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REVIEW

Catching the WAVEs of Plant Actin Regulation

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ABSTRACT

Plants, as all other eukaryotic organisms, depend on a dynamic actin cytoskeleton for proper function and development. Actin dynamics is a complex process, regulated by a number of actin-binding proteins and large multiprotein complexes like ARP2/3 and WAVE. The ARP2/3 complex is recognized as a nucleator of actin filaments, and it generates a highly branched network of interlaced microfilaments. Results from multiple organisms show that ARP2/3 activity is regulated through multiple pathways. Recent results from plants point to a signaling pathway leading from the small GTPase RAC/ROP through a protein complex containing the ARP2/3-activating protein WAVE. This signaling pathway appears to be evolutionarily conserved. Support for this regulatory mechanism comes from studies of mutations in genes encoding subunits of the putative ARP2/3 complex and the WAVE complex in *Arabidopsis*. Several such mutants have defects of actin filament organization, leading to a conspicuous ''distorted'' trichome phenotype. Multiple growth and developmental phenotypes reported for *napp/gnarled/atnap, pirp/pirogi/atpir*, and *distorted3* mutants reveal that these WAVE proteins are also required for a wider variety of cellular functions in addition to regulating trichome cell growth. These results have implications for the current view on cell morphogenesis in plants.

Key words: Actin; ARP2/3; Cell morphology; WAVE; Cytoskeleton; Morphogenesis

INDRODUCTION

The shape of a plant cell is often tightly coupled to its function. In general, directional growth in plant cells is achieved by mechanisms of diffuse growth and tip growth (Martin and others 2001; Smith 2003). Most cell types expand by diffuse growth, in which cell wall extension occurs over the entire cell surface. Turgor pressure is the driving force of diffuse growth, and growth directionality is determined by the deposition of cellulose microfibrils transverse to the growth axis. Cortical microtubules in diffusively growing cells are oriented in parallel with the microfibrils, and current models indicate that microtubules are important for generation of long microfibrils that restrain radial expansion. In contradiction to what was assumed earlier, microtubules appear not to be important for orientation of microfibrils (Wasteneys 2004). Tip growth occurs at single spots on the cell surface. Pollen tubes and

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root hairs are cell types believed to grow exclusively by tip growth. Actin filaments are aligned longitudinally along most of the cell body, but they reorganize to short, highly dynamic actin filaments in the subapical region. A popular model for the role of subapical actin is that the filaments facilitate targeting and fusion of post-Golgi vesicles to the apical region, thereby delivering plasma membrane and cell wall material to the cell tip (Mathur and Hülskamp 2002).

Some epidermal cell types with complex growth patterns, such as leaf pavement cells and trichomes, are thought to employ both diffuse growth and tip growth during cell expansion. Trichomes, which are large, unicellular cells protruding from the leaf epidermis, have become a popular model system for the study of cell morphogenesis in plants (Hülskamp and others 1994; Szymanski 2001). Actin filaments and microtubules are both necessary for normal trichome morphogenesis. Inhibitor studies indicate that microtubules are involved in initiation and positioning of trichome branches (Mathur and Chua 2000), whereas the actin cytoskeleton is important for the subsequent branch elongation (Mathur and others 1999; Szymanski and others 1999).

Although the importance of the actin cytoskeleton in plant cells has been recognized for some years, the mechanisms regulating actin filament organization remained unknown. Over the last two years, reports from a number of groups have led to the emergence of the ARP2/3 and the WAVE regulatory protein complexes as important modulators of actin dynamics during plant cell morphogenesis. ARP2/3 and WAVE complex components identified so far are listed in Table 1. This review will briefly summarize the present knowledge on the WAVE regulatory complex in general. Subsequently, recent studies that have highlighted the role of the WAVE and ARP2/3 complexes in regulating the actin cytoskeleton will be discussed.

The ARP2/3 Complex and Regulation of the Actin Cytoskeleton

During the last 10 years, the ARP2/3 complex has emerged as an important initiator of actin polymerization. The ARP2/3 complex was first identified in *Acanthamoeba* as a protein complex binding to profilin (Machesky and others 1994), and it appears to be conserved in all eukaryotes (Machesky and Gould 1999). The complex consists of seven different subunits (Machesky and others 1994; Mullins and others 1997). Two of the subunits, ARP2 and ARP3, belong to the family of actin-related proteins (ARPs) and are predicted to share the same protein fold as actin, although the amino acid sequence is divergent from that of conventional actins (Kelleher and others 1995). ARPC1 contains seven putative WD40 motifs that may be involved in protein–protein interaction (Welch and others 1997). The other four subunits, ARPC2, ARPC3, ARPC4, and ARPC5, are novel proteins without any described protein domains.

An important feature of the ARP2/3 complex is the ability to nucleate actin filaments and promote polymerization at the filament's barbed end (Mullins and others 1998). The ARP2/3 complex binds both to the sides and barbed ends of actin filaments (Amann and Pollard 2001; Pantaloni and others 2000). Although kinetic and microscopic experiments indicate that the activated ARP2/3 complex preferentially nucleates branches along the sides of pre-existing filaments (Amann and Pollard 2001; Carlsson and others 2004), barbed-end nucleation cannot yet be ruled out. At the side of a ''mother'' filament, the ARP2/3 complex will nucleate a new ''daughter'' filament at an angle of 70° (Mullins and others 1998). In motile eukaryotic cells, the ARP2/3 complex is central in the formation of the highly branched actin filament structures at the leading edge of lamellipodia (Svitkina and Borisy 1999).

WASP/WAVE Family Proteins: Activators of the ARP2/3 Complex

Although several different activators of the ARP2/3 complex have been discovered (Higgs and Pollard 2001; Weaver and others 2003), the WASP/WAVE family of proteins has been the most intensively studied protein class. The WASP/WAVE protein family can be divided into two groups: the Wiskott-Aldrich syndrome proteins (WASP) and the WASP family Verprolin-homologous protein (WAVE). WASP was identified as the target of mutation in Wiskott-Aldrich syndrome, a rare, X-linked disease characterized by eczema, bleeding, and recurrent infections (Derry and others 1994). The first WAVE group protein, originally called SCAR (Suppressor of cAMP receptor), was identified in a genetic screen in *Dictyostelium* for suppressors of the phenotype of a mutant strain in which the cAMP receptor was disrupted (Bear and others 1998). In mammals, the WAVE group appears to consist of three members, WAVE1–WAVE3.

WASP and WAVE proteins (Figure 1) share two main regions of homology: a central, proline-rich region, and a C-terminal module containing a verprolin-homology (V), a central (C) region, and an acidic (A) region, collectively called the VCA region (Miki and others 1996). The proline-rich region

Table 1. Subunits of the ARP2/3 and WAVE Complexes and their Corresponding Genes and Mutants

Gene	Other names	At locus ID	Mutant	Interacts with	References
ARP2	*WURM*	At3g27000	*wurm*		Le and others 2003; Li and others 2003; Mathur and others 2003a
ARP3	*DISTORTED1*	At1g13180	*distorted 1*		Le and others 2003; Li and others 2003; Mathur and others 2003a
ARPC1a		At2g30910			
ARPC1b		At2g31300		WAVE4	
ARPC2a	*DISTORTED2*	At1g30825	*distorted2*	ARPC4	El-Din El-Assal and others 2004b; Saedler and others 2004
ARPC2b		At2g33385			
ARPC3		At1g64030			
ARPC4		At4g14147?	*alien?*	ARPC2a	
ARPC5	*CROOKED*	At4g01710	*crooked*		Li and others 2003; Mathur and others 2003b
NAP1	*NAPP, GNARLED, AtNAP*	At2g35110	*gnarled*	PIRP	Brembu and others 2004; Deeks and others 2004; El-Din El-Assal and others 2004a; Li and others 2004; Zimmermann and others 2004
SRA1	*PIRP, AtPIR*	At5g18410	*pirogi klunker*	NAPP, ROP2	Basu and others 2004; Brembu and others 2004; Li and others 2004; Szymanski 2004
BRK1		At2g22640	*brick1* (maize)	WAVE1, WAVE2	Frank and Smith 2002
SCAR1	*WAVE1*	At2g34150		BRK1, actin	Frank and others 2004
SCAR2	*DISTORTED3, WAVE4*	At4g38440	*distorted3*	NAPP, ABIL1, ARPC3	Basu and others 2005
SCAR3	*WAVE2*	At1g29170		BRK1	Frank and others 2004
SCAR4	*WAVE3*	At5g01730			
WAVE5		At4g18600			
ABIL1		At5g24310			
AB1L2		At3g49290			
ABIL3		At5g42030			
ABIL4		At2g46225			

binds several SH3 domain–containing proteins as well as profilin (Bompard and Caron 2004; Finan and others 1996). The VCA region is essential for actin binding and activation of the ARP2/3 complex (Machesky and others 1999; Rohatgi and others 1999; Yarar and others 1999). The V region binds G-actin, whereas the A region appears to be the main site of ARP2/3 complex binding (Marchand and others 2001). Upon binding the ARP2/3 complex, the C region forms an amphipathic helix that is required for activation (Panchal and others 2003). Binding of the VCA region enhances the affinity of the ARP2/3 complex for the side of an actin filament and promotes the formation of a quaternary complex of VCA, an actin monomer, the ARP2/3 complex, and an actin filament. A subsequent activation step promotes the nucleation of a daughter filament from the side of the mother filament (Higgs and Pollard 2001). Furthermore, actin filaments increase the affinity of the VCA region for the ARP2/3 complex, implying that nucleation by filament-bound ARP2/3 is favored over that by free ARP2/3 (Marchand and others 2001).

The N-terminal parts of WASP and WAVE proteins are quite divergent. At the N-terminal end, WASP proteins contain a domain similar to the Ena/VASP homology 1 (EVH1) domain. EVH1 domains generally recognize and bind specific proline-rich sequences, and they are found in many proteins involved in reorganization of the actin cytoskeleton (Ball and others 2002). Both WASP and N-WASP EVH1 domains interact with WASP interacting protein (WIP), which also binds actin (Ramesh and others 1997; Volkman and others 2002). The corresponding region in WAVE proteins constitutes a unique domain called the Scar homology domain (SHD). The SHD of mammalian WAVE2 has been reported to interact with Abil (Abelson interactor), a subunit of the WAVE regulatory complex (Innocenti and others 2004), which will be further discussed

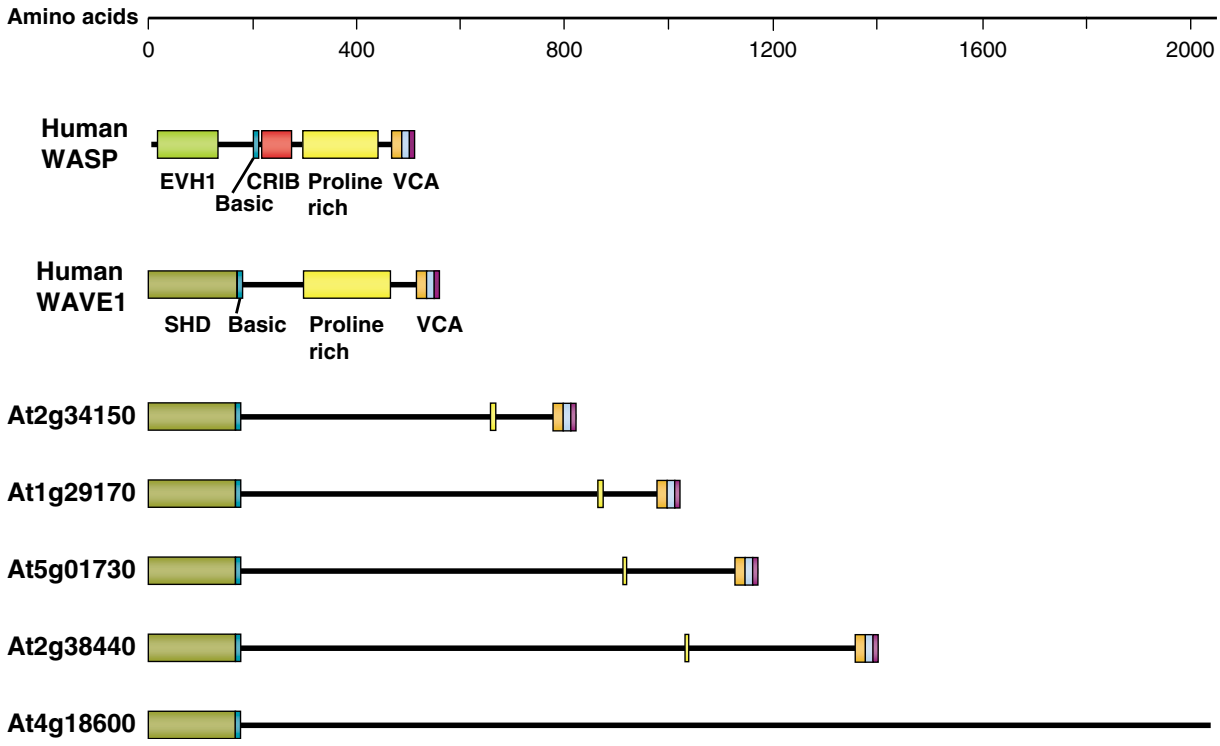

Figure 1. Domain structure of mammalian and plant WASP/WAVE-like proteins. CRIB, Cdc42/Rac-interactive binding motif; EVH1, Ena/VASP homology 1; SHD, SCAR homology domain; VCA, Verprolin homology-Central-Acidic.

below. Recent studies indicate that a leucine zipper-like motif in the SHD is important in localization of WAVE at the tips of filopodia in the growth cone of neuronal cells (Nozumi and others 2003). WASP proteins also contain a CRIB domain, positioned next to the proline-rich region, that binds Cdc42 with high affinity (Abdul-Manan and others 1999; Rudolph and others 1998).

WAVE Activity is Controlled by Interacting Protein Factors

The WAVE proteins lack a CRIB domain, suggesting that they are neither binding any Rho GTPases directly nor being regulated by autoinhibition. In accordance with these presumptions, WAVE is constitutively active *in vitro* (Machesky and others 1999). Eden and co-workers (2002) purified a mammalian WAVE 1-containing protein complex, and identified the proteins in the complex as WAVE1, PIR121, Nap125, HSPC300, and Abi2. Although recombinant WAVE1 was constitutively active, the WAVE1 protein complex was unable to stimulate actin polymerization, indicating that WAVE1 in the complex is kept inactive. Interestingly, when active Rac or the SH3 domain–containing adapter protein Nck was added to the inactive WAVE1 complex, PIR121, Nap125, and Abi2 dissociated from HSPC300-WAVE1, which regained the ability to activate the Arp2/3 complex. Thus, WAVE1 appears to be kept inactive through interaction with a regulatory protein complex, and its activation by Rac is indirect rather than direct, through the binding and dissociation of the regulatory complex controlled by Rac (Figure 2a). The role of HSPC300 in WAVE1-mediated activation of the Arp2/3 complex is still uncertain.

A recent publication (Steffen and others 2004) suggests an alternative model for the role of the WAVE regulatory complex in controlling WAVE activity (Figure 2b). According to this model, Nap1 and the PIR121 ortholog Sra-1 stay associated with WAVE2 upon activation by Rac1 and are essential components of a protein complex necessary for formation of lamellipodia (Steffen and others 2004). The discrepancy between the two models is difficult to explain, but it may be caused by the fact that Eden and others (2002) purified the WAVE1 complex from brain, whereas Steffen and others (2004) reconstituted the WAVE2 complex using purified components.

Recently, the molecular architecture of the mammalian WAVE complex was resolved (Gautreau and others 2004; Innocenti and others 2004).

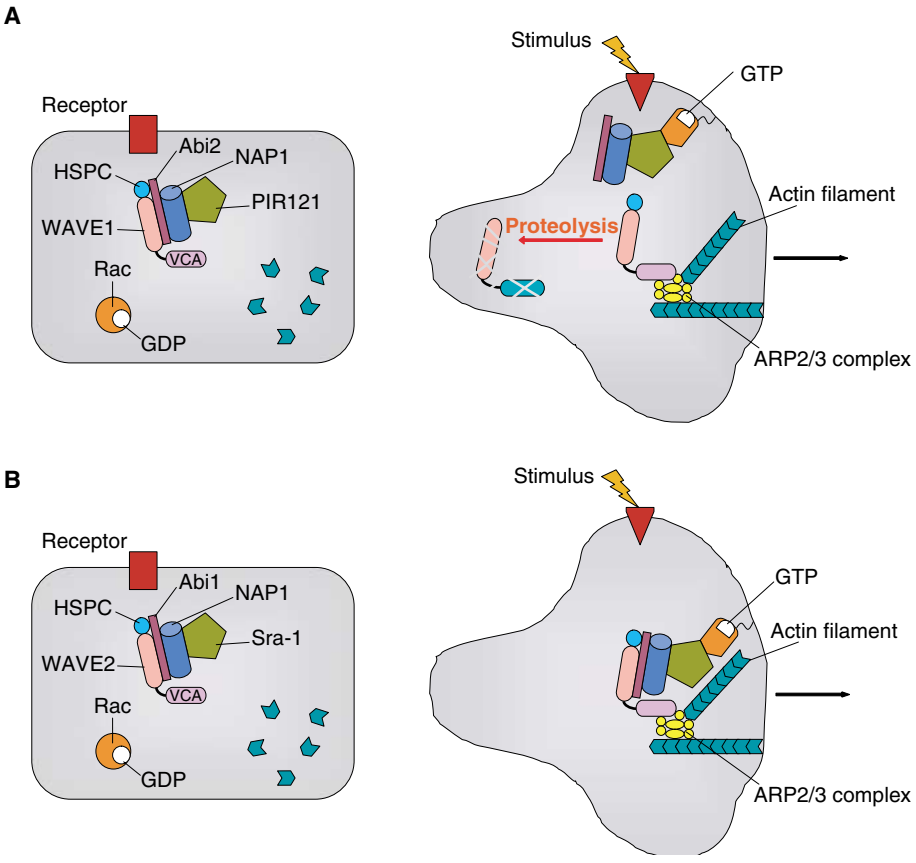

Figure 2. Two models for the regulation of WAVE activity. (**A**) In the dissociation model (Eden and others 2002; Gautreau and others 2004), WAVE1 is kept inactive by the WAVE complex. Binding of active Rac to PIR121 leads to the dissociation of the complex. WAVE is released together with HSPC300 and activates the ARP2/3 complex. The signal is terminated by the degradation of uncomplexed WAVE by a yet-unknown proteolytic system. (**B**) In the alternative model (Innocenti and others 2004; Steffen and others 2004), the WAVE complex is necessary for activation of WAVE *in vivo*. Active Rac recruits the whole complex to the plasma membrane, thereby ensuring proper localization.

Abil and Nap1 appear to constitute the core of the WAVE complex. Sra-1 is a peripheral subunit interacting with Nap, whereas WAVE interacts with Abi through its SHD domain, as well as with HSPC300.

The ARP2/3 Complex in Plants

Until recently, very little was known about the ARP2/3 complex and its regulation in plants. The sequencing of the *Arabidopsis* genome (The Arabidopsis Genome Initiative 2000) revealed that all of the ARP2/3 complex subunits apparently were present and evolutionarily conserved in plants. Interestingly, each of the subunit homologs is represented by a single copy gene in the genome, except from the ARPC1 and ARPC2 subunits, which are represented by two genes.

A class *of Arabidopsis* trichome mutants collectively called the ''distorted'' mutants have been central in resolving/understanding the function and regulation of the putative ARP2/3 complex in plants. The trichomes of the *alien*, *crooked*, *distorted1*, *distorted2*, *gnarled*, *klunker*, *spirrig*, and *wurm* mutants display randomized cell expansion, leading to reduced branch growth, especially of the secondary and tertiary branches, swelling of the stalk, and a generally irregular shape (Hülskamp and others 1994). Treatment with actin-interfering drugs phenocopied the ''distorted'' mutants, indicating that the actin cytoskeleton is affected (Mathur and others 1999; Szymanski and others 1999). Visualization of the actin cytoskeleton using either GFP protein fusions of actin filament-binding proteins (Mathur and others 1999) or phalloidin staining (Szymanski and others 1999) supported this observation, with the notable exception of *spirrig*, which appears to contain a normally organized actin cytoskeleton (Schwab and others 2003). Comparison of the different ''distorted'' mutants'' indicates that the class can be divided into subgroups on the basis of phenotype strength: *dis1* and *dis2* show the strongest phenotype, whereas *wrm*, *cro*, *ali*, *grl*, and *klk* show intermediate phenotypic strength (Basu and others 2005; Schwab and others 2003). A newly characterized mutant in this class, *distorted3* (discussed below), shows an even weaker phenotype (Basu and others 2005). Mapping of these mutants, in combination with characterization of T-DNA knockouts, led to the identification of the *WURM*, *DISTORTED1*, *CROOKED*, and *DISTORTED2* genes as homologs of ARP2, ARP3, ARPC5, and ARPC2,

respectively (El-Din El-Assal and others 2004b; Le and others 2003; Li and others 2003; Mathur and others 2003a, 2003b; Saedler and others 2004).

WAVE Regulates ARP2/3 in Plants

In a number of articles published over the last year, a mechanism regulating ARP2/3 complex activity in plants has been revealed. Putative homologs of all the subunits of the mammalian WAVE regulatory complex have been identified in *Arabidopsis* (Brembu and others 2004; Deeks and others 2004). Furthermore, T-DNA insertion mutants of the *Arabidopsis* Nap1 and PIR121 homologs (named NAP1/NAPP/GNARLED/AtNAP and SRA1/PIRP/AtPIR, respectively) display a phenotype similar to the putative ARP2/3 complex mutants (Basu and others 2005; Brembu and others 2004; Deeks and others 2004; E1-Din E1-Assal and others 2004a; Li and others, 2004; Zimmermann and others 2004). Not surprisingly, several *gnarled* alleles have been mapped to *NAP1* (Brembu and others 2004; E1-Din El-Assal and others 2004a). In maize, the *brick1* (*brk1*) mutation was mapped to the maize HSPC300 homologs (Frank and Smith 2002). The *brk1* mutation causes defects in lobe formation of epidermal cells, similar to the *distorted* phenotype. Although no *distorted* mutants have been mapped to the *Arabidopsis BRK1* homologs, the gene shares high similarity with mammalian homologs.

A small family of four genes encoding SCAR/WAVE-like proteins has been identified in *Arabidopsis* (Brembu and others 2004; Deeks and others 2004). The plant WAVE homologs have retained the N-terminal Scar homology domain (SHD), which interacts with Abi, and the C-terminal VCA domain/region, which activates the ARP2/3 complex (Figure 1). However, the central, proline-rich region of metazoan WAVE homologs does not appear to be well conserved in plants; only a small proline-rich motif (8–12 residues) is present (Brembu and others unpublished observations). This is not very surprising, as only five SH3 domain-containing proteins have been identified in *Arabidopsis* (Lam and others 2001; personal observation). Instead, the central part of the plant WAVE homologs contains a large region without similarity to any known domain. The central region is also highly divergent within the different family members, but some pair-wise similarity is found between SCAR1/At2g34150 and SCAR3/At1g29170 and also between SCAR2/At2g38440 and SCAR4/At5g01730. Because of the large central region, the plant WAVE homologs are substantially larger than identified WAVE/SCAR proteins from other organisms. The role of this region is unknown. A fifth *Arabidopsis* gene encodes a protein with a conserved SHD domain, but without a VCA region (Brembu and others 2004). The unique domain composition of WAVE5/At4g18600 is interesting; it is the only known protein that contains an SHD but lacks a VCA domain/region. This indicates that WAVE5 localization, and possibly activation, is regulated by the WAVE regulatory complex, and that other effectors than the putative ARP2/3 complex are activated by WAVE. If this assumption is correct, plants apparently have developed a novel RAC signaling pathway based on the WAVE pathway. T-DNA inactivation of one of the *Arabidopsis* WAVE homologs, SCAR2/DISTORTED3/WAVE4, results in defects of epidermal cell morphogenesis similar to the characterized *distorted* mutants, although with a milder phenotype (Basu and others 2005). Thus, functional data also connect the WAVE homologs to the ARP2/3 and the WAVE regulatory complex in *Arabidopsis*.

The last component of the WAVE regulatory complex, Abi is very poorly conserved in plants. Four genes encoding proteins with low similarity to the N-terminal part of mammalian Abi have been identified in *Arabidopsis* (Deeks and others 2004). The putative *Arabidopsis* Abi-like proteins lack a C-terminal SH3 domain, indicating that there are differences in the interaction partners of mammalian and plant Abi homologs. Thus, all of the subunits of the putative ARP2/3 and WAVE complexes appear to be evolutionarily conserved in plants.

Two of the ''*distorted*'' mutants, *ali* and *klk*, have still not been mapped to a specific gene. Could these two mutants be disrupted in proteins related to the *Arabidopsis* ARP2/3 complex or WAVE regulatory complex? *KLK* maps to markers very close to the *PIRP/PIROGI* locus (Hülskamp and others 1994), suggesting that *KLK* may be allelic to *PIRP/PIROGI*. *ALI* maps close to *CRK* on chromosome 4. The *ARPC4* locus lies at some distance from the mapped *ALI* locus; however, Li and others (2003) reported as unpublished data that a T-DNA insertion mutant for *ARPC4* showed a ''distorted'' phenotype. Preliminary observations at our laboratory support these data. Therefore, *ARPC4* could be identical to *ALI*.

Although neither the ARP2/3 complex nor the WAVE regulatory complex have been reconstituted in plants, protein–protein interaction experiments between pairs of the different subunits suggest that these complexes are present and intact. Yeast two-hybrid experiments and GST pull-down assays indicate that interactions appear to exist between ARPC2 and ARPC4 (El-Din El-Assal and others 2004b), NAP1 and SRA1 (Basu and others 2004; El-Din EI-Assal and others 2004a), SRA1 and ROP2/AtRAC4 (Basu and others 2004), SCAR2/DIS3 and

the Abi homolog ABIL1, and between SCAR2/DIS3 and ARPC3 (Basu and others 2005). Importantly, both the ROP/AtRAC-SRA1 and the NAP1-SRA1 interaction are highly conserved, because each of the partners in the interaction pairs can be substituted with the human homolog and still remain in an active interaction. Actin in a tobacco BY2 cell extract associates with the VCA region of SCAR1/WAVE1 fused to GST, in accordance with results from other organisms (Deeks and others 2004). Frank and others (2004) have shown that the SHD domain of SCAR1/WAVE1 and SCAR3/WAVE2 interacts *in vitro* with AtBRK1. The ability of *Arabidopsis* WAVE homologs to activate the bovine ARP2/3 complex *in vitro* has also been tested (Basu and others 2005; Frank and others 2004). The VCA domains of SCAR1, SCAR2, and SCAR3, as well as a maize WAVE/SCAR homolog, all induce significant actin polymerization in the presence of the bovine ARP2/3 complex, although SCAR1 and SCAR3 are less potent activators. Thus, the biological activity of the plant WAVE/SCAR homologs appears to be conserved.

Phenotypes of the ARP2/3 and WAVE Complex Mutants

A closer phenotypic analysis of the ''*distorted*'' mutants has revealed that other epidermal cell types are affected in addition to trichomes. Pavement cells of cotyledons and rosette leaves have a highly lobed, jigsaw puzzle shape in wild-type plants. By comparison, the pavement cells of ARP2/3 and WAVE complex mutants have reduced lobe length, resulting in a more regular cell shape (Brembu and others 2004; Le and others 2003; Li and others 2003; Mathur and others 2003a). Gaps between adjacent pavement cells have also been reported (Basu and others 2004, 2005; El-Din El-Assal and others 2004a, 2004b; Le and others 2003). Epidermal cells are also affected in the rapidly elongating hypocotyls of etiolated seedlings; mutant cells become unlinked at their ends, which subsequently curl out from the surface (Mathur and others 2003a, 2003b). When challenged into rapid growth, mutant root hairs become shorter and also appear somewhat curled compared to wild-type root hairs (Mathur and others 2003a, 2003b). Li and others (2004) report additional phenotypes for the *gnarled/napp/Atnap* and *pirogi/pirp/Atpir* mutants, including enhanced skotomorphogenesis and sugar responses and longer roots of dark-grown plants, as well as reduced chlorophyll content.

How is the actin cytoskeleton affected in the ''*distorted*'' mutants? Different methods for visualizing actin filaments have yielded slightly different results. When the actin-binding domain of mouse talin fused to GFP (Mathur and others 2003a, 2003b; Saedler and others 2004) or YFP (Brembu and others 2004) is used, actin filaments in wild-type trichomes generally appear to be longitudinally oriented from the initiation of branch elongation; a cap of dense actin staining is observed at the branch tips throughout branch elongation. In contrast, actin filaments in young trichomes of distorted mutants show a more random orientation, often terminating against the side of the branch, and dense actin at the branch tips is often absent. Actin filament bundling is observed to increase throughout trichome development in ''*distorted*'' mutants, and transversely linked actin bundles are also reported. Using phalloidin staining, anti–actin antibodies, or the actin-binding domain of *Arabidopsis* Fimbrin 1 fused to GFP, a lower level of actin filament organization is observed in mutant trichomes, similar to the observations made using GFP-Talin. However, increased actin bundling is not seen when these markers are used (Basu and others 2004, 2005; Deeks and others 2004; El-Din El-Assal and others 2004a, 2004b; Le and others 2003). Comparisons of actin filament density throughout elongating trichome branches instead indicate that the density of actin bundles in the branch core is lower in mutant trichomes than in wild-type trichomes.

It has been assumed that the microtubule cytoskeleton is not affected in the ''*distorted*'' mutants. On closer inspection, Saedler and co-workers (2004) found that sub-cortical or endoplasmic microtubules (EMTs) show increased clustering near sites of actin aggregation in mutant trichomes. This interesting observation led the authors to suggest a model (Mathur 2004) in which microtubules and actin filaments cooperate during polarized growth in plant cells (Figure 3). According to this model, transport of Golgi-derived vesicles to the plasma membrane is normally hindered by a dense and relatively stable cortical actin mesh. Polar growth is initiated by weakening of the cortical actin cytoskeleton by an ARP2/3 complex-mediated increase in actin polymerizing activity, probably in concert with other actin-regulating proteins. As a result, cortical F-actin becomes more dynamic and labile, facilitating transport of exocytic vesicles carrying plasma membrane and cell wall material to the growth site. Endoplasmic microtubules respond to the weakened cortical F-actin by establishing a ''reinforcing patch'' around the site, thereby restricting the area of expansion. In support of this model, the SRA1-interacting GTPase ROP2/AtRAC4

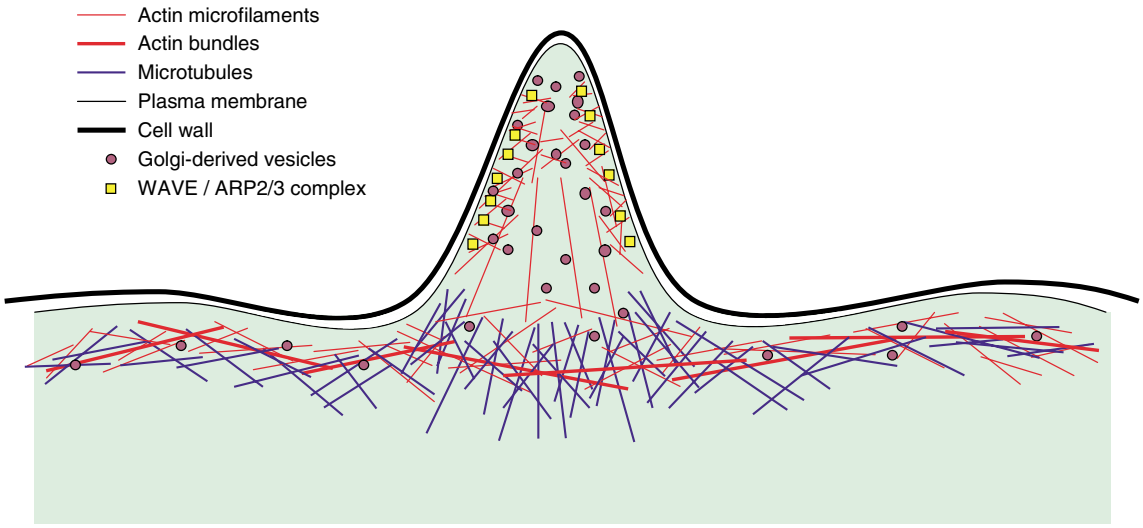

Figure 3. A hypothetical model for the cooperation between actin filaments and microtubules during polarized growth. In the model, proposed by Mathur (2004), transport of Golgi-derived vesicles carrying cell wall and plasma membrane material to the cell cortex is normally restricted by a dense and relatively stable actin mesh containing thick actin cables. Localized activation of ARP2/3 by the WAVE complex leads to the production of more dynamic and labile actin filaments, resulting in a loosening of the cortical actin mesh. Other proteins regulating actin dynamics also take part in this process. The weakened cortical actin facilitates transport of Golgi-derived vesicles to the cell cortex, possibly along actin filaments. To restrict loosening of the actin mesh to the site of polar growth, endoplasmic microtubules accumulate around the site as a ''reinforcing patch.''

localizes to expanding regions of the plasma membrane in leaf pavement cells and root hairs (Fu and others 2002; Jones and others 2002). Furthermore, immunolabeling in the brown alga *Silvetica compressa* showed that ARP2 is localized to the subapex of the elongating rhizoid tip (Hable and Kropf 2005).

Based on their observations that trichomes of ''*distorted*'' mutants have disorganized and, in later stages of development, reduced levels of core cytoplasmic actin bundles, Szymanski and others have presented an alternative hypothesis for ARP2/3 complex function in plants (Basu and others 2004, 2005; El-Din El-Assal and others 2004b; Le and others 2003; Szymanski 2005). They argue that plant cell expansion generally is accomplished by turgor pressure. Rather than exerting its function at the plasma membrane, they hypothesize that the ARP2/3 complex regulates actin filaments involved in positioning of vacuole compartments or the trafficking to these compartments. The dynamic movement of vacuolar structures is inhibited by Cytochalasin D, an inhibitor of actin polymerization (Uemura and others 2002), suggesting a role for F-actin in this process. Using anti-Rop1Ps antibodies, other investigators have localized RAC/ROP proteins to the tonoplast in growing tapetal cells of pea anthers (Lin and others 2001).

Both hypotheses for ARP2/3 function in plants suggest that a direct or indirect connection between the ARP2/3 complex and the cell vesicular system exists. In mammalian cells, Cdc42 participates in regulation of Golgi-to-ER transport and exocytosis through N-WASP and the ARP2/3 complex (Fucini and others 2002; Gasman and others 2004). Thus, it is not unlikely that several steps of vesicle transport in plant cells may be facilitated by ARP2/3-driven actin polymerization.

WAVE and ARP2/3 Mutations in Plants Are Not Lethal

Although a number of plant cell types are affected by mutations in subunits of the putative ARP2/3 complex or the WAVE regulatory complex, mutant plants are viable and fertile and overall morphology is not affected. In contrast, mutations in any of these genes in other eukaryotes affect viability (Vartiainen and Machesky 2004). In yeast, gene disruptions of ARP2/3 complex subunits led to defects in assembly and polarization of cortical actin patches (Winter and others 1999); a similar phenotype is observed in a yeast strain disrupted in the WASP/WAVE-like gene BEE1 (Li 1997). Mutations of *Drosophila* Scar/Wave, Cyfip/PIR121 and Kette/Nap are all lethal and display indistinguishable neuronal defects (Hummel and others 2000; Schenck and others 2003, 2004; Zallen and others 2002). The mild phenotype of the *Arabidopsis* ARP2/3 and

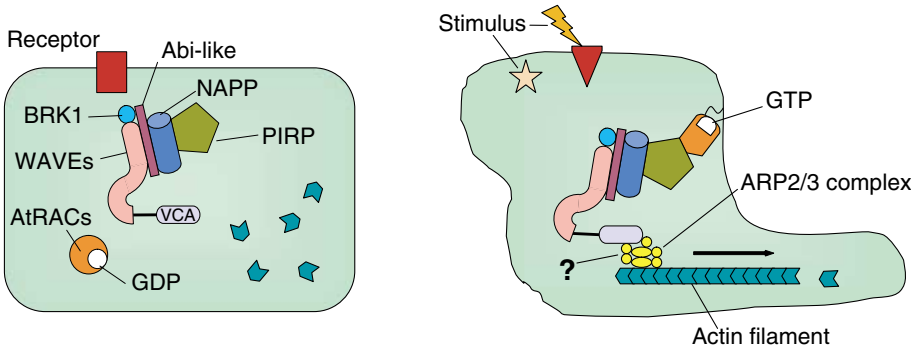

Figure 4. A hypothetical model for regulation of the ARP2/3 complex in plants. The illustrated model is based on the mechanism proposed by Innocenti and others (2004), but the dissociation model (Gautreau and others 2004) may also be valid. The signal activating RAC/ROP GTPases can be intracellular or extracellular. Active RAC/ROP binds PIRP/PIROGI/AtPIR, thereby translocating and activating the WAVE complex. The large central domain of plant WAVEs may interact with other proteins. Other, yet unidentified activators of the ARP2/3 complex might exist in plants.

WAVE complex mutants clearly indicates that the ARP2/3 pathway of actin filament nucleation is not essential for normal development and function of most plant cell types. In accordance with these results, plants grown in the presence of the actin depolymerizing agent latrunculin B were able to develop morphologically normal organs. However, the plants were highly stunted, indicating that actin is essential for cell elongation (Baluska and others 2001). Consequently, one or several other protein classes must be responsible for some of the actin-nucleating activity in plants. The only known protein family in plants with this activity besides the ARP2/3 complex is the formin family (Deeks and others 2002; Zigmond 2004). The *Arabidopsis* formin gene family is large, with 21 members divided into two subfamilies (Deeks and others 2002). Yeast formins associate in dimers that bind G-actin and induce nucleation at the barbed end. During filament elongation, the formin dimer stays associated with the barbed end as a protective cap while permitting assembly of actin monomers onto the barbed end (Kovar and Pollard 2004; Zigmond and others 2003). Formins produce long, unbranched actin bundles, in contrast to the highly branched filament network produced by the ARP2/3 complex. Zigmond (2004) proposes that formin-nucleated filament bundles may sustain tension for cell contraction, whereas ARP2/3 complex-nucleated filament networks may sustain compression for cell protrusion. Whereas this hypothesis might hold true for migrating metazoan cells, the relationship between formins and the putative ARP2/3 complex could be different in plant cells, whose movement is restricted by the cell wall. The recent discovery of a novel actin-nucleating factor in *Drosophila*, Spire (Quinlan and others 2005), indicates that unknown proteins with actin polymerizing activity may exist in plants.

Recently, phosphatidylinositol 3,4,5 phosphate (PtdIns(3,4,5)P_3) has been shown to bind the basic region of human WAVE2 (Oikawa and others 2004). PtdIns(3,4,5)P_3 appears to be important for proper localization of WAVE2 at the plasma membrane. In contrast, mammalian WASP (and N-WASP) has been found to be bound by PtdIns (4,5)P_2 (Prehoda and others 2000; Rohatgi and others 2000). A basic region is found at a conserved position in plant WAVE/SCAR homologs, suggesting that phosphoinositides may take part in localization and/or activation of plant WAVEs. PtdIns(3,4,5)P_3 has never been observed in plants (Mueller-Roeber and Pical 2002), but PtdIns(4,5)P_2 has been shown to take part in signaling to the actin cytoskeleton during pollen tube growth (Kost and others 1999). PtdIns(4,5)P2 localizes to the plasma membrane at the pollen tube tip; furthermore, the RAC/ROP GTPase At-Rac2/ROP5/AtRAC6 interacts physically with a PIP kinases, which produces PtdIns(4,5)P_2. A gene encoding a type II inositol polyphosphate 5-phosphatase was recently identified as the target of the *fragile fiber3* (*fra3*) mutations in *Arabidopsis* (Zhong and others 2004). The actin cytoskeleton in *fra3* interfascicular fiber cells show increased bundling compared to wild-type fiber cells, suggesting that phosphoinositides play a role in plant actin organization.

Conclusions/Perspectives

An evolutionary conserved signaling pathway for regulation of actin polymerization and filament branching exists in plants. A hypothetical model for this pathway is shown in Figure 4. Unknown

extracellular or intracellular signals may activate one or several RAC/ROP GTPases. Active RAC/ROP binds the SRA1/PIRP/ AtPIR subunit of the WAVE regulatory complex, thereby recruiting the complex to the site of activation. RAC/ROP binding probably leads to a conformational change or dissociation of the complex, resulting in exposure of the SCAR VCA domain, which binds and activates the ARP2/3 complex. Mutational studies of the ARP2/3 complex and the WAVE regulatory complex suggest that this pathway is important for morphogenesis of epidermal cell types with complex growth patterns, such as pavement cells and trichomes, and to a lesser extent, for tip-growing cell types such as root hairs. Because none of these mutants are lethal, other protein classes with actin nucleating activity, such as formins or other still-to be-discovered proteins, likely perform functions essential for plant viability.

Still, many questions remain unanswered. The presence of a large, non-conserved region in the WAVE homologs and the lack of a SH3 domain in the Abi-like proteins in *Arabidopsis* suggest that the WAVE regulatory complex in plants may interact with novel proteins. Identifying these interaction partners might reveal plant-specific mechanisms for modulation of WAVE activity. At what intracellular sites does the ARP2/3 complex exert its function? How do actin filaments and microtubules cooperate during polarized growth? Is there a physical interaction via cross-linking proteins, or is the interplay of a more indirect nature? What is the role of the ARP2/3 complex in vesicle transport in plant cells? What is the labor division between the ARP2/3 complex and other protein classes with actin-nucleating activity in plants, and how do they cross-talk? Clearly, we have a long way to go before the role of the WAVE and ARP2/3 complexes in plants is fully understood.

Note Added in Proof

In a screen for *Arabidopsis* mutants with trichome branching defects, Zhang and others (2005) isolated the mutant *irregular trichome branch1* (itb1). Positional mapping shows that *itb1* is allelic to dis3 (Basu and Others, 2005). In addition to actin defects, the dyamics of cortical microtubules also appears to be altered in the *itbl* mutant. Pull-down assays indicates that the SHD domain of ITB1/ SCAR2 interacts with BRK1, as previously shown for SCAR1 and SCAR3 (Frank and others, 2004).

A study of the ARP2/3 complex in the moss *Physcomitrella patents* has also recently been published (Harries and others, 2005). RNAi suppression of ARPC1 expression results in defects in cell morphology and abnormal cell division. *arpc1* RNAi plants are defective in bud formation due to the lack of rapidly elongating caulonemal cells. *arpc1* protoplasts show increased sensitivity to osmotic shock, and also fail to form polar extensions through tip growth, indicating the importance of the ARP2/3 complex in this process in *Physocmitrella*.

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