 2000b; Chen et al. 2002) has reinforced many of the in vitro observations.

As expected, cells expressing fluorescent protein fused to actin monomer interacting proteins such as profilin (Kovar et al. 2000a) exhibit a diffuse fluorescence (Ramachandran et al. 2000; Fu et al. 2002). However, overexpression of fluorescent protein fusions with different actin depolymerizing factors, also actin monomer interacting proteins, labels filamentous actin (Dong et al. 2001; Chen et al. 2002). Labeling experiments have confirmed that the proteins fimbrin (Kovar et al. 2000b) and villin (Klahre et al. 2000; Tominaga et al. 2000) bind to and bundle F-actin at different strengths. The ARP2/3 complex, an efficient enhancer of actin polymerization, is known to initiate side branches on actin filaments, creating a dendritic actin network (Mullins et al. 1998; Amann and Pollard 2001). Though immunological detection of ARP3 (one of the large subunits of the ARP2/3 complex) has been carried out (Van Gestel et al. 2003), and despite the demonstrated presence of its seven subunits (Mathur 2005a; Szymanski 2005), the complex has not been isolated from plants thus far. Inferences on ARP2/3 complex activity in relation to the actin cytoskeleton in plants are thus largely based on observations made in heterologous systems and actin organization observed in arp2/3 mutants.

More than 20 formins are recognized in plants, and as profilin-binding poly(L-proline)-containing proteins these have important roles in cytoskeletal organization (Cheung and Wu 2004; Deeks et al. 2005; Michelot et al. 2005; Ingouff et al. 2005). A major regulator of actin nucleation and polymerization through its control of the pool of unpolymerized ATP-actin is the adenyl cyclase associated protein (AtCAP1; Chaudhry et al. 2007; Deeks et al. 2007). A gelsolin-related actin binding protein PrABP80 has been identified in poppy pollen tubes (Huang et al. 2004). Biochemical assays indicate that the protein can nucleate actin polymerization from monomers, block the assembly of profilin-actin complex onto actin filament ends, enhance profilin-mediated actin depolymerization, and also sever actin filaments (Huang et al. 2004).

Since the number of proteins that can interact and affect actin organization continues to increase, clearly the key to actin's pivotal role as a subcellular responder lies in the way it is organized through the combinatorial and cumulative activities of its various regulatory proteins.

2.2 Local Ionic Interactions Modulate Actin Regulators

The plant cell functions by maintaining a basal level of ionic homeostasis. Any extrinsic or intrinsic cues that perturb this homeostasis are dealt with by the release or sequestration of relevant ionic species (Foreman et al. 2003; Shabala et al. 2006). This provides the cell with a fine-tuned mechanism for sensing environmental cues as well as responding to them. Since proteins affecting actin nucleation, polymerization, and subsequent organizational activities respond rapidly to alterations in their ionic environment (Day et al. 2002), they are direct mediators in the stimulus perception-response elicitation machinery. Many actin-regulating proteins are themselves regulated by ionic conditions in their environment (Bretscher and Weber

1980; Matsudaira and Burgess 1982; Harris and Weeds 1983; Kovar et al. 2000a; Huang et al. 2003; Drøbak et al. 2004; Gu et al. 2004; Jones et al. 2007). The best studied ionic pools playing a role in signaling to actin include Ca^{2+} (Hetherington and Brownlee 2004; Bothwell and Ng 2005), NADPH oxidases (Navazio et al. 2000; Kwak et al. 2003; Foreman et al. 2003) and ROS, such as hydrogen peroxide (H_2O_2), superoxide anion (O^{2-}), hydroxyl ions (OH) and nitric oxide (NO) (Miller et al. 2008; Mori and Schroeder 2004). Low, basal levels of cytosolic free calcium ($[Ca^{2+}]_{cyt}$) are sustained by Ca^{2+}-ATPases, and through the activity of a Ca^{2+}/H^+ antiporter that removes Ca^{2+} from the cytosol. Elevation of $[Ca^{2+}]_{cyt}$ during signaling occurs through the opening of different classes of plasma membrane, vacuolar membrane, or endoplasmic reticulum localized Ca^{2+}-permeable ion channels. Whereas Ca^{2+}-and H^+-mediated signaling in plants is usually achieved through storage and release mechanisms, ROS signaling is usually controlled through rapid production and scavenging.

A major approach adopted for linking subcellular ionic distribution to actin organization and cell growth characteristics has been to observe and quantify ionic gradients and site-specific localizations (Felle and Hepler 1997). This approach has been successfully applied to single-celled, tip-growing cells such as pollen tubes (Cárdenas et al. 2008; Kroeger et al. 2008) and root hairs (Carol and Dolan 2006; Monshausen et al. 2007). In *Arabidopsis*, ROS stimulate the entry of Ca^{2+} into the cell through a calcium plasma membrane hyperpolarization-activated Ca^{2+} channel (Foreman et al. 2003). Recently, Takeda et al. (2008) have elucidated an elegant mechanism involving local positive feedback, where ROS derived through an RHD2 NADPH oxidase stimulates a Ca^{2+} influx into the cytoplasm. Though the targeted actin regulators were not identified in this study, the regulatory loop is clearly involved in cell shape determination of root hair cells. Similar interdependent local loops between ROS and Ca^{2+} gradients have been uncovered during polarized growth of *Fucus serratus* zygotes (Coelho et al. 2008).

Details pertaining to integrated calcium-mediated signaling (see "Integrated Calcium Signaling in Plants" by Narendra Tuteja, this volume), the role of apoplastic and cell-wall mediation in signaling events (see "Signaling and Cell Walls" by Ewelina Rodakowska et al., this volume), and signaling during plant-pathogen interactions utilizing localized ROS production (see "Signaling via Plant Peroxidases" by Claude Penel and Christophe Dunand, this volume) are not further elaborated upon here.

2.3 Rho-like GTPases of Plants: Master Switches Responding to Environmental Cues

In tracing back many of the ionic alterations brought about in response to environmental cues, a conserved set of proteins constantly comes up. Rho-like GTPases of plants (ROPs) localize to the cell membrane (Bischoff et al. 2000; Molendijk et al. 2001; Cheung et al. 2003) and are involved in multiple signal transduction events in plants. In *Arabidopsis*, 11 ROPs have been described and extensive reviews have

been published on signaling mediated by ROPs and their regulators (Zheng and Yang 2000; Gu et al. 2004; Xu and Scheres 2005; Nibau et al. 2006; Kost 2008). Direct links between ROP activity and $Ca2^{+}$ spiking (Li et al. 1999; Jones et al. 2002), and ROP mediation in localized ROS activation (Jones et al. 2007) have been demonstrated. ROP-mediated signaling is also one of the best elucidated chains of molecular links directed to the actin cytoskeleton in plants (Fu et al. 2005; Fig. 1).

Specific ROPs and their regulators (RICs, ROP-GEFs/ROP-GDI) have an effect on the actin cytoskeleton but these proteins, high up in the response hierarchy of

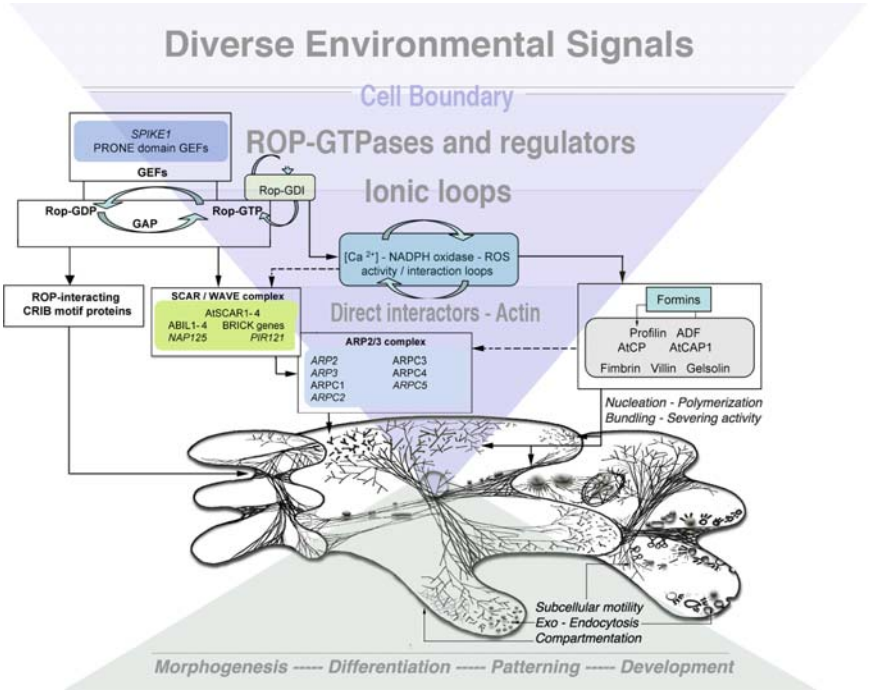

Fig. 1 A generalized depiction of events involved in signaling to the actin cytoskeleton in plants. The leaf epidermal pavement cells drawn here are considered to represent all cell types since the vast majority of actin organizations and actin-mediated processes can be observed in them. Moreover, whereas many different signal cascades might operate in different plant cells, presently pavement cells provide one of the best characterized systems for dissecting signals to the actin cytoskeleton (Fu et al. 2005). An inverted pyramid of events suggests the gradual parsing of signals through the molecular apparatus to produce an impact on the actin cytoskeleton. Diverse environmental cues perceived at the cell boundary (cell wall and plasma membrane) initiate feedback loops of Rho-like GTPases of plants (ROP)-GTPases and ionic activity, which subsequently affect several proteins that bind directly to actin (such as actin monomer interacting, nucleating, bundling, and severing proteins) and bring about changes in its organization. Local actin organization has implications for subcellular motility, exocytosis-endocytosis, cytomembrane organization, and structural reinforcement. The cumulative results of these activities lead to cell differentiation, morphogenesis, and functional diversification during plant development. *Solid lines* and *arrows* depict experimentally demonstrated links between protein complexes, whereas *broken lines* imply putative interactions

the plant cell, do not cause their effects through direct binding to actin. Their effects impact multiple proteins and processes, influencing both the microtubule and the actin cytoskeleton (Fu et al. 2005). From a phenotype-based viewpoint an interference with ROPs and associated machinery does not produce cell forms that can be solely linked to actin defects (e.g., root hair phenotypes depicted in Fig. 2).

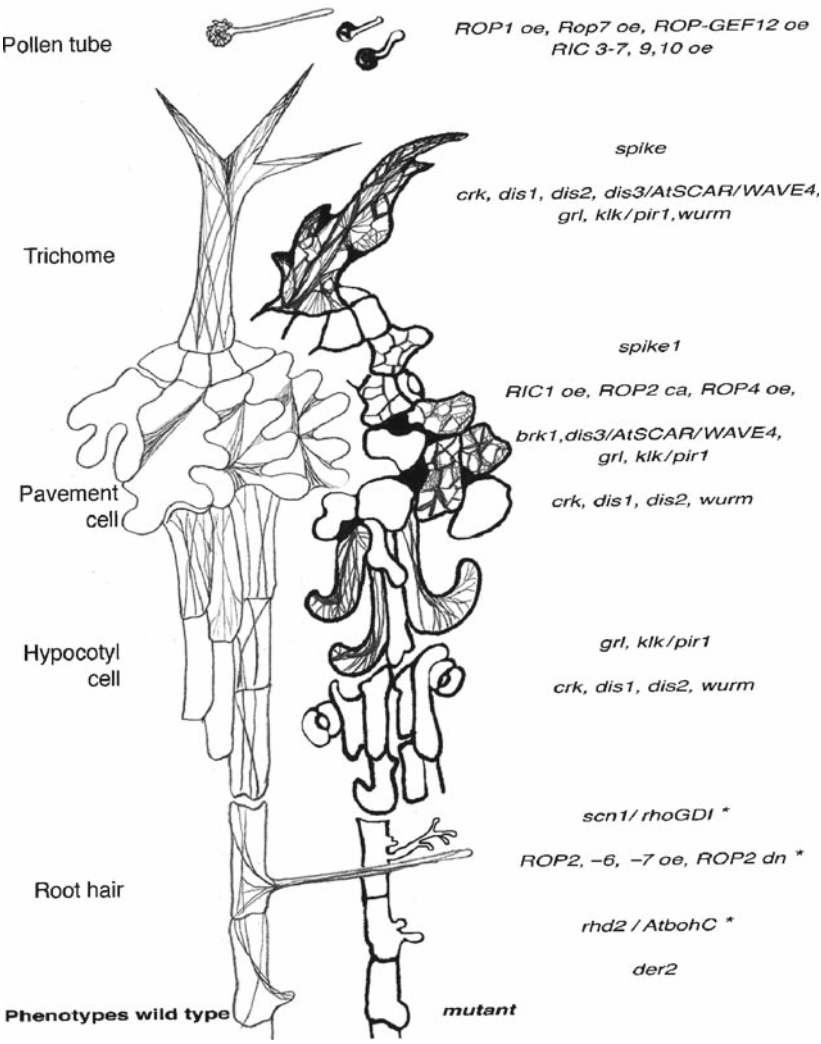

Fig. 2 Salient cellular phenotypes in model cell types of *Arabidopsis thaliana*. The *left side* shows cell morphology displayed by wild-type plants, while the phenotypes drawn in *bold lines* are exhibited by different mutants. In general, cells with an aberrant actin cytoskeleton are smaller in size. Phenotypes displayed by ROPs and their regulators do not strictly follow the pattern obtained by an interference with the actin cytoskeleton only. These phenotypes suggest a strong disturbance in the microtubule cytoskeleton too and could reflect actin-microtubule interactivity during cell shape development

However, neither are the ROP-associated phenotypes indicative of microtubule cytoskeletal defects only. Live imaging of the actin and microtubule cytoskeletons in *Arabidopsis* plants possessing aberrant activity of different ROPs and their regulators (e.g., ROP-GDI) suggests that they exhibit both actin and microtubule defects (Mathur, unpublished data).

3 Developmental Consequences of Different Actin Organizations

Whereas it has been possible to draw correlations between a small region of growth and its ionic environment, such as the one provided by tip-growing cells (Gu et al. 2005; Ovecka et al. 2008; Kroeger et al. 2008), establishing similar correlations has been difficult for diffuse-growing cells that make up the bulk of higher plants. The very nature of diffuse growth involves widespread, possibly transient signaling events that preclude precise localization of ionic concentrations and gradients. Therefore, a reverse approach has been adopted for understanding molecular mechanisms involved in unraveling the signaling to and the roles of actin in diffuse-growing cells. This approach (Mathur et al. 1999; Szymanski et al. 1999; Fu et al. 2002) has largely been based on visualizing defects in actin organization in particular cell types and then tracing them to a molecular lesion involved in creating similar aberrant cellular activity. In this approach, the use of inhibitors such as cytochalasin D, latrunculin, phalloidin, and jasplakinolide has been very helpful in uncovering specific actin-associated cellular phenotypes (Mathur et al. 1999; Baluska et al. 2001; Collings et al. 2006; Foissner and Wasteneys 2007) and hinting at possible processes involved. The actin defective phenotype based approach, when combined with live imaging, has been successful in uncovering new protein classes and complexes involved in mediating signals to the actin cytoskeleton (Mathur et al. 1999, 2003a, b).

The occurrence of the actin organizations discussed above has been demonstrated in different model cell types in plants (representative images in publications: root hair – Ryan et al. 2001; pollen tube – Lovy-Wheeler et al. 2005; hypocotyl cells –Mathur et al. 2003b; pavement cells – Fu et al. 2002, 2005; trichomes – Mathur et al. 1999). Section 3.1 associates actin cytoskeleton organization to a developmental consequence in different cell types.

3.1 Actin Patch Indicative of Cell Polarization

Polar growth is an established facet of plant development. However, the analysis of early events leading to the establishment of polarity in the asymmetric environment inhabited by land plants is a difficult task. Observations on the early development of fucoid zygotes have been informative in highlighting a role for the actin cytoskeleton during polarity establishment in a nearly apolar cell (Kropf et al. 1989; Bisgrove 2007). Upon release from the mother frond, and following their fertilization, spherical fucoid zygotes rely on several vectorial inputs such as

blue light, gravity, electrical-ionic impulses, and temperature and osmotic gradients for subsequent development (Brownlee and Bouget 1998). A distinct actin patch has been associated with zygote polarization and creation of the rhizoidal pole (Kropf et al. 1989). A cortical localization of the actin modulator ARP2/3 appears to be intimately involved in actin patch formation (Hable and Kropf 2005). Actin depolymerizing substances disrupt cell polarization and subsequent development (Hable et al. 2003). In later stages of zonation of the zygote, asymmetric localization of ARP2 and actin results in distinct actin arrays (Hable and Kropf 2005).

3.2 Actin Organization Indicative of Focused Growth

Pollen tubes and root hairs have been the most extensively studied tip-growing cells and numerous publications have described the actin cytoskeleton in these cells and reviewed the implications of the polar F-actin observed in them (Geitmann and Emons 2000; Ryan et al. 2001; Ringli et al. 2005; Geitmann 2006). In recent years the moss *Physcomitrella patens* has emerged as an equally informative model system for understanding polarized tip growth.

In *Physcomitrella*, single apical cells of moss filaments undergo polarized tip growth by perceiving and aligning themselves to gravity and light inputs (Cove 2005). Several recent studies point to specialized roles for actin dynamics in modulating these events, prompting the hypothesis that the ARP2/3 complex is pivotal in translating environmental cues by acting as a downstream target for signals regulating polar tip growth (Perroud and Quatrano 2006). Subsequently, there has been a strong focus on evaluating the roles of both the ARP2/3 complex and its upstream regulators Scar/WAVE complexes in polar growth of the apical cell of *P. patens* filament cells. When the arpc4 subunit of the ARP2/3 complex is deleted, the resulting null mutant is viable. However, while tissue morphogenesis in the mutant proceeds normally from filamentous growth to leafy shoot formation, it displays inefficient perception of polarized white light and a marked reduction in apical cell growth (Perroud and Quatrano 2006). Additionally, RNA interference lines knocking down expression of another ARP2/3 member, ARPC1, result in a similar, albeit more severe, phenotype as displayed by the arpc4 mutant. Overexpression of the respective ARP2/3 members (ARPC4, ARPC1) rescues the mutant phenotypes. Moreover, insertion of a yellow fluorescent protein–ARPC4 localizes ARPC4 exclusively to the site of polarized extension in the apical cell tip (Perroud and Quatrano 2006). Moreover, the deletion of BRK1, an upstream regulator of the ARP2/3 complex (Frank and Smith 2002), results in a mutant that though viable and responsive to polarized white light shows significant reduction in apical cell growth (Perroud and Quatrano 2008). BRK1 also localizes to the apical tip. A role for BRK1 in ARP2/3recruitment is supported as the BRK1 mutant of physcomitrella is unable to localize both ARPC4 and AGP (a cell wall proteoglycan) to the apical cell tip (Perroud and Quatrano 2008).

3.3 Actin Organization Indicative of Diffuse Growth

The majority of plant cells grow by diffuse growth. Different model cell types have been informative about the actin-associated molecular–cellular machinery involved in spreading growth occurring over large regions of the cell. These include trichomes (epidermal hair) that grow outward from the aerial epidermis, interconnected jigsaw puzzle shaped pavement cells, and axially elongating epidermal cells of the hypocotyl, cotyledon, and leaf petiole. Each of these epidermal cell types offers a different surface to the environment and their growth responses differ accordingly.

In *Arabidopsis*, trichomes are single-celled and display a rapid expansion phase in the later stage of their development (Mathur 2006b). As the trichome cell grows out of the epidermal plane, unencumbered by growth of neighboring cells, a large portion of it is exposed directly to the environment. In contrast, both pavement and hypocotyl/petiole cells achieve accommodative growth by maintaining connectivity with their neighbors. All three epidermal cell types exhibit long F-actin cables that stretch from end to end, as well as a fine cortical F-actin mesh. In addition, in both trichomes and pavement cells, areas of indention (valleys) display bundled or aggregated F-actin. In general, the presence of bundled F-actin is plant cells has come to be associated with regions of low expansion, whereas the presence of a fine cortical F-actin meshwork suggests regions of increased expansion.

3.3.1 Diffuse Growth in Trichomes

The use of actin inhibitors resulted in random shape alterations in *Arabidopsis* trichomes (Mathur et al. 1999; Szymanski et al. 1999). The drug-induced trichome distortion is phenocopied by mutations in several *Arabidopsis* genes. Molecular characterization of these genes has identified the ARP2/3 complex, an efficient modulator of actin polymerization that initiates the formation of a fine dendritic F-actin mesh (reviewed in Mathur 2005a, b; Szymanski 2005; Hussey et al. 2006), and its upstream regulators, starting from ROPs, ROP-GEFs such as SPIKE1 (Qiu et al. 2002; Basu et al. 2008; Zhang et al. 2008), and including different SCAR/WAVE, HSPC300-like/BRICK proteins (Frank and Smith 2002; Frank et al. 2004; Zhang et al. 2005; Basu et al. 2005; Le et al. 2006; Djakovic et al. 2006), NAP125-like and PIR121-like proteins (reviewed by Szymanski 2005; Smith and Oppenheimer 2005). An elegant interaction map of SCAR genes and their interactions with ARP2/3 proteins obtained through mutant studies, yeast two-hybrid, and bimolecular fluorescence complementation proteins experiments has been presented (Uhrig et al. 2007). In addition to the ROP-SPIKE-SCAR/WAVE-ARP2/3 pathway mentioned above, several other signaling pathways to the actin cytoskeleton might be activated through the involvement of novel ROP-GEFs, termed “plant-specific ROP nucleotide exchanger (PRONE), which are exclusively active towards members of the ROP subfamily (Berken et al 2005).

3.3.2 Interdigitating Growth of Pavement Cells

The final "jigsaw puzzle" shape of epidermal pavement cells arises from multiple local projections (lobes) of a polygonal initial. During expansion, the lobes of one pavement cell fit into the indentions of its neighbors to produce an epidermal surface with an interdigitating pattern. Fu et al. (2005) revealed a ROP-GTPase signaling network underlying the creation of these lobes and indentions by pavement cells. Local activation of a ROP (AtROP2) activates ROP-interactive CRIB-motif-containing protein 4 (RIC4) to enhance actin dynamics and promote localized growth. However, ROP2 activity leads to the inactivation of another target, RIC1, that localizes to cortical microtubules and promotes their ordering into parallel arrays. RIC1-dependent microtubule organization not only inhibits cell outgrowth locally but also suppresses ROP2 activation in the indention zone. Thus, while the ROP2–RIC4 interaction promotes cell outgrowth, the ROP2–RIC1 interaction restrains outgrowth. Such coordinated activity in epidermal cells creates the interdigitations between adjacent pavement cells.

3.3.3 Accommodative Growth of Cylindrical Cells

The growth of cells of the seedling hypocotyl and petioles relies on efficient actin-based communication between cell ends for producing elongation along the longitudinal axis (Baluska et al. 2003). These cells have a diffuse F-actin mesh but display cytoplasmic aggregation at the cell ends. Mutants in different subunits of the ARP2/3 complex, hypocotyl, and petiole cells often display increased F-actin aggregation at their ends and consequently lose cell-cell connectivity (Mathur et al. 2003a, b). The reduced actin polymerization activity in elongating cylindrical cells observed in the arp2/3 mutants has implications for cell-cell communication and polar transport of growth regulators. It thus offers a novel system to understand the initiation and maintenance of multicellularity in higher plants.

4 The Diverse Roles of the Actin Cytoskeleton in Plants

An appraisal of cellular phenotypes associated with different actin cytoskeleton organizations, the nature of actin regulatory proteins that can create the organizations, and the diversity of environmental cues that trigger subcellular activity suggests that actin can be involved in many different kinds of roles inside the plant cell. Some of these roles are discussed in Sect. 4.1.

4.1 Actin as a Barrier

The regional accumulation of actin correlates with increased aggregation and decreased motility of small organelles such as Golgi bodies, peroxisomes, and mitochondria

(Mathur et al. 2003a, b). Upon extrapolation, the observations could suggest that F-actin acts as an intracellular barrier for subcellular trafficking of vesicles. The notion was tested by looking for areas of actin aggregation versus those with fine cortical actin meshwork in different cell types (Mathur et al. 2003a). Regions with actin aggregation invariably displayed lesser growth as compared with regions with a fine meshwork of F-actin (Mathur et al. 2003a; Fu et al. 2005). According to the "actin barrier" concept, a fine F-actin meshwork resulting from increased actin dynamics mediated by the ARP2/3 complex and formins would allow and perhaps even aid the rapid motility of organelles and exocytotic vesicles (Mathur 2004). This leads to a promotion of local growth. In contrast, a dense web comprising F-actin aggregates and bundles in an intracellular locality might hinder organelle motility and vesicular trafficking and could thereby display a lower rate of regional growth. The idea of F-actin behaving as a flexible barrier is appealing when considering the phenotypes described above for pavement and hypocotyl cells impaired in their actin organization. The role of cortical F-actin as a "fence" has also been suggested through three-dimensional electron-tomographic reconstructions of the membrane cytoskeleton in normal rat kidney fibroblast cells (Morone et al. 2006).

4.2 Actin as a Propulsive Force

Asymmetric actin polymerization mediated through the ARP2/3 complex has been implicated in the rocketing motility of enteropathogenic organisms such as *Listeria monocytogenes* and *Shigella* species, as well as vesicles and organelles such as endosomes, phagosomes (Higgs and Pollard 2001; Gouin et al. 2005), and mitochondria (Boldogh et al. 2001). The polymerization of actin is also believed to act as the driving force at the leading edge of motile animal cells (Goley and Welch 2006). In plants, direct evidence for actin polymerization acting as a propulsive force for subcellular structures or as a protrusive force for cell membranes has not been observed. However, given the role of actin in vesicle movement during auxin efflux and translocation (Dhonukshe et al. 2007), the rapid movement of endocytic vesicles (Voigt et al. 2005), and cytoplasmic streaming, the possibility of actin polymerization mediated force generation within the plant cell cannot be totally ruled out (Mathur 2005b).

4.3 Actin as an Interactive Support System

The actin cytoskeleton interacts with microtubules. During root elongation in *Arabidopsis* the hypersensitivity to cytoskeletal antagonists has been used to demonstrate microtubule-microfilament cross-talk (Blancaflor 2000; Collings et al. 2006). One of the molecular mechanisms controlling these interactions has been traced to the antagonistic activities of ROP-RIC proteins (Fu et al. 2005) and elegantly described for the morphogenesis of pavement cells of *Arabidopsis*.

Interestingly, observations in trichome cells suggest that F-actin aggregates coincide with regional clusters of cytoplasmic microtubules (Saedler et al. 2004). The observation suggests that microtubules, with their own set of interactors and regulators, might act to confine F-actin-based regional expansion in a cell (Mathur 2004, 2006a). The role of F-actin in sculpting the cortical microtubule cytoskeleton has also been suggested (Schwab et al. 2003).

4.4 Actin as a Track

Observations on different organelles in plant cells have shown that their motility is actin-based (Ovecka et al. 2008) and involves interactions with myosin motors (Samaj et al. 2000; Reisen and Hanson 2007; Sparkes et al. 2008). In this situation, actin filaments act as subcellular tracks for the motor molecules. How cargo specificity and the choice of a specific actin track is decided by the different myosins in plant cells still remains an enigma. There also remains the question of interactivity and switching between myosin motors and microtubule-based motors such as kinesins.

5 Conclusions

While reviews on the minutiae of signaling to the actin cytoskeleton will continue being published, one of the long-term goals of the field is to uncover the mechanisms whereby this global perceiver of, and responder to, myriad environmental cues is able to create local influences within a cell. As described in this chapter, the key to actin's responsiveness lies in its interactivity with numerous regulatory proteins as well as with other components and compartments of cell. Signaling to actin thus becomes an exercise in attempting to understand and sum up everything that goes on inside a living cell. Realistically, we are far from the goal, but given the tremendous scope of actin interactions, the increased availability of novel mutants in model plant species, and the emergence of new tools and techniques for live imaging (Dhanoa et al. 2006), the prospects of unraveling the tangled web around actin looks very promising.

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