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Cell polarity: ROPing the ends together

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Cell polarity plays an important role in plant development, but the mechanisms that first establish polarity cues remain obscure. By contrast, a flurry of information has recently emerged on the elaboration of cell shape from such unknown initial cell-polarity cues. Recent studies suggest that Rho-related GTPases in plants (ROPs), and their effector targets among the ROP-interactive CRIB motif-containing proteins (RICs), mediate two antagonistic pathways that have opposing action on cell polarization. ROP proteins appear to interact directly with upstream regulators of the ARP2/3 complex, which are conserved modulators of the actin cytoskeleton. ROP function is dependent on the class 1 ADP-ribosylation factors (ARFs), which are core components of the vesicle transport machinery that are also involved in the polar localization of PIN-FORMED (PIN) family auxin efflux facilitators.

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Introduction

Cells can be viewed along three orthogonal axes. When one axis can be clearly distinguished from the others a cell is said to be 'axialized'. The distribution of subcellular structures and molecules may be asymmetric along one or more particular axes, making one end of the cell different from the others. This often stunning internal asymmetry is known as 'cell polarity'.

Cell polarity plays important roles in both animal and plant development. For example, in *Caenorhabditis elegans*, *Drosophila melanogaster* and *Arabidopsis thaliana*, the main body axis is defined by the polarity of the single-celled zygote and polar information is used for patterning and cell specification along this axis.

In plants, single-cell systems such as pollen tubes or root hairs have been used to reveal molecular components that are required for apical–basal cell polarity and polar outgrowth. At the multicellular level, the polar localization of the PIN-FORMED (PIN) family auxin transport facilitators has yielded valuable molecular markers for cell polarization and better understanding of coordinated cell polarity in plant growth and development.

In this review, we can discuss only brief highlights of recent findings that have contributed to our understanding of the mechanisms that control cell polarity in plants. Several recent accounts provide further reading [1•–3•].

ARFs, ARF–GEFs and vesicle trafficking

ADP-ribosylation factors (ARFs) are core factors for vesicle trafficking. 'Class 1' ARF proteins regulate intracellular vesicular trafficking at multiple stages of the secretory and lysosomal/vacuolar transport pathways in mammalian and plant cells [4–8]. In particular, they participate in the formation of transport vesicles and the selection of transmembrane protein cargo from donor compartments in mammalian cells [9,10].

The vesicle transport inhibitor Brefeldin A (BFA) interferes with ARF action in mammalian, fungal, and plant cells [11,12]. BFA treatment compromises specific cell polarization processes such as the establishment of apical–basal polarity in root-hair-bearing epidermal cells (trichoblasts), reflected by root hair position defects and by defects in polar outgrowth [13], indicating that ARF-dependent vesicle trafficking is involved in cell polarity.

Consistent with a potential role for the ARF proteins in the establishment of plant cell polarity, mutations in the *GNOM/EMB30* gene, which encodes a BFA-sensitive guanine nucleotide exchange factor for ARFs (ARF–GEF) [14,15], lead to aberrant cell shape and orientation of cell division [15,16]. GNOM/EMB30 localizes to endocytic organelles, where it controls the polarized vesicle trafficking of the BFA-sensitive auxin efflux facilitator PIN1 to the basal end of the plasma membrane [17,18]. Weak alleles show auxin-related defects that support a specialized function for the GNOM ARF–GEF in polar-auxin transport [19•].

Plants have many class 1 ARF proteins [18,20] and *Arabidopsis* ARF1 complements the ARF1 ARF2 yeast double mutant [7], suggesting that class 1 ARF proteins in *Arabidopsis* have canonical cellular functions. Co-localization studies reveal that *Arabidopsis* ARF1 is localized to the Golgi apparatus and endocytic organelles in both onion and *Arabidopsis* cells [21•]. Manipulation of *Arabi-*

dopsis ARF1 function influences cytokinesis and polar outgrowth [21•]. Moreover, the position of root hair outgrowth is affected when GTP- and GDP-locked mutants of ARF1 are expressed at early stages of cell differentiation but after they exit mitosis, revealing specific modulation of apical–basal epidermal polarity by ARF1-dependent vesicle trafficking machinery in a distinct phase of plant development [21•].

ROPs and RICs

Targets for ARF-dependent vesicle trafficking are suggested by the observation that BFA inhibits early polar localization of RHO-of-plants proteins (ROPs) at the root hair initiation site [22]. ROPs are homologs of RHO/RAC/CDC42 RHO-family GTPases, which include key regulators of cell polarity in yeast and animals [23]. Among 11 *Arabidopsis ROP* genes [24], *ROP1*, *ROP2* and *ROP4* are essential for pollen tube or root hair outgrowth [21•,22,25,26,27••]. Consistent with its role in plant cell polarity, the expression of *ROP2* is highly enriched in the apical plasma membranes of the root meristematic cells [28], and ROP2 localizes to the future site of root hair formation as well as to the tips of growing root hairs [21•,25]. ROP2 partially co-localizes with the endocytic marker FM4-64 upon BFA treatment, and both polar ROP2 localization to the hair tip and the activity of ROP2 in this region requires ARF1 function [21•], supporting the idea that root hair outgrowth involves ARF-dependent polar localization of ROP proteins [22].

A genome-wide search for putative ROP effector targets led to the identification of genes that encode ROP-interactive CRIB (for CDC42/RAC-interactive binding) motif-containing proteins (RICs) [29]. The CRIB motif is required for the specific interaction of RICs with GTP-bound ROPs. RICs share little sequence homology with each other outside of the conserved ROP-interactive domain. The overexpression of *RIC* genes in pollen causes various degrees of growth inhibition in pollen tubes, implying that various RICs have distinct functions.

Like ROP1, both RIC3 and RIC4 cause depolarized growth. Furthermore, they display ROP1-enhanced localization to the tip of pollen tubes, suggesting that these RICs might be targets of ROP1 [29]. Indeed, RIC4 promotes F-actin assembly, whereas RIC3 activates calcium ion signaling that leads to F-actin disassembly. Polar outgrowth defects that are caused by the overexpression or depletion of either *RIC3* or *RIC4* can be rescued by the overproduction or depletion of the other protein. Thus, ROP1 modulates actin cytoskeletal dynamics and polar outgrowth in pollen tube by coordinating two counteracting downstream pathways that are controlled by the ROP1 targets RIC3 and RIC4 [27••]. Similar ROP-mediated regulatory mechanisms appear to account for the jigsaw-puzzle shape of *Arabidopsis* leaf pavement cells, in which locally activated ROP2 activates RIC4 to promote the assembly of cortical actin microfilaments that are required for the localized outgrowth of lobes [30••]. At the same time, ROP2 inactivates RIC1, promoting the formation of well-ordered cortical microtubules and the microtubule-dependent suppression of ROP2–RIC4 interaction in the indentation zone [30••]. Although the underlying mechanism by which microtubules regulate ROP–RIC4 interaction is not known, these findings suggest a fascinating precisely regulated cross-talk between ROP-mediated signaling pathways, which might serve as a general molecular mechanism for the polar outgrowth of plant cells.

ARP2/3 and its upstream regulatory factors

The inhibitory activity of the potent actin filament-disrupting drug latrunculin B suggests that actin cytoskeletal dynamics play an important role during axis establishment and polar outgrowth [31,32]. Recent genetic studies in *Arabidopsis* demonstrate that the major actin protein ACTIN2 is essential for both apical–basal root hair polarity and polar outgrowth [33,34], further supporting the importance of the actin cytoskeleton in plant cell polarity.

The seven-subunit actin-related protein (ARP)2/3 complex is a conserved modulator of the actin cytoskeleton. The identification of different subunit homologs in *Arabidopsis* suggests the existence of a functional ARP2/3 complex in plants [3•]. Mutations in genes that encode subunits of the ARP2/3 complex lead to increased F-actin bundling and aberrant actin patches, which misdirect the expansion of various cell types including trichomes, pavement cells, hypocotyl cells and root hair cells [35–40]. These mutations reveal a pivotal role for the ARP2/3 complex and the actin cytoskeleton in controlling polar cell expansion in plants.

In animal cells, the activity of the ARP2/3 complex is regulated by a variety of proteins of the WAVE (for Wiskott–Aldrich syndrome protein family verprolin homologous)/SCAR (for suppressor of cAMP receptor) complex [41–44]. In response to signals from the RHO family small GTPase Rac1, alterations in the composition and/or subcellular localization of the WAVE/SCAR complex lead to ARP2/3 activation [41,43]. Putative homologs for proteins of these upstream regulatory complexes were identified recently in *Arabidopsis* and maize [45,46••,47–49,50••,51–55]. In *brick1* mutants of maize, the failure of lobes to form along the margins of expanding leaf pavement cells is associated with the loss of local enrichments of cortical F-actin that are found at sites of lobe outgrowth in wildtype cells [45]. *Arabidopsis Atscar2*/*distored3*, *pirogi* (*pir*)/*Atpir*/*Atsra1*, *gnarled*/*Atnap1* and *klunker* mutants have trichome F-actin alterations and trichome morphology defects that are nearly identical to those of Arp2/3 complex mutants and to those of plants that express constitutively active ROP2 [46••,47–49,51,53,56]. Moreover, PIR/AtPIR/AtSRA1 directly interacts with ROP2

with isoform specificity and with selectivity for active forms of the protein [46••], although *in vivo* interaction and its significance has yet to be investigated. Together, these findings strongly suggest that ROP signaling, the ARP2/3 complex and its upstream regulatory complexes play essential roles in the local modulation of actin cytoskeletal dynamics, which in turn regulate axis establishment and polar outgrowth in plants.

Polar PIN protein localization

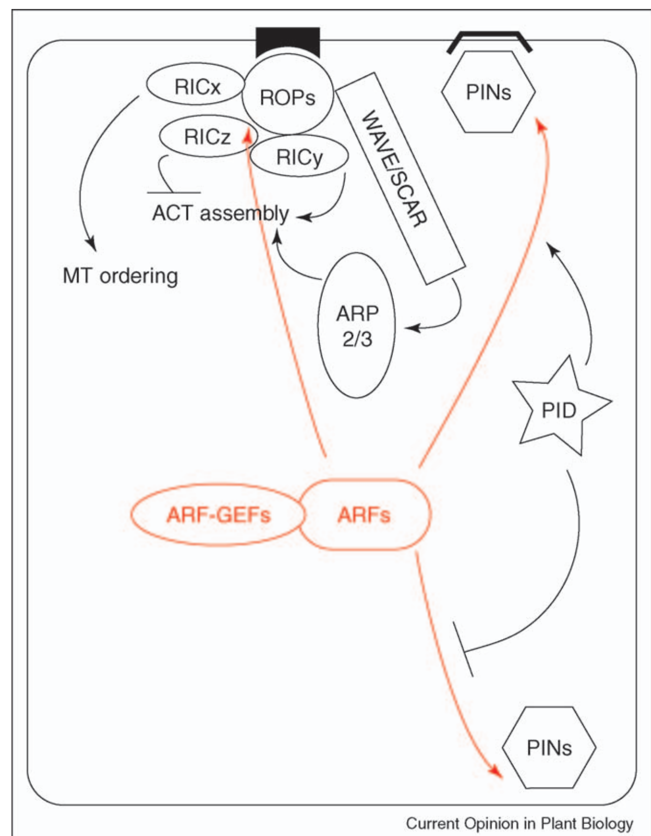
Polar transport of auxin plays crucial roles in axis establishment and polar growth during both embryonic and post-embryonic development [2•,57]. Both the presumptive auxin influx carrier AUX1 and the PIN efflux facilitators display an asymmetrical subcellular localization at the plasma membranes of auxin-transporting cells, which correlates with the presumed direction of auxin flow [58,59]. PIN proteins act redundantly, and their expression and localization undergo dynamic changes in response to developmental regulation [60,61••] or environmental stimuli, such as light or gravity [62], which makes them excellent read-outs for cell polarity.

Besides the earlier-mentioned role of vesicle-trafficking machinery in polar PIN protein localization, the serine-threonine kinase PINOID (PID) acts as a major determinant of PIN protein localization [63••]. In cells in which PID is present above a threshold level, PIN proteins are targeted to the apical membrane, whereas low levels of PID activity lead to the basal localization of the PIN proteins. The dependence on kinase activity of the precise switching between two alternative localizations suggests that PID mediates the choice of PIN trafficking or docking to alternative localization cues.

Interestingly, the accumulation of *PID* transcript is strongly and rapidly upregulated by auxin, the early expression of *PID* mRNA in the embryo is dependent on PIN1, and the recycling of PIN proteins from the membrane appears to be auxin dependent [64,65,66•], suggesting a tight connection among auxin, polar auxin transport, PINs and PID. Moreover, PID interacts with two calcium-binding proteins TOUCH3 and PID-BINDING PROTEIN 1 (PBP1) [67], supporting a role for calcium ion signaling in a PID-dependent signaling pathway for the control of PIN protein localization. PID protein localization should reveal whether ARF-dependent vesicle-trafficking machinery is involved in the localization or function of PID, for which a mechanism has been proposed that resembles insulin-stimulated GLUT4 transport in mammalian insulin-responsive tissues [68].

Polar PIN protein localization also appears to require sterol function because mutations in the *Arabidopsis* sterol biosynthesis gene *ORC/Sterol methyl-transferase 1* (*SMT1*) lead to reduced auxin transport and mis-localization of PIN proteins [69]. The underlying mechanisms, however, remain to be discovered. The internalization of sterols and PIN2 exhibited similar kinetics and pharmacological sensitivities, raising the possibility that the redistribution of sterols plays a role during the endocytic recycling of PIN proteins [70]. Sterol transport to the plasma membrane is actively mediated by P-glycoproteins (PGP)/multiple drug resistance (MDR) proteins in animal cells [71]. Mutations in *PGP/MDR*-related genes from plants result in the mislocalization of PIN proteins and in defective auxin transport [72–74], suggesting a potential mechanism by which PGP/MDR-related proteins in plants mediate the transport of sterols to distinct membrane domains in response to yet unknown signals, resulting in the polar localization of PIN proteins. Alternatively, PGP/MDR-related proteins might interact with PIN proteins and regulate their localization directly.

Figure 1



Processes contributing to cell polarization. Vesicle trafficking that is mediated by Class 1 ARFs is required for the polar localization of ROP GTPases, which control actin (ACT) assembly through RICs and WAVE/SCAR–ARP2/3 pathways and microtubule (MT) bundling through other RICs. ARF-mediated vesicle trafficking and a specific ARF–GEF regulator of this process also control the localization of PIN proteins, and the polarity of this localization is controlled by the PID kinase, which functions as a binary switch. Polar localization cues for ROP localization or activation and for PIN localization (black bodies) remain unknown. Red arrows: vesicle trafficking control. Black arrows: protein activity control.

In summary, the subcellular localization of PIN proteins is known to rely on targeted vesicle transport, on PID kinase activity, and on the function of sterol and PGP/MDR-related proteins but the initial cues for the polar localization of PIN proteins are as yet unknown.

Conclusions

Many of the components that are involved in polarized growth and polar protein distribution have recently been discovered and are beginning to be connected within pathways that lead from regulators to cytoskeletal elements (Figure 1). Vesicle trafficking plays a central role in polarized growth, but the specific controls of this process during cell polarization need to be identified, for example by studies on regulators of vesicle fusion [75,76]. However, the nature of initial polarity cues remains unknown. Emerging connections between lipid signaling and cell polarity might help in the identification of such cues [77–79]. After that, the challenge will be to find out whether diverse cues regulate the polar activity and localization of diverse proteins in distinct or in related pathways, or whether a very small set of initial polar marks are used by all systems that elaborate plant cell polarity. Can we rope these ends together?

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Lindsey K (Ed): *Polarity in Plants*. Blackwell Publishing;
• 2004.
This volume provides an account of current research into the mechanisms by which polarity is generated at the levels of the cell, organ and organism in plants, drawing especially on recent work with model organisms.

2. Grebe M: **Ups and downs of tissue and planar polarity in plants**.
• *Bioessays* 2004, **26**:719-729.
An insightful review that summarizes recent progress in research on vascular tissue and planar epidermal polarity in the *Arabidopsis* root, and compares this new information to findings on planar polarity in animals and cell polarity in yeast.

3. Mathur J: **The ARP2/3 complex: giving plant cells a leading**
• **edge**. *Bioessays* 2005, **27**:377-387.
A comprehensive review of the recent advances in understanding and the implications of the ARP2/3 complex in plants.

4. Balch WE, Kahn RA, Schwaninger R: **ADP-ribosylation factor is required for vesicular trafficking between the endoplasmic reticulum and the *cis*-Golgi compartment**. *J Biol Chem* 1992, **267**:13053-13061.

5. Presley JF, Ward TH, Pfeifer AC, Siggia ED, Phair RD, Lippincott-Schwartz J: **Dissection of COPI and Arf1 dynamics *in vivo* and role in Golgi membrane transport**. *Nature* 2002, **417**:187-193.

6. Lee MH, Min MK, Lee YJ, Jin JB, Shin DH, Kim DH, Lee KH, Hwang I: **ADP-ribosylation factor 1 of *Arabidopsis* plays a critical role in intracellular trafficking and maintenance of endoplasmic reticulum morphology in *Arabidopsis***. *Plant Physiol* 2002, **129**:1507-1520.

7. Takeuchi M, Ueda T, Yahara N, Nakano A: **Arf1 GTPase plays roles in the protein traffic between the endoplasmic reticulum and the Golgi apparatus in tobacco and *Arabidopsis* cultured cells**. *Plant J* 2002, **31**:499-515.

8. Pimpl P, Hanton SL, Taylor JP, Pinto-DaSilva LL, Denecke J: **The GTPase ARF1p controls the sequence-specific vacuolar sorting route to the lytic vacuole**. *Plant Cell* 2003, **15**:1242-1256.

9. Goldberg J: **Decoding of sorting signals by coatomer through a GTPase switch in the COPI coat complex**. *Cell* 2000, **100**:671-679.

10. Spang A: **ARF1 regulatory factors and COPI vesicle formation**. *Curr Opin Cell Biol* 2002, **14**:423-427.

11. Chardin P, McCormick F: **Brefeldin A: the advantage of being uncompetitive**. *Cell* 1999, **97**:153-155.

12. Nebenführ A, Ritzenthaler C, Robinson DG: **Brefeldin A: deciphering an enigmatic inhibitor of secretion**. *Plant Physiol* 2002, **130**:1102-1108.

13. Grebe M, Friml J, Swarup R, Ljung K, Sandberg G, Terlou M, Palme K, Bennett MJ, Scheres B: **Cell polarity signaling in *Arabidopsis* involves a BFA-sensitive auxin influx pathway**. *Curr Biol* 2002, **12**:329-334.

14. Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G: **The *Arabidopsis* GNOM ARF–GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth**. *Cell* 2003, **112**:219-230.

15. Shevell DE, Leu WM, Gillmor CS, Xia G, Feldmann KA, Chua NH: **EMB30 is essential for normal cell division, cell expansion, and cell adhesion in *Arabidopsis* and encodes a protein that has similarity to Sec7**. *Cell* 1994, **77**:1051-1062.

16. Mayer U, Buettner G, Jürgens G: **Apical–basal pattern formation in the *Arabidopsis* embryo: studies on the role of the gnom gene**. *Development* 1993, **117**:149-162.

17. Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, Paris S, Gälweiler L, Palme K, Jürgens G: **Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF**. *Science* 1999, **286**:316-318.

18. Jürgens G, Geldner N: **Protein secretion in plants: from the trans-Golgi network to the outer space**. *Traffic* 2002, **3**:605-613.

19. Geldner N, Richter S, Vieten A, Marquardt S, Torres-Ruiz RA,
• Mayer U, Jürgens G: **Partial loss-of-function alleles reveal a role for GNOM in auxin transport-related, post-embryonic development of *Arabidopsis***. *Development* 2004, **131**:389-400.
gnom/emb30 allele combinations with weak phenotypes display phenotypes that are compatible with a primary role for an ARF–GEF in polar auxin transport.

20. Vernoud V, Horton AC, Yang Z, Nielsen E: **Analysis of the small GTPase gene superfamily of *Arabidopsis***. *Plant Physiol* 2003, **131**:1191-1208.

21. Xu J, Scheres B: **Dissection of *Arabidopsis* ADP-**
• **RIBOSYLATION FACTOR 1 function in epidermal cell polarity**. *Plant Cell* 2005, **17**:525-536.
This study provides evidence that the *Arabidopsis* ADP-RIBOSYLATION FACTOR 1 localizes to both Golgi apparatus and endocytic organelles, has multiple cell-polarity-related functions and is required for both apical–basal root hair polarity and polar root hair outgrowth.

22. Molendijk AJ, Bischoff F, Rajendrakumar CS, Friml J, Braun M, Gilroy S, Palme K: ***Arabidopsis thaliana* Rop GTPases are localized to tips of root hairs and control polar growth**. *EMBO J* 2001, **20**:2779-2788.

23. Etienne-Manneville S, Hall A: **Rho GTPases in cell biology**. *Nature* 2002, **420**:629-635.

24. Christensen TM, Vejlupkova Z, Sharma YK, Arthur KM, Spatafora JW, Albright CA, Meeley RB, Duvick JP, Quatrano RS, Fowler JE: **Conserved subgroups and developmental regulation in the monocot rop gene family**. *Plant Physiol* 2003, **133**:1791-1808.

25. Jones MA, Shen JJ, Fu Y, Li H, Yang Z, Grierson CS: **The *Arabidopsis* Rop2 GTPase is a positive regulator**

of both root hair initiation and tip growth. *Plant Cell* 2002, **14**:763-776.

26. Gu Y, Vernoud V, Fu Y, Yang Z: **ROP GTPase regulation of pollen tube growth through the dynamics of tip-localized F-actin**. *J Exp Bot* 2003, **54**:93-101.

27. •• Gu Y, Fu Y, Dowd P, Li S, Vernoud V, Gilroy S, Yang Z: **A Rho family GTPase controls actin dynamics and tip growth via two counteracting downstream pathways in pollen tubes**. *J Cell Biol* 2005, **169**:127-138.

The *Arabidopsis* RHO family GTPase, ROP1, had previously been shown by the same Yang group to control pollen tube growth by regulating apical F-actin dynamics. This excellent manuscript now provides the underlying mechanism by which ROP1 activates two counteracting pathways to modulate actin cytoskeletal changes and polar outgrowth.

28. Li L, Xu J, Xu ZH, Xue HW: **Brassinosteroid stimulates plant tropisms through modulation of polar auxin transport**. *Plant Cell* 2005, in press.

29. Wu G, Gu Y, Li S, Yang Z: **A genome-wide analysis of *Arabidopsis* Rop-interactive CRIB motif-containing proteins that act as Rop GTPase targets**. *Plant Cell* 2001, **13**:2841-2856.

30. •• Fu Y, Gu Y, Zheng Z, Wasteneys G, Yang Z: ***Arabidopsis* interdigitating cell growth requires two antagonistic pathways with opposing action on cell morphogenesis**. *Cell* 2005, **120**:687-700.

This paper provides comprehensive evidence that a ROP GTPase-mediated regulatory mechanism is required to produce the jigsaw-like appearance of *Arabidopsis* leaf pavement cells. This evidence shows that precisely regulated crosstalk between ROP-mediated signaling pathways functions to coordinate microtubule and actin cytoskeletal systems and cytoskeleton-dependent cell polarization in plants.

31. Baluska F, Salaj J, Mathur J, Braun M, Jasper F, Samaj J, Chua NH, Barlow PW, Volkmann D: **Root hair formation: F-actin-dependent tip growth is initiated by local assembly of profilin-supported F-actin meshworks accumulated within expansin-enriched bulges**. *Dev Biol* 2000, **227**:618-632.

32. Hable WE, Miller NR, Kropf DL: **Polarity establishment requires dynamic actin in fucoid zygotes**. *Protoplasma* 2003, **221**:193-204.

33. Gilliland LU, Kandasamy MK, Pawloski LC, Meagher RB: **Both vegetative and reproductive actin isovariants complement the stunted root hair phenotype of the *Arabidopsis act2-1* mutation**. *Plant Physiol* 2002, **130**:2199-2209.

34. Ringli C, Baumberger N, Diet A, Frey B, Keller B: **ACTIN2 is essential for bulge site selection and tip growth during root hair development of *Arabidopsis***. *Plant Physiol* 2002, **129**:1464-1472.

35. Mathur J, Mathur N, Kernebeck B, Hülskamp M: **Mutations in actin-related proteins 2 and 3 affect cell shape development in *Arabidopsis***. *Plant Cell* 2003, **15**:1632-1645.

36. Mathur J, Mathur N, Kirik V, Kernebeck B, Srinivas BP, Hülskamp M: ***Arabidopsis CROOKED* encodes for the smallest subunit of the ARP2/3 complex and controls cell shape by region specific fine F-actin formation**. *Development* 2003, **130**:3137-3146.

37. Le J, El-Assal Sel-D, Basu D, Saad ME, Szymanski DB: **Requirements for *Arabidopsis* ATARP2 and ATARP3 during epidermal development**. *Curr Biol* 2003, **13**:1341-1347.

38. Li S, Blanchoin L, Yang Z, Lord EM: **The putative *Arabidopsis* arp2/3 complex controls leaf cell morphogenesis**. *Plant Physiol* 2003, **132**:2034-2044.

39. El-Din El-Assal S, Le J, Basu D, Mallery EL, Szymanski DB: ***DISTORTED2* encodes an ARPC2 subunit of the putative *Arabidopsis* ARP2/3 complex**. *Plant J* 2004, **38**:526-538.

40. Saedler R, Mathur N, Srinivas BP, Kernebeck B, Hülskamp M, Mathur J: **Actin control over microtubules suggested by *DISTORTED2* encoding the *Arabidopsis* ARPC2 subunit homolog**. *Plant Cell Physiol* 2004, **45**:813-822.

41. Eden S, Rohatgi R, Podtelejnikov AV, Mann M, Kirschner MW: **Mechanism of regulation of WAVE1-induced actin nucleation by Rac1 and Nck**. *Nature* 2002, **418**:790-793.

42. Stradal TE, Rottner K, Disanza A, Confalonieri S, Innocenti M, Scita G: **Regulation of actin dynamics by WASP and WAVE family proteins**. *Trends Cell Biol* 2004, **14**:303-311.

43. Steffen A, Rottner K, Ehinger J, Innocenti M, Scita G, Wehland J, Stradal TE: **Sra-1 and Nap1 link Rac to actin assembly driving lamellipodia formation**. *EMBO J* 2004, **23**:749-759.

44. Vartiainen MK, Machesky LM: **The WASP-Arp2/3 pathway: genetic insights**. *Curr Opin Cell Biol* 2004, **16**:174-181.

45. Frank MJ, Smith LG: **A small, novel protein highly conserved in plants and animals promotes the polarized growth and division of maize leaf epidermal cells**. *Curr Biol* 2002, **12**:849-853.

46. •• Basu D, El-Assal Sel-D, Le J, Mallery EL, Szymanski DB: **Interchangeable functions of *Arabidopsis* PIROGI and the human WAVE complex subunit SRA1 during leaf epidermal development**. *Development* 2004, **131**:4345-4355.

The authors report that the 'distorted group' gene *PIR* encodes a homolog of the WAVE complex subunit SRA1. The similar cell shape and actin phenotypes of *pir* and ARP2/3 complex subunit mutants suggest that PIR positively regulates ARP2/3. Moreover, PIR directly interacts with ROP2 with isoform specificity and with selectivity for active forms of the protein, indicating a tight connection between ROP signaling and the WAVE complex.

47. Brembu T, Winge P, Seem M, Bones AM: ***NAPP* and *PIRP* encode subunits of a putative wave regulatory protein complex involved in plant cell morphogenesis**. *Plant Cell* 2004, **16**:2335-2349.

48. Deeks MJ, Kaloriti D, Davies B, Malho R, Hussey PJ: ***Arabidopsis* NAP1 is essential for Arp2/3-dependent trichome morphogenesis**. *Curr Biol* 2004, **14**:1410-1414.

49. El-Assal Sel-D, Le J, Basu D, Mallery EL, Szymanski DB: ***Arabidopsis GNARLED* encodes a NAP125 homolog that positively regulates ARP2/3**. *Curr Biol* 2004, **14**:1405-1409.

50. •• Frank M, Egile C, Dyachok J, Djakovic S, Nolasco M, Li R, Smith LG: **Activation of Arp2/3 complex-dependent actin polymerization by plant proteins distantly related to Scar/WAVE**. *Proc Natl Acad Sci USA* 2004, **101**:16379-16384.

The authors describe the identification of a family of Scar/WAVE-related plant Arp2/3 activators. Analysis of gene expression patterns suggests functional redundancy among members of the AtSCAR family. AtSCAR1 and ATSCAR3 interact with AtBRICK1, the *Arabidopsis* homolog of HSPC300. This suggests that AtSCAR proteins, together with AtBRICK1, and most probably with other *Arabidopsis* homologs of WAVE complex components, regulate the activation of the ARP2/3 complex *in vivo*.

51. Li Y, Sorefan K, Hemmann G, Bevan MW: ***Arabidopsis* NAP and PIR regulate actin-based cell morphogenesis and multiple developmental processes**. *Plant Physiol* 2004, **136**:3616-3627.

52. Saedler R, Zimmermann I, Mutondo M, Hülskamp M: **The *Arabidopsis KLUNKER* gene controls cell shape changes and encodes the AtSRA1 homolog**. *Plant Mol Biol* 2004, **56**:775-782.

53. Zimmermann I, Saedler R, Mutondo M, Hülskamp M: **The *Arabidopsis GNARLED* gene encodes the NAP125 homolog and controls several actin-based cell shape changes**. *Mol Genet Genomics* 2004, **272**:290-296.

54. Basu D, Le J, El-Essal Sel-D, Huang S, Zhang C, Mallery EL, Koliantz G, Staiger CJ, Szymanski DB: **DISTORTED3/SCAR2 is a putative *Arabidopsis* WAVE complex subunit that activates the Arp2/3 complex and is required for epidermal morphogenesis**. *Plant Cell* 2005, **17**:502-524.

55. Zhang XG, Dyachok J, Krishnakumar S, Smith LG, Oppenheimer DG: ***IRREGULAR TRICHOME BRANCH1* in *Arabidopsis* encodes a plant homolog of the actin-related protein2/3 complex activator Scar/WAVE that regulates actin and microtubule organization**. *Plant Cell* 2005, **17**:2314-2326.

56. Fu Y, Li H, Yang Z: **The ROP2 GTPase controls the formation of cortical fine F-actin and the early phase of directional cell expansion during *Arabidopsis* organogenesis**. *Plant Cell* 2002, **14**:777-794.

57. Weijers D, Jürgens G: **Auxin and embryo axis formation: the ends in sight?** *Curr Opin Plant Biol* 2005, **8**:32-37.

58. Swarup R, Kargul J, Marchant A, Zadik D, Rahman A, Mills R, Yemm A, May S, Williams L, Millner P *et al.*: **Structure–function analysis of the presumptive *Arabidopsis* auxin permease AUX1**. *Plant Cell* 2004, **16**:3069-3083.

59. Paponov IA, Teale WD, Trebar M, Blilou I, Palme K: **The PIN auxin efflux facilitators: evolutionary and functional perspectives**. *Trends Plant Sci* 2005, **10**:170-177.

60. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, Hamann T, Offringa R, Jürgens G: **Efflux-dependent auxin gradients establish the apical–basal axis of *Arabidopsis***. *Nature* 2003, **426**:147-153.

61. •• Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B: **The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots**. *Nature* 2005, **433**:39-44.
The authors show that five *PIN* genes display an unusual level of functional redundancy and collectively control auxin distribution in the root, thereby defining spatial domains for the expression of major determinants of root stem cell specification, for cell division and for cell expansion.

62. Friml J, Wisniewska J, Benkova E, Mendgen K, Palme K: **Lateral relocation of auxin efflux regulator PIN3 mediates tropism in *Arabidopsis***. *Nature* 2002, **415**:806-809.

63. •• Friml J, Yang X, Michniewicz M, Weijers D, Quint A, Tietz O, Benjamins R, Ouwerkerk PB, Ljung K, Sandberg G *et al.*: **A PINOID-dependent binary switch in apical–basal PIN polar targeting directs auxin efflux**. *Science* 2004, **306**:862-865.
This paper reveals that PID protein kinase can determine the apical or basal localization of PIN proteins, thus acting as a binary localization switch. These observations explain the observed phenotypes of *pid* mutants and provide a new molecular entry into the regulation of polar protein localization.

64. Benjamins R, Quint A, Weijers D, Hooykaas P, Offringa R: **The PINOID protein kinase regulates organ development in *Arabidopsis* by enhancing polar auxin transport**. *Development* 2001, **128**:4057-4067.

65. Furutani M, Vernoux T, Traas J, Kato T, Tasaka M, Aida M: **PIN-FORMED1 and PINOID regulate boundary formation and cotyledon development in *Arabidopsis* embryogenesis**. *Development* 2004, **131**:5021-5030.

66. • Paciorek T, Zazimalova E, Ruthardt N, Petrasek J, Stierhof YD, Kleine-Vehn J, Morris DA, Emans N, Jürgens G, Geldner N, Friml J: **Auxin inhibits endocytosis and promotes its own efflux from cells**. *Nature* 2005, **435**:1251-1256.
The authors provide evidence that biologically active auxins inhibit the internalization of PIN proteins, a mechanism by which auxin accumulation could promote its own efflux.

67. Benjamins R, Ampudia CS, Hooykaas PJ, Offringa R: **PINOID-mediated signaling involves calcium-binding proteins**. *Plant Physiol* 2003, **132**:1623-1630.

68. Muday GK, Murphy AS: **An emerging model of auxin transport regulation**. *Plant Cell* 2002, **14**:293-299.

69. Willemsen V, Friml J, Grebe M, van den Toorn A, Palme K, Scheres B: **Cell polarity and PIN protein positioning in *Arabidopsis* require STEROL METHYLTRANSFERASE1 function**. *Plant Cell* 2003, **15**:612-625.

70. Grebe M, Xu J, Mobius W, Ueda T, Nakano A, Geuze HJ, Rook MB, Scheres B: ***Arabidopsis* sterol endocytosis involves actin-mediated trafficking via ARA6-positive early endosomes**. *Curr Biol* 2003, **13**:1378-1387.

71. Garrigues A, Escargueil AE, Orlowski S: **The multidrug transporter, P-glycoprotein, actively mediates cholesterol redistribution in the cell membrane**. *Proc Natl Acad Sci USA* 2002, **99**:10347-10352.

72. Noh B, Murphy AS, Spalding EP: **Multidrug resistance-like genes of *Arabidopsis* required for auxin transport and auxin-mediated development**. *Plant Cell* 2001, **13**:2441-2454.

73. Multani DS, Briggs SP, Chamberlin MA, Blakeslee JJ, Murphy AS, Johal GS: **Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants**. *Science* 2003, **302**:81-84.

74. Noh B, Bandyopadhyay A, Peer WA, Spalding EP, Murphy AS: **Enhanced gravi- and phototropism in plant *mdr* mutants mislocalizing the auxin efflux protein PIN1**. *Nature* 2003, **423**:999-1002.

75. Muller I, Wagner W, Volker A, Schellmann S, Nacry P, Kuttner F, Schwarz-Sommer Z, Mayer U, Jürgens G: **Syntaxin specificity of cytokinesis in *Arabidopsis***. *Nat Cell Biol* 2003, **5**:531-534.

76. Preuss ML, Serna J, Falbel TG, Bednarek SY, Nielsen E: **The *Arabidopsis* Rab GTPase RabA4b localizes to the tips of growing root hair cells**. *Plant Cell* 2004, **16**:1589-1603.

77. Samaj J, Baluska F, Menzel D: **New signalling molecules regulating root hair tip growth**. *Trends Plant Sci* 2004, **9**:217-220.

78. Fischer U, Men S, Grebe M: **Lipid function in plant cell polarity**. *Curr Opin Plant Biol* 2004, **7**:670-676.

79. Wang X: **Lipid signaling**. *Curr Opin Plant Biol* 2004, **7**:329-336.