The ARP2/3 complex: giving plant cells a leading edge

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Summary
The seven-subunit ARP2/3 complex is an efficient modulator of the actin cytoskeleton with well-recognized roles in amoeboid locomotion and subcellular motility of organelles and microbes. The recent identification of different subunit homologs suggests the existence of a functional ARP2/3 complex in higher plants. Mutations in some of the subunits have revealed a pivotal role for the complex in determining the shape of walled cells and focused attention on the interlinked processes of cortical-actin organization, growth-site selection, organelle motility and actin–microtubule interactions during plant cell morphogenesis. The findings supporting a global conservation of molecular mechanisms for membrane protrusion have been further strengthened by the identification of plant homologs of upstream regulators of the complex such as PIR121, NAP125 and HSPC300. As discussed here, the recent studies suggest that there might be hitherto unappreciated molecular and cell-biological commonalities between protrusion mediated motility of animal cells and polarized, expansion-mediated growth of plant cells. ***BioEssays*** **27:377–387, 2005. © 2005 Wiley Periodicals, Inc.**

Introduction

A living cell can be thought of as an elastic, fluid-filled balloon capable of being molded into any form. As long as it remains just membrane-bound, as in the case of most animal cells, it retains a plasticity of form that allows it to change shape according to its requirement. For animate cells, these changes in shape are essential for whole-body displacement (locomotion).[1] However, when a cell becomes encased in an exocyst or a cell wall, as occurs in many fungi and plant cells, it loses both its flexibility of form as well as the capacity for locomotion. Walled cells, therefore, unless equipped with special locomotor organs, can only grow towards or away from a stimulus. Animal cell locomotion and growth-dependent extension of plant cells thus clearly appear to be very different processes.

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Abbreviations: ARP2/3, Actin Related Protein 2/3; *DIS*, *DISTORTED* class of *Arabidopsis* genes; F-actin, Filamentous actin; GFP, Green Fluorescent Protein; HSPC300, Hematopoietic stem progenitor clone 300 protein; NAP125, Nck-associated protein 125; PIR121, p53-121F-induced; ROP, Rho-like GTPases of plants; SCAR, Suppresor of cAMP receptor from *Dictyostelium*; SRA1, Specifically Rac1-associated; VCA, verproline homology connecting acidic domain; WAVE, Wiskott-Aldrich syndrome protein family VErprolin-homologous protein.

Animal cell locomotion relies upon localized membrane protrusion and is intimately linked to cytoskeletal dynamics.[1] Molecular and cell-biological dissection of interactions at the leading edge of an animal cell suggests that membrane protrusion could be related to a more fundamental actin polymerization-based form of motility that is exhibited by certain microorganisms and endosymbiotic subcellular organelles.[2–4] Recent studies reveal that certain molecular components in expanding, non-motile, plant cells[5–18] bear strong similarity to those found in motile, non-plant cells.[19–21]

One such molecular component, the ARP2/3 complex, which hitherto had largely been implicated in motility,[2–4] has now emerged as a pivotal player in cell shape determination for higher plants.[5–10] Although the plant ARP2/3 complex has not been isolated and biochemically characterized, and it is still unclear whether its composition and function precisely match those described for other organisms, sequence homologies (Table 1), complementation of *Arabidopsis* mutants by respective animal homologs,[5] and rescue of yeast mutants by plant homologs,[8,9] nevertheless, suggest a high degree of functional conservation of the complex in plants. Can this singular finding serve to diffuse the basic boundaries between animal cell motility and the expansion of walled cells? Here I first summarize the recent findings on the putative ARP2/3 complex and its key role in plant cell morphogenesis. Subsequently, I address the contextual similarities between motile animal cells and non-motile plant cells to discuss whether motility and growth by cell expansion, apparently two very different processes, operate on fundamentally similar mechanisms.

The ARP2/3 complex: a conserved modulator of the actin cytoskeleton

The ARP2/3 complex, consisting of seven subunits of differing molecular sizes[22–24] was first discovered in *Acanthamoeba castellanii*[25] and is now known from diverse organisms.[19]

Table 1. Comparison of amino-acid similarity of *Arabidopsis* ARP2/3 complex subunits with their counterparts from other organisms

		% amino-acid identity					
Subunit	**AtDB Acc. No**	**Ce**	**Dd**	**Dm**	**Hu**	**Sc**	**Sp**
ARP2	At3g27000	60	63	61	62	57	53
ARP3	At1g13180	56	59	57	59	53	55
*ARPC1a	At2g30910	39	43	37	41	34	34
*ARPC1b	At2g31300	38	43	24	42	34	34
**ARPC2	At1g30825	—	33	26	26	28	26
ARPC3	At1g60430	41	41	40	47	39	43
ARPC4	At4g14140	48	68	61	57	—	49
ARPC5	At4g01710	32	31	35	32	43	25

Ce, *Caenorhabditis elegans*; Dd, *Dictyostelium discoideum*; Dm, *Drosophila melanogaster*, Hu, Humans; Sc, *Saccharomyces cerevisiae*; Sp, *Schizosaccharomyces pombe*.
*The ARPC1/p41 homolog is encoded by 2 genes (designated a and b) arranged in reverse orientation and in close proximity.[6,7]
**Two genes are also reported for the ARPC2 Subunit in *Arabidopsis* (ATARPC2B) and rice (OSARPC2B).[9]

The complex is an enhancer of actin nucleation and polymerization[22] and, through its binding to a parent actin filament, it initiates the formation of a dynamic, dendritic array of F-actin.[22,26,27] It localizes to well-characterized regions of dynamic actin cytoskeleton activity, such as macropinocytotic cups, the leading edges of lamellipodia in animal cells and to motile actin patches in yeasts.[28–30] The ARP2/3 complex is also renowned for its role in actin-polymerization-based rocketing motility of enteropathogenic organisms like *Listeria monocytogenes* and *Shigella*,[31–33] and the intracellular motility of endosomes, lysosomes, pinocytic vesicles and mitochondria.[34–36] Malfunctioning of the complex leads to a variety of cellular disorders in different organisms (Table 2), including, in severe cases, to non-viable cells.[37–40]. In animate cells, the functional role attributed to the complex is usually in the context of motility. However, yeast mutants for different ARP2/3 complex subunits, though limited in the range of shape alterations that they are able to exhibit, have also implicated the complex in cell morphogenesis.[41] Irrefutable evidence for its involvement in mechanisms relating to cell morphogenesis (Figs 1, 2) now comes from higher plants, where both cell function and whole plant survival are intimately linked to the shape acquired by a cell during its development.

In wild-type *Arabidopsis*, epidermal hairs or trichomes are unicellular, stellate, 2–4 branched, 300–500 μm tall cells (Fig. 1A). Purely on the basis of randomly misshapen trichomes (Fig. 1B), eight genes, *ALIEN, CROOKED, DISTORTED1, DISTORTED2, GNARLED, KLUNKER, SPIRRIG* and *WURM* were grouped together into a *DISTORTED* class.[42] Subsequently it was shown that treatment of wild-type *Arabidopsis* leaves with actin-interacting drugs like cytochalasins, latrunculins, phalloidin and jasplakinolide phenocopied the distorted mutant trichomes.[43,44] The drug-based association with actin strongly suggested that the *DIS* class of genes could be

Table 2. Some cellular defects attributed to compromised ARP2/3 complex activity in different organisms

Organism	Salient phenotypes	Key references
Arabidopsis	Shape defects: Mutations in ARP2, ARP3, ARPC2, ARPC5 lead to random shape alterations in epidermal cells due to misdirected expansion. Display defects in actin organization.	(5–10)
Budding yeast	Shape defects: Mutants in ARPC1non-viable. Mutants in other subunits conditional; usually display defects in cortical actin cytoskeleton. Mutants in ARPC5 and ARP3 subunits exhibit aberrant mitochondrial behavior.	(36,41)
C. elegans	Motility defect: RNAi mediated depletion of different subunit leads to defects in ventral closure.	(74)
Drosophila	Motile cell behavior defective: Loss-of-function mutants frequently embryo/juvenile stage lethal. Affect blastoderm organization, ring canal expansion, axon development and eye morphogenesis.	(39,76)
Fission yeast	Shape defects: Mutants in ARPC1non-viable. Mutants in other subunits conditional; display vacuolation and actin cytoskeleton defects. Defects in contractile ring formation during cytokinesis.	(37,40,75)
Mammals	Motile cell behavior affected: RNAi of the ARPC3 subunit is lethal in mouse.	(38)

involved in regulation of the actin cytoskeleton and resulted in a candidate gene approach for the cloning of these genes. *CROOKED* was the first *DIS* class gene to be cloned.[5]

CROOKED encodes the smallest subunit (p16/ARPC5) of the ARP2/3 complex.[5,6] Soon after its identification, *DIS1, DIS2* and *WRM* were identified as the ARP3, ARPC2/p35 and ARP2 subunits of the complex, respectively.[6–10] A single report[6] mentions finding a distorted trichome phenotype in a T-DNA insertion mutant line for the ARPC4/p20 gene. The strong phenotypic similarities among mutants identified for the five different subunit homologs (Fig. 2; Table 3) strongly suggests that, in higher plants, the complex functions as a whole and that each of the subunits plays a crucial role in maintaining its integrity. However, mutants corresponding to ARPC1/p41 and ARPC3/p21 subunits are conspicuously missing since none of the remaining four distorted mutants map to the same chromosomal locations as these genes. Perhaps in plants too, the situation for these two genes is similar to that observed in yeasts where the ARPC1/p41 subunit is essential for cell viability.[37,41] Mutations in these two subunits might result in embryo lethality in plants. Alternatively, specific, and hitherto unidentified, growth conditions might be required for eliciting a mutant phenotype for these genes. Nevertheless, as discussed below, studies on mutants in four subunits (hereafter referred to as ARP mutants) have provided some interesting insights into plant cell morphogenesis.

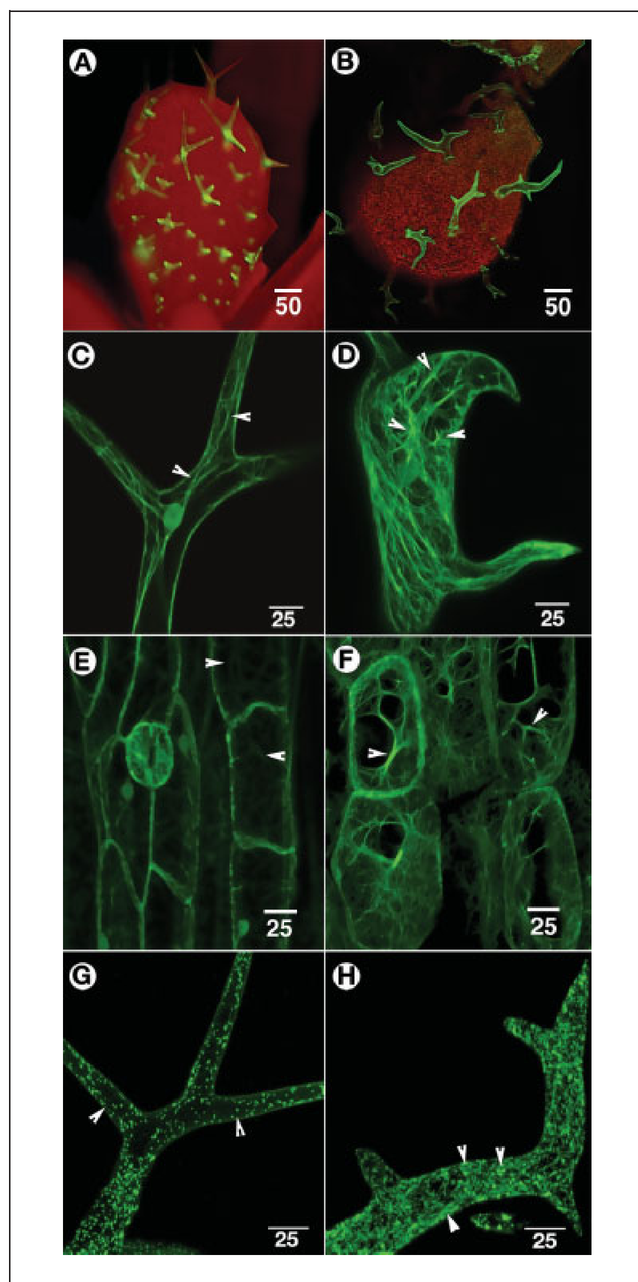

Figure 1. Comparison of some characteristic morphological and intracellular features between wild-type and ARP mutant epidermal cells. **A:** Branched, stellate, epidermal trichomes on a wild-type *Arabidopsis* leaf. **B:** Distorted trichomes on the leaf of *arp2/wurm* mutant. **C,D:** Expression of a GFP-mTalin fusion protein[77] visualizes the F-actin organization in **C:** a mature wild-type trichome cell with characteristic longitudinally stretched actin cables (arrowheads) and **D:** a mature *arp2/wurm* trichome that exhibits greatly increased F-actin bundling and aberrant lateral connections between the actin bundles (arrowheads). **E:** Hypocotyl cells from a light-grown wild-type seedling display well-stretched actin cables (arrowheads) and a diffuse actin mesh visualized using the GFP-mTalin probe. **F:** Hypocotyl cells from a *crooked* mutant seedling grown under identical conditions as the wild-type and expressing the GFP-mTalin marker show altered, swollen shapes and a massive increase in F-actin bundles (arrowheads). **G:** Golgi bodies visualized using an ERD2-GFP fusion protein[78] are seen as single entities in a wild-type trichome (arrowheads) whereas in **H:** an *arpc5/crooked* trichome shows increased numbers and aggregation (arrowheads) of Golgi bodies.

Emergent phenotypes in *ARP* mutants

Although initially identified and grouped together on the basis of their distorted trichome phenotype[42] subsequent transcript analysis for *CRK, DIS1, DIS2* and *WRM* showed that these genes are expressed ubiquitously at low levels in the plant.[5,6,8,9] Consistent with this observation a closer examination of the epidermal surface in these mutants identified additional phenotypic characters (Fig. 2). Shape defects were observed for tip-growing root hair cells that in wild-type plants can elongate up to ten times their initial length of 60–80 μm. In the *crk* and *wrm* mutants, root-hair elongation is considerably reduced and the resultant thick hairs display an increased degree of waviness or nutation.[5,6] Forcing mutant root hairs to elongate rapidly by increasing their distance from the substratum further augments the wavy phenotype.[5] Similarly,

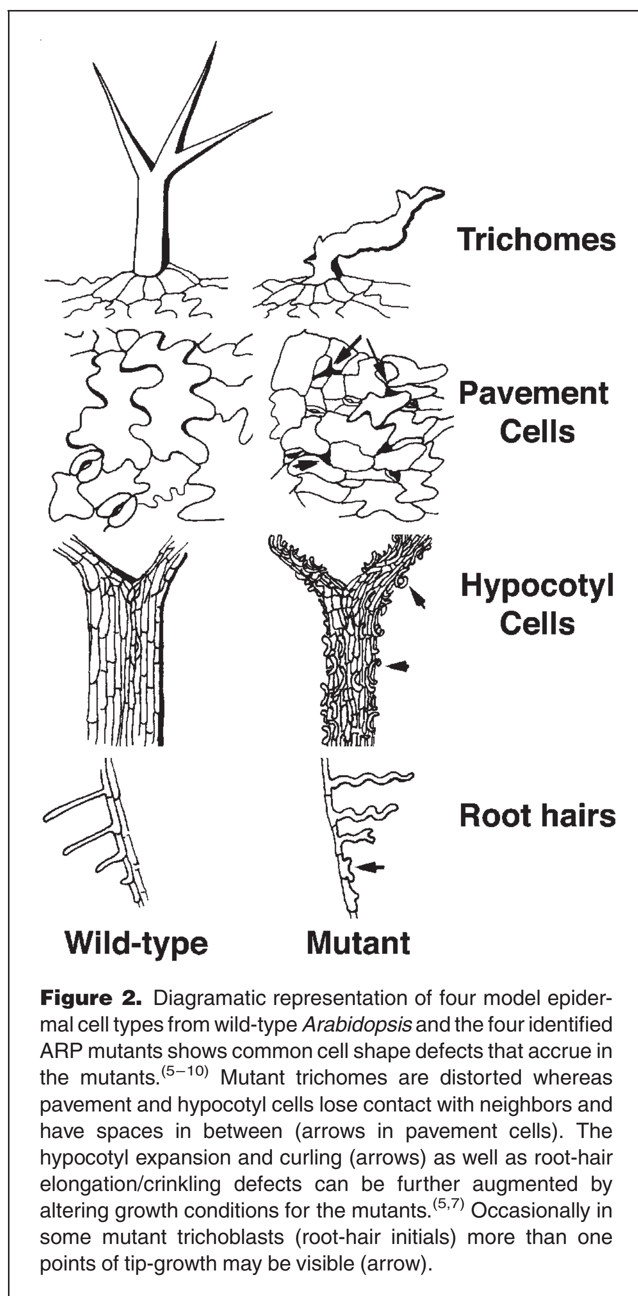

Figure 2. Diagramatic representation of four model epidermal cell types from wild-type *Arabidopsis* and the four identified ARP mutants shows common cell shape defects that accrue in the mutants.[5–10] Mutant trichomes are distorted whereas pavement and hypocotyl cells lose contact with neighbors and have spaces in between (arrows in pavement cells). The hypocotyl expansion and curling (arrows) as well as root-hair elongation/crinkling defects can be further augmented by altering growth conditions for the mutants.[5,7] Occasionally in some mutant trichoblasts (root-hair initials) more than one points of tip-growth may be visible (arrow).

hypocotyl cells, which in the wild-type usually elongate rapidly following seed germination, display increased lateral, rather than longitudinal, expansion in the mutants. This produces fat, misshapen hypocotyl cells.[5–10] Upon being stimulated to elongate further under low-light conditions, mutant hypocotyl cells lose contact with their neighbors along the cross-walls and curl out randomly.[5,7] Frequent and substantial gaps in the hypocotyl epidermis thus result from different elongation rates exhibited by neighboring mutant cells. In a similar manner, the petioles of cotyledons and the first pair of leaves also display cells that lose contact with each other and curl out, giving the mutant petioles a rugged, disorganized look. Further, in wild-type plants cotyledon epidermal cells expand laterally to produce characteristic lobed, jig-saw-puzzle-shaped pavement cells. In the ARP mutants, lobe formation in cotyledon cells and their general expansion is seriously impaired.[5–10] The formation and patterning of stomatal complexes on the epidermis also appears disturbed in the mutants.[5,7]

Cells in layers underlying the epidermis might also display subtle defects but these have been more difficult to observe and quantify due to the compensating influences between neighboring cells present on all sides. Epidermal cells displayed clear phenotypes mostly on the side exposed to the external environment. The observations on rapidly elongating cells suggest that the complex is required during active growth when actin-cytoskeleton dynamics might play pivotal roles in intracellular trafficking of vesicles and subcellular components for achieving a rapid increase in the cell volume.[5–10] Consistent with this view, an overall reduction in plant size has been observed for some of the mutant alleles (eg *wrm1–2*) and a decrease in fresh weight has been documented for *dis1*[8] and *dis2*.[9] The fact that the ARP mutants complete their life cycle and are fully viable suggests that, in higher plants, the complex works more as an enhancer of an intracellular phenomenon linked to growth rather than as a strictly essential component of basic life processes.

In an effort to understand the processes that might be mediated by the complex during plant cell morphogenesis, all the studies on the ARP mutants (Table 3) have also involved extensive cell biological observations.

Cell biological observations on ARP mutants

The actin cytoskeleton

Observations on the F-actin organization have played an important part in characterizing the ARP mutants[43–45] The first two independent studies[43,44] utilized different actin visualization methods and, based on observations on six of the eight mutants, reached a common conclusion: that the actin cytoskeleton is intimately involved in trichome cell morphogenesis and is aberrant in the mutants. Descriptions of the F-actin organization in mutant trichomes have ranged from randomly localized dense aggregates of F-actin,[5,6] highly bundled F-actin[6,7] and randomly located cortical actin patches,[10] to detailed differences between immunolocalized core cytoplasmic F-actin and total cytoplasmic F-actin.[8,9] Two of the major aberrations in actin organization consistently observed in mutant trichomes are the occurrence of random F-actin patches, and an apparent increase in the degree of lateral connections between actin cables, especially in non-expanding regions of the cell. However, areas between dense actin patches frequently display very clear F-actin strands with no signs of bundling. Thus, as compared to wild-type trichomes, the F-actin organization in mutant trichomes

Table 3. The *DISTORTED* class of *Arabidopsis* genes

Gene	Chr	AtDB Ac. No	Homolog for	T-DNA lines**	References
ALIEN	4	unknown	unknown	Unknown	(41)
CROOKED	4	At4g01710	ARPC5	SALK_123936	(5,6)
DISTORTED1	1	At1g13180	ARP3	SALK_010045	(6–8)
DISTORTED2	1	At1g30825	ARPC2	Unknown	(9,10)
GNARLED	2	At2g35110	NAP135	SALK_014298, SALK_058074 SALK_038799, SALK_135634 SALK_009695	(11–13,15)
*KLUNKER**	5	At5g18410	PIR121	SALK_106757	(13,14)
SPIRRIG	1	Unknown	Unknown	Unknown	(41)
WURM	3	At3g27000	ARP2	SALK_03448, SALK_077920	(6–8)

*Based on the same *Arabidopsis* database gene accession number [13,14,16] for the published *PIRP* [13] and *PIROGI* [14] genes and pre-publication information from M. Hülskamp these genes appear to be the *KLK* gene.
**For additional alleles and insertion lines please see original papers.

appears patchy (Fig. 1C versus D). Amongst the different characterized mutants, the greatest amount of F-actin bundling appears in *crk* trichomes and the least in *dis2* (Refs 5,10 and personal observations).

Increased F-actin bundling and aberrant actin patches are also seen in other cell types in the ARP mutants.[5–7,10] Changes in F-actin organization have been best followed in the aberrantly elongating hypocotyl cells of *wrm* and *dis1* mutants.[7] Whereas F-actin organization in wild-type hypocotyl cells becomes increasingly diffuse as they elongate; in mutant *wrm* and *dis1* cells, F-actin starts aggregating at the ends of elongating cells. Consequently cell expansion appears to cease in the regions with actin accumulation and apparently causes neighboring cells to separate from each other.[7] Similar observations have been made for mutant petiole and cotyledon cells, which exhibit a patchy actin organization rather than a regular, diffuse F-actin mesh.[6,8]

The microtubule cytoskeleton

Only two published reports describe the microtubule organization in ARP mutants. Basing their conclusions primarily on trichomes of the *dis2* mutant and distorted trichomes obtained through actin-drug treatment, Schwab et al found that, despite differences in actin organization, cortical microtubules followed the general contours of the distorted cell.[45] However, Saedler et al[10] observed that, whereas cortical microtubule arrays were as described by Schwab et al., endoplasmic microtubules formed relatively stable clusters whose positioning coincided with that of the aberrant dense actin patches in *dis2* and *crk* trichomes. They concluded that actin patches might guide the distribution and dynamics of endoplasmic microtubules.[10] Although the validity of these observations needs to be investigated for other cells in the ARP mutants, it is interesting that, in budding yeast, the ARPC2/p35/DIS2 subunit has been shown to be involved in two genetically separable calmodulin-mediated functions that independently regulate the actin and microtubule cytoskeletons.[46] Similar interactions of ARP2/3 complex subunits with the microtubule cytoskeleton remain a possibility in plants.

Organelle distribution and motility in the mutants

The ARP 2/3 complex has been identified as a critical component for the rocketing actin polymerization-propelled motility exhibited by different microbes and subcellular structures in yeast and animal cells.[2–4] Observations on the *crk* mutant have suggested that the complex might have a role in organelle motility in plants as well.[5] In wild-type cells, Golgi bodies are independent and highly motile organelles that move along actin tracks.[47] Trichomes in *crk*, however, display aggregates of up to 8–10 Golgi bodies (Fig. 1G versus H). In addition, Golgi bodies and peroxisomes in *crk* trichomes exhibit localized fluctuations in velocity that are not usually observed in wild-type cells.[5] Low rates of organelle motility in the mutant were generally associated with intracellular regions with dense F-actin aggregation, whereas areas with a diffuse F-actin mesh, displayed organelle motility rates comparable to wild-type cells.[5] At this stage, it is unclear whether, as has been demonstrated for mitochondrial motility in budding yeast cells,[36] the p15/ARPC5/CRK subunit of the complex is directly involved in organelle motility or the observations of reduced velocity result merely from the regional alterations in F-actin density observed in *crk* trichomes.

ARP2/3 complex mutants in yeasts have vacuole biogenesis defects.[48] Similar observations of an abnormal increase in the proportion of unfused mini-vacuoles suggesting compromised membrane fusion capability have been made for *wrm, dis1*[7] and *dis2* mutants.[9] A role for the ARP2/3 complex in vesicle trafficking and endosome motility in plants, similar to that described in yeasts,[3,4,35] has so far only been a matter of speculation.

Putative regulatory molecules for the complex in plants

In animals and fungi, the ARP2/3 complex is regulated by a variety of proteins and protein complexes.[19,49,50] One such regulatory complex involves the activator WAVE (Wiskott-Aldrich syndrome protein family VErprolin-homologous protein) /SCAR (Suppresor of cAMP receptor from *Dictyostelium*) proteins.[51,52] These proteins are characterized by a VCA (verproline homology connecting acidic domain) region that binds to the ARP2/3 complex and induces actin filament branching,[21,50] In one of the proposed regulatory models[50] SCAR1 associates with an HSPC300 (Hematopoietic stem progenitor clone 300) protein and its VCA region, in the default state, is kept repressed by three proteins: SRA1 (Specifically Rac1-associated[53]) also known as PIR121 (p53–121F-induced),[54] NAP125 (Nck-associated protein 125[51,55]) and Abl-interactor2 (Abi2[50]). Activation of a Rac-GTPase and its binding to the inhibited pentameric complex releases SCAR1-HSPC300 from the rest of the complex and allows it to activate the ARP2/3 complex.[50]

Whereas clear homologs (see note added in proof) of the ABI2 or the ARP2/3 activator SCAR/WAVE have not been identified, several *Arabidopsis* proteins exhibit sequence homology with the domains of AB12 and WAVE/SCAR that are specifically required for the assembly of the pentameric complex.[12,18,20,21] Moreover, *GNARLED* encodes a NAP125 homolog,[11–16] and a PIR121/SRA1[13,14,16] homolog might be the *KLUNKER* gene (M. Hülskamp; personal communication). The external distorted trichome phenotype of both *grl* and *klk* mutants is similar to that of the ARP2/3 complex mutants and strongly suggests that products of these two genes feed into the ARP2/3 complex regulatory pathway. In addition, both the human and plant NAP125 homologs have been shown to interact with *Arabidopsis* ATSRA1[11] suggesting they might work as part of a complex. The third member of the proposed SCAR/WAVE complex, a putative homolog of the HSPC300 protein, has also been identified as the *BRICK1* gene of maize.[17] Like the ARP mutants, the maize *brk1* mutant exhibits reduced lobe formation in leaf epidermal cells and aberrant morphogenesis of stomatal complexes.[17] An *Arabidopsis BRICK1* homolog (At2g22640) exists but a mutant phenotype has not been reported so far. Finally, the requirement for a small GTPase that should initiate the dissociation of the SCAR1–HSPC300–NAP125–PIR121–Abi2 complex[50] appears to have been met, as Basu et al[14] document interactions between a SRA1/PIR121 homolog and AtROP2 a Rho GTPase of plants.[14,56] This interaction provides an enviable explanation for the *in-planta* localization pattern of different ROP-GFP fusion proteins to the extending tips of root hairs and pollen tubes as well as to regions of increased expansion in diffuse-growing cells[56–59] and is a crucial link for the transduction of environmental cues to the actin cytoskeleton through ARP2/3 complex activation.

Based on the function of the ARP2/3 complex suggested from studies in non-plant systems, it is believed that the complex acts as a potent actin nucleator in plants as well. However, ARP mutant phenotypes possess an actin organization, which, even though aberrant, suggests the presence of another actin nucleator. It is noteworthy that overexpression of the *Arabidopsis* formin AFH1 in tobacco pollen tubes induces a considerable increase in cytoplasmic actin cables, leading to depolarization and eventual arrest of tube extension.[60] Moreover, there are at least 21 formin-like proteins in higher plants.[61] An interesting hypothesis has therefore been suggested by Brembu et al.,[13] who propose that there may be an equilibrium between formin activity and ARP2/3 complex activity, with the former being responsible for production of actin cables and the latter for fine, cortical F-actin meshwork. A compromised ARP2/3 complex, as in the ARP mutants, would then be expected to favor increased formin activity to produce more actin cables and reduce cortical F-actin.[13]

Presently, though the details of interactions between the putative regulators and the ARP2/3 complex are far from clear, there are unambiguous indications that these different proteins are active in regions of acute cell expansion, in a manner strikingly similar to that observed at the leading edge of motile cells (Fig. 3).

Insights from the discovery of the ARP2/3 complex in plants

Actin polymerization activity in a system is dependent upon nucleation rates and the number of available barbed ends.[62] Free barbed ends can arise by the uncapping of existing filaments, by cleaving existing filaments, or by nucleation of new filaments.[62,63] The ARP2/3 complex has been identified as an actin nucleator as well as a minus-end capping factor.[22,26] Its binding to an existing actin filament enhances the rate of polymerization and results in an extensive dendritic array of daughter filaments. For motile cells, the creation of this dendritic actin array at the leading edge is critical, as it is believed to generate the force required for driving membrane protrusion.[27,29]

In an interesting parallel to the localization of the complex in motile cells, a recent study immunolocalizes the large ARP3 subunit of the complex to the tips of extending root hairs.[64] Why should a major subunit of the complex, ostensibly signifying the presence of the entire complex, be localized at the very tip of an extending non-motile plant cell? Independent observations on tip-extension and actin dynamics in tip-growing pollen-tubes have also suggested a major role for actin-polymerization in tube extension.[65,66] Moreover, the effects of mutations in ARP2/3 complex subunits are most apparent in cells that undergo a rapid growth phase during their morphogenesis (epidermal trichomes, elongating hypocotyls and petiole cells).[67] Which processes involving the ARP2/3 complex in motile cells could be equally important for expansion of non-motile cells?

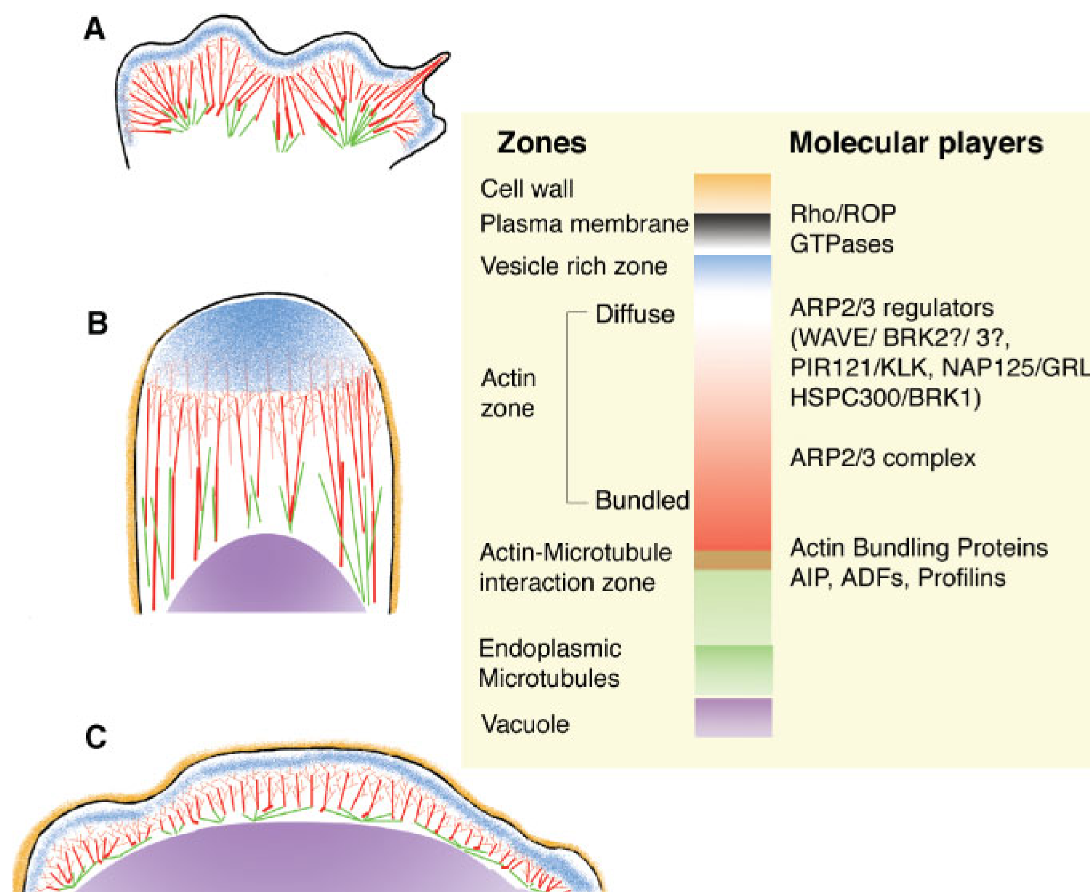

Figure 3. Diagramatic representation of intracellular and molecular features that appear to be common between **A:** an animal cell lamellipodium (based on Ref. 1), and **B:** a tip-growing (based on Refs 57–59,64–66,68) and **C:** a diffuse-growing (based on Refs 5,56,67) plant cell. In contrast to plant cells, the animal cell does not have a cell wall and is devoid of a large central vacuole. However, the zones extending from the plasma membrane to the endoplasmic microtubules are strikingly similar in all the three cells. In the plant cells, an expanding vacuole is a major component of the cell and plays an important role in its expansion. One suggested role for the vacuole is to provide and maintain a strong outward directed pressure against the cell wall. Under such a condition, regions of the plasma membrane underlying a stretched or nascent cell wall would likely exhibit a regional protuberance. Turgor pressure could thus drive membrane protrusion. However, general turgor appears insufficient for focusing membrane protrusion and growth to a specific region of the plant cell for accomplishing polarized growth. The molecular players (right side of figure) that have been implicated in providing pertinent cues for localizing membrane protrusion during amoeboid locomotion are very similar to those being discovered now in plant cells. Considering that achieving membrane protrusion is the common aim for both motile animal cells and non-motile expanding plant cells this schematic comparison suggests a conservation of the fundamental molecular mechinery between animal and plant cells.

Do rapidly growing plant cells have a 'leading edge'?

Both amoeboid locomotion and plant cell expansion rely on membrane protrusion. The differences in cytoskeletal arrays between the leading edge and the rest of the cell body have long been appreciated in relation to amoeboid locomotion. The advancing lamellipodium in motile cells displays a characteristic dynamic actin region just behind the very edge.[1] This is the region where the ARP2/3 complex localizes.[28,29] Plant cells, like root hairs[68] and pollen-tubes[66] that extend by tip-focused localized growth, display a very similar zonation pattern (Fig. 3). At the very tip, a thin plasma membrane lies under a minimal, apparently stretched and weakened, cell wall. A vesicle-enriched zone follows and merges into a diffuse F-actin mesh. The actin filaments become increasingly bundled as their distance from the apex increases.[66–68] In a behavior similar to that displayed by an extending lamellipodium, tip-growing plant cells treated with actin polymerization inhibitors rapidly stop their extension.[69] The significance of dynamic actin at the very tip is also highlighted by the fact that mature cells that have stopped tip extension, display bundled F-actin that extends all the way to the apex. Regional

membrane dynamics, general actin organization and the demonstrated presence of at least one of the major components of the actin regulatory machinery, the ARP2/3 complex, in this extending region allow it to be considered as the leading edge of a tip-growing plant cell (Fig. 3).

Such similarities are not as obvious when we consider that a majority of plant cells do not extend by tip-directed growth, but expand in a diffuse manner such that the process of growth is spread over much larger areas of the cellular surface.[67] The expansion of diffuse-growing plant cells appears to go hand in hand with the enlargement of vacuoles and an increase in turgor pressure in these cells. This presses the cytoplasm into a thin layer against the plasma membrane and has given rise to the belief that diffuse growth is largely driven by turgor pressure. In that case, would diffuse growing plant cells also utilize the same mechanisms for membrane protrusion as tip-growing cells? If so, how is the difference between localized (tip) growth and global (diffuse) growth generated? Recent studies provide some thought-provoking observations in this context.

Broadening the leading edge and creating a diffuse growing cell

Though turgor does exert a general force on the cell membrane and can push it outwards into a bulbous or spherical shape, it appears difficult to imagine how the general turgor force can be regulated locally to create cell shapes as diverse as branched trichomes, cylindrical hypocotyl cells and the puzzle-shaped pavement cells. Consistent with this line of reasoning, though highly fragmented or unfused vacuoles have been observed in some ARP mutants, many of the diffuse growing mutant cells do possess large central vacuoles that fill the cell interior.[7] More strikingly, branches of trichomes in ARP mutants fail to extend and often remain as small spikes.[5–10] If turgor force and vacuolar expansion were to be major forces that determine the shape of a diffuse growing cell, the mutant branches would expand out, since there are usually well-developed vacuolar compartments beneath the branch initials. Another factor is clearly involved in guiding internal forces to produce regions of expansion and non-expansion in a plant cell. Observations on the actin cytoskeleton in wild-type and ARP mutants suggest an explanation. At maturity, diffuse growing wild-type cells retain only some small regions with a dense F-actin organization, while most of their well-expanded regions display a diffuse F-actin mesh.[5,67] By contrast, mature diffuse-growing cells in ARP mutants exhibit numerous randomly located patches of dense F-actin and intervening areas with a fine F-actin mesh.[5–7] Distorted shapes apparently result from random areas of expansion and non-expansion. Clearly the ability of a diffuse-growing cell to expand uniformly in response to internal turgor pressure needs to be matched by an ability of its actin cytoskeleton to stretch out evenly. ARP2/3 complex mediation resulting in a dendritic, dynamic actin organization just below the plasma membrane can achieve this.

Accordingly, in diffuse growing cells, the additional force provided by turgor does play a very important role since it helps in spreading the cytoplasm, the cortical actin cytoskeleton and its regulatory machinery over a larger internal surface area of the cell. The small area of a tip-growing cell with its well-defined zones (Fig. 3B), if compressed, can easily become a broad diffuse growth area (Fig. 3C). This reasoning also implies that it is not the ARP2/3 complex per se, but its localized activation that is important for cell-shape development and draws attention towards upstream regulators of the complex.

Regional activation of the ARP2/3 complex: a link to microtubules?

As discussed earlier the activation of the complex through the proposed SCAR/WAVE-mediated pathway is initiated by triggering of molecular switches like Rho-GTPases.[50] In non-plant systems, Rho-like proteins are activated by specific GTPase-activators (such as Rho GEFs—Guanine nucleotide Exchange Factors).[70,71] In *Drosophila*, the delivery of a Rho-GEF to cortical sites has been shown to involve an intimate interaction with microtubules.[72] For higher plants, AtROP2 has already been shown to interact directly with upstream regulators of the ARP2/3 complex[14] and an *Arabidopsis SPIKE1* gene is a candidate ROP-GEF.[73] If, as demonstrated for animal cells,[71,72] microtubules are involved in transporting and targeting of ROP-GEFs in plants also, then either a breakdown in the microtubule cytoskeleton or an increase in ROP levels in a cell should ultimately lead to a general increase in ARP2/3 complex activity and produce a similar phenotype of abnormally expanded cells. This is precisely what is observed upon the overexpression of the *Arabidopsis* AtROP2 gene and its constitutively active form.[56,57] The cells expand more and display diffuse F-actin, suggesting an increase in global actin polymerization activity.[56] Also, as expected, compromising microtubule activity by drug treatments has the same effect on cell morphology.[67] Coincidental localization patterns for F-actin patches and endoplasmic microtubules observed in wild-type and *dis2, crk* trichomes have already suggested[10] an intimate relationship between these two major cytoskeletal elements (Fig. 4). Based on presently available molecular and cell biological evidence, it appears that the pathway leading to localized activation of the ARP2/3 complex might ultimately depend upon intimate interactions between its activators and the microtubule cytoskeleton.

Conclusions and perspectives

The molecular characterization of major subunits of the ARP2/3 complex and components of a possible regulatory network have firmly established the presence of this important modulator of the actin cytoskeleton in plants. The findings highlight the cross-kingdom molecular and functional con-

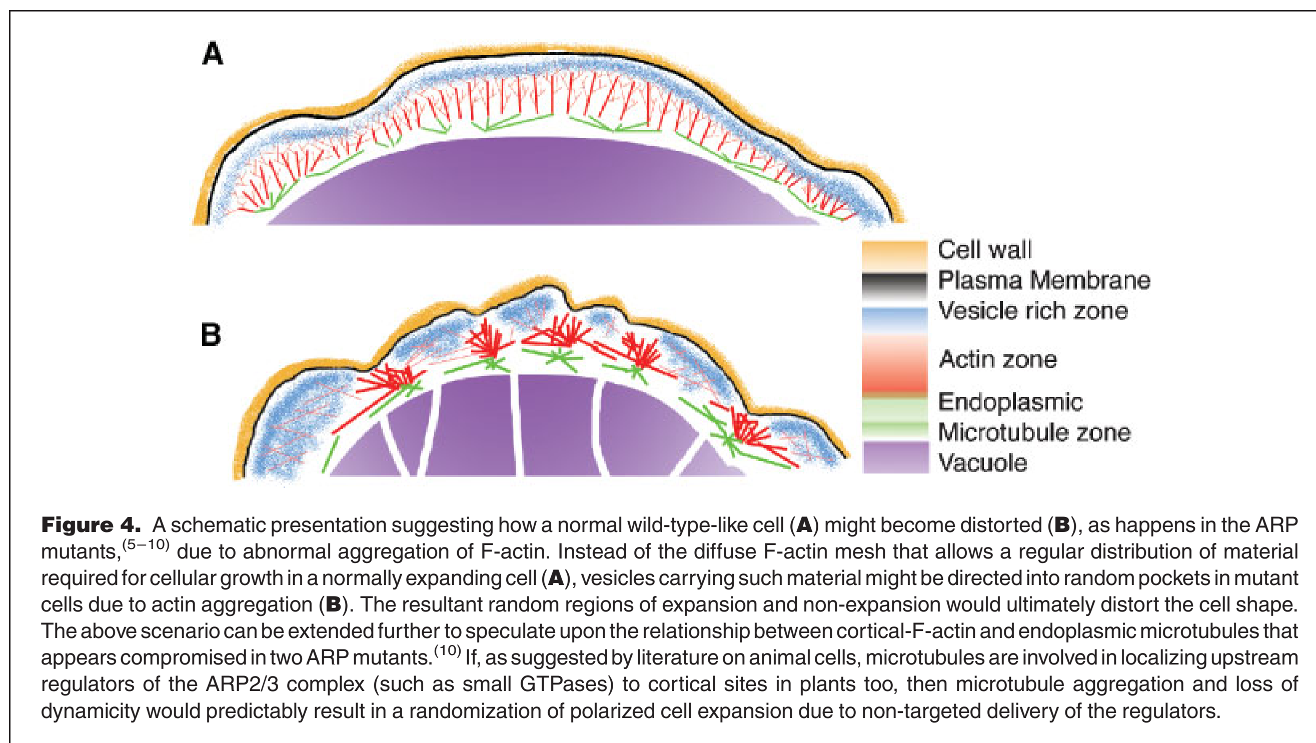

Figure 4. A schematic presentation suggesting how a normal wild-type-like cell (**A**) might become distorted (**B**), as happens in the ARP mutants,[5–10] due to abnormal aggregation of F-actin. Instead of the diffuse F-actin mesh that allows a regular distribution of material required for cellular growth in a normally expanding cell (**A**), vesicles carrying such material might be directed into random pockets in mutant cells due to actin aggregation (**B**). The resultant random regions of expansion and non-expansion would ultimately distort the cell shape. The above scenario can be extended further to speculate upon the relationship between cortical-F-actin and endoplasmic microtubules that appears compromised in two ARP mutants.[10] If, as suggested by literature on animal cells, microtubules are involved in localizing upstream regulators of the ARP2/3 complex (such as small GTPases) to cortical sites in plants too, then microtubule aggregation and loss of dynamicity would predictably result in a randomization of polarized cell expansion due to non-targeted delivery of the regulators.

servation of actin polymerization as a fundamental process for membrane protrusion that is required for both amoeboid motility and expansion of all plant cells. The isolation and biochemical characterization of the putative plant ARP2/3 complex is undoubtedly the next major achievement to look forward to as it will allow us to compare and contrast animal and plant systems at a new level of detail. Nevertheless, the present studies have already generated a plethora of exciting questions relating to actin-cytoskeleton dynamics, actin–microtubule interactions and intracellular motility in plants.

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Note added in proof

Recent publications[79,80] have identified plant specific SCAR/WAVE homologs and an interacting Abi-1-like bridging protein from *Arabidopsis*.[80]

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