Cell axiality and polarity in plants – adding pieces to the puzzle

Markus Grebe, Jian Xu and Ben Scheres*

Plant cell polarity is important for cellular function and multicellular development. Classical physiological and cell biological analyses identified cues that orient cell polarity and suggested molecules that translate a cue into intracellular asymmetry. A range of proteins that either mark or are involved in the establishment of a (polar) axis are now available, as are many relevant mutants. These tools are likely to facilitate a dissection of the molecular mechanisms behind cell and organ polarity in the near future.

Addresses
Department of Molecular Cell Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
*e-mail: b.scheres@bio.uu.nl

Current Opinion in Plant Biology 2001, 4:520–526

1369-5266/01/$ – see front matter

Abbreviations

ARF	ADP-ribosylation factor
AUX1	AUXIN1
BFA	brefeldin A
[Ca^{2+}]	concentration of calcium ions
ER	endoplasmic reticulum
GAP	GTPase-activating protein
GDI	guanine-nucleotide dissociation inhibitor
GEF	guanine-nucleotide exchange factor
GFP	green fluorescent protein
GPI	glycosylphosphatidylinositol
LRX1	*LEUCINE-RICH REPEAT/EXTENSIN1*
PIN1	PIN-FORMED1
Rop	Rho of plants
SAB	short actin bundles
sim	*siamese*
TRH1	*TINY ROOT HAIR1*

Introduction

Cells frequently exhibit an ordered distribution of components along one or more axes. When one axis differs from others a cell is said to be 'axialized'. The distribution of organelles, proteins or cytoskeletal components may be asymmetric along a particular axis, making one end of the cell different from the other. This stunning internal organization is referred to as 'cell polarity' [1] (Figure 1a). Analyses of cell polarity in easily accessible plants, such as mosses and algae, revealed that it could be influenced by environmental factors, such as light and gravity, and suggested important roles for ion fluxes, the plasma membrane, and the cytoskeleton in the establishment and maintenance of cell polarity [1,2]. Moreover, earlier studies revealed that polarity at the single-cell level contributes to multicellular plant development [2,3].

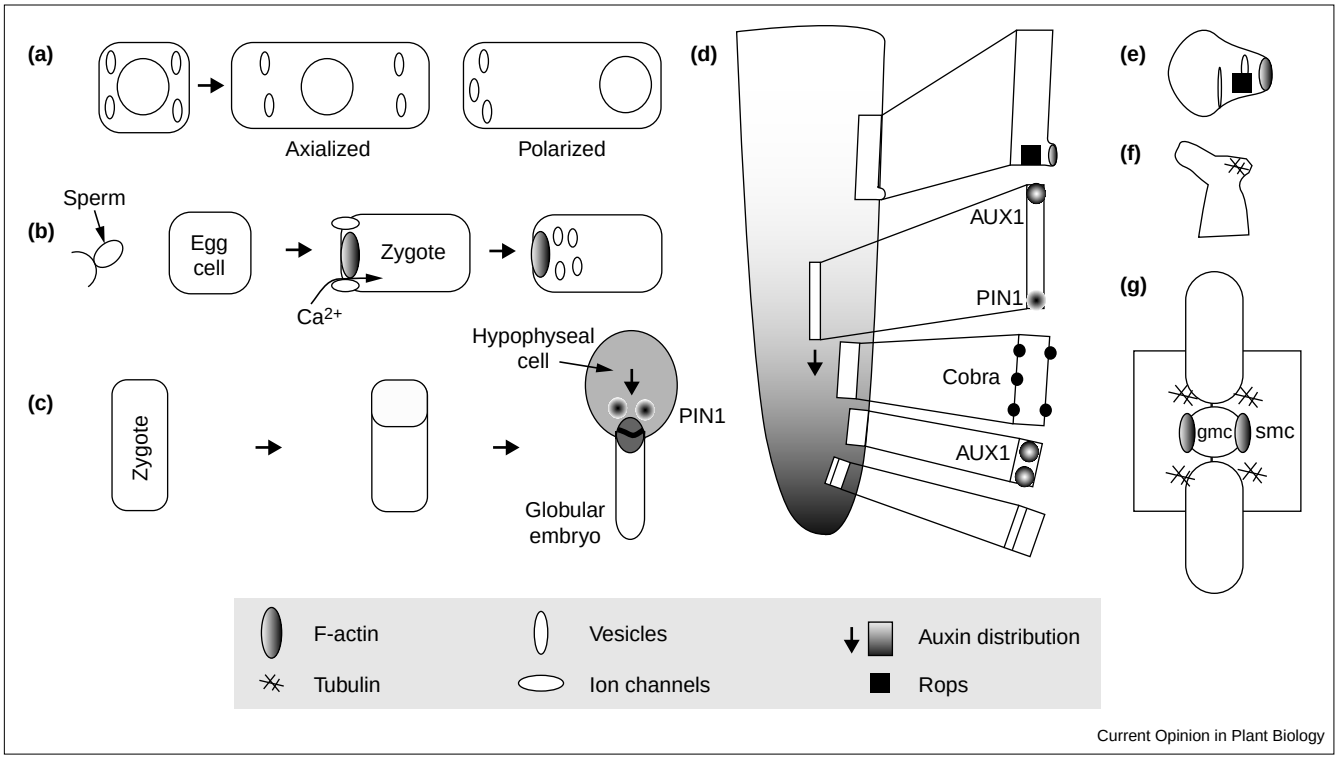
More recently, cell polarity has been studied using molecular genetic tools. At the single-cell level, suitable systems for the analysis of tip growth, such as growing pollen tubes or root hairs, have been used to reveal components required for polar growth. Genetic analysis of trichome development provided information on how multiple axes of polarity are established during cell morphogenesis [4]. The role of cell polarity in multicellular development, especially during embryo development, has also received increasing attention [5–7]. Here, we highlight recent progress in the plant cell polarity field, both at the single-cell level and in the multicellular context.

Cell polarity during embryo development

Polarity cues can be conveniently studied in single-cell systems such as egg cells and zygotes of fucoid brown algae [5]. In *Fucus* egg cells, sperm entry triggers local calcium influx at the penetration site, which has been implicated in directing egg activation [8]. In the absence of light, polar rhizoid initiation in *Pelvetia* occurs at the site of sperm entry, suggesting that sperm entry may provide a default pathway for zygote polarization in the absence of strong environmental cues [9••] (Figure 1b). Soon after fertilization, the entry site is marked by an actin patch and, later, by polarized secretion. Similar responses occurring after light-induced zygote polarization suggest the convergence of signals from two distinct polarity cues [9••]. Whether sperm-entry-induced polar site selection involves local calcium influx in *Pelvetia*, and which other factors regulate early polar actin assembly and secretion, awaits further studies. Intriguingly, brefeldin A (BFA), an inhibitor of vesicle transport, interferes with the fixation of light-induced polarity in *Fucus* zygotes [10]. This finding suggests that known BFA targets, such as guanine-nucleotide exchange factors (GEFs) on ADP-ribosylation factor (ARF)-type small G-proteins [11], are required for polar axis fixation. In the future, the functions of ion channels, the cytoskeleton, and BFA targets will have to be connected to improve our understanding of the mechanisms executing zygote polarization.

In *Arabidopsis*, the zygote and the hypophysis (a derivative required for root formation) divide asymmetrically (Figure 1c). Asymmetric zygote division and hypophysis formation are perturbed in *gnom/emb30* mutants. The embryos and seedlings of *gnom/emb30* mutants lack roots and display variable defects in apical–basal cell and tissue polarity [12,13]. *GNOM/EMB30* encodes a BFA-sensitive ARF GEF [13], which in other systems is required to coat coatomer vesicles during membrane trafficking [11]. By analogy with the BFA-sensitive processes of polarity fixation in *Fucus* zygotes, GNOM/EMB30-dependent vesicle trafficking may stabilize zygotic and embryonic cell and tissue polarity. Consistent with this view, *gnom/emb30* interferes with the coordinated polar localization of an auxin efflux carrier (PIN-FORMED1 [PIN1]) during early embryogenesis, for example, in cells localized apical to the hypophysis [13] (Figure 1c). Thus, the polarity defects

Figure 1



Examples of axialized and polarized cells in plants. (a) The concepts of axiality and polarity. (b) Sperm entry and axis fixation in algae. (c) Asymmetric division of *Arabidopsis* zygote and hypophyseal cell. (d) Axialized and polarized cells in the multicellular context of the *Arabidopsis* root. Note polarity at the organ level associated with distal auxin accumulation. (e) Pollen tube growth. (f) Trichome branch initiation. (g) Cytoskeletal components guiding the asymmetric subsidiary mother cell division (smc) in maize. gmc, guard mother cell. Black or shaded circles represent the localization of the proteins indicated next to these symbols.

observed in *gnom/emb30* mutants could be caused, in part, by perturbation of polar auxin transport that may contribute to the formation or asymmetric division of the hypophysis. Consistent with a scenario involving auxin perception and response during the establishment of the hypophyseal region, oriented asymmetric hypophysis division is disturbed in the *auxin binding protein 1* [14•] and auxin-insensitive *bodenlos* mutants [15]. Moreover, the hypophyseal cell appears to be lacking in *auxin resistant 6* mutants [16•]. The question of whether the formation or asymmetric division of the hypophysis involves a polarizing auxin cue remains open. Similarly, it remains to be determined whether the defects in PIN1 localization observed in *gnom/emb30* embryos reflect a direct cellular consequence of the *gnom/emb30* mutation or result from earlier zygotic polarity defects. Post-embryonically, *gnom/emb30* mutants display altered lectin and pectin secretion [17•], but it remains to be tested whether these changes in the secretion of cell wall components are early cellular responses caused by the *gnom/emb30* mutation. The subcellular localization of GNOM-interacting proteins [18•], coatomer components [19•], cell wall components [17•], and auxin-binding proteins and transporters [14•,20••], together with analyses of available auxin-responsive reporters [20••,21] in the mutant backgrounds mentioned above, may identify some of the primary polarity cues in *Arabidopsis* zygote and early embryo development. Such studies should provide clues to the integration of auxin action, membrane trafficking, and cell wall formation during the establishment of embryonic cell and organ polarity.

Axial and polar events in post-embryonic root development

The post-embryonic *Arabidopsis* root harbors several manifestations of cell and organ axiality and polarity (Figure 1d). Meristem initials act as stem cells and, by asymmetric division, give rise to daughters that undergo cell expansion along specific axes. In the root epidermis, cell elongation is followed by initiation of a polar outgrowth from hair cells (trichoblasts). At the multicellular level, an auxin maximum accumulates at the distal end of the root tip, providing a cue that regulates aspects of differential cell division, expansion, and polarity [21]. The *Arabidopsis* auxin influx carrier protein AUXIN1 (AUX1) localizes to the apical ends of protophloem cells and, in concert with basally localized PIN1, may deliver auxin to the maximum by acropetal transport [20••]. Thus, polar localization of carriers at the cellular level appears to contribute to organ polarity. Consistent with this hypothesis,

auxin concentrations and responses are reduced in the root tips of agravitropic *aux1* mutants [20••]. AUX1 localization in additional cell types suggests that this carrier protein may regulate gravitropism by facilitating basipetal auxin transport. Indeed, auxin responses are altered in the lateral root cap and epidermis cells of *aux1* mutants. In contrast to the auxin influx carrier substrate 2,4-D, the membrane permeable auxin 1-NAA ([1-naphthaleneacetic acid]; which does not require functioning influx carriers) restores these auxin responses in *aux1* mutants and rescues *aux1* gravitropism defects. Thus, AUX1-mediated auxin influx contributes to auxin response activation and gravitropic responses [20••]. Axial AUX1 localization to the apical–basal membranes of epidermal cells may mediate differential auxin-regulated cell elongation in response to a gravitropic stimulus.

In the root elongation zone, different cell types, including epidermal cells, undergo a phase of concomitant longitudinal and radial expansion, followed by a phase of rapid longitudinal expansion. The *Arabidopsis COBRA* gene mediates longitudinal expansion: *cobra* mutant cells undergo only rapid radial expansion [22••]. Conditional defects in cell wall components in *cobra* mutants, and localization of COBRA protein to longitudinal cell walls, suggest that COBRA acts on longitudinal cell axialization through an interaction with the cell wall. COBRA has homology to glycosylphosphatidylinositol (GPI)-linked proteins, and its localization in plants is consistent with findings in animal cells in which GPI-linkage can confer polar protein localization to membrane rafts [22••]. COBRA is the first potentially GPI-anchored protein with an associated mutant phenotype in plants. Its identification provides an excellent perspective from which to study the function of GPI-anchored proteins in plant cell-wall–plasma-membrane interactions during cell axialization.

After initial elongation, *Arabidopsis* trichoblasts initiate a single hair outgrowth (i.e. a 'bulge') at the basal end of their outer longitudinal wall. The recent localization of Rop (Rho of plants) GTPases (see below) to the incipient position of hair initiation in the elongation zone indicates that apical–basal polarity is established much earlier than the stage at which it can be distinguished morphologically [23•]. Polar localization of RopGTPases is abolished by BFA treatment, suggesting that BFA targets act on trichoblast polarity before Rop localization [23•]. The functional role of Rops at this developmental stage, whether BFA affects the fixation of polarity, and the directional cues signaling apical–basal polarity are not yet known, though it has been noted that the phytohormones auxin and ethylene promote polar hair initiation [24].

Although information about polar apical–basal site selection remains scarce, hair-bulge formation (i.e. swelling) has been characterized in more detail. At the onset of swelling, expansins [25•], Rop-like proteins [23•], F-actin, the actin-binding protein profilin [25•,26], a myosin [25•], phosphatidylinositol-4,5-bisphosphate (PIP_2) [26], endoplasmic reticulum (ER) [25•], and calcium [23•] all accumulate within the bulge. The accumulation of profilin mRNA, profilin and ER is actin-dependent as it is sensitive to treatment with actin-depolymerizing drugs [25•]. None of the other components mentioned above has been functionally characterized during bulge formation.

A screen for new root hair morphogenesis mutants and analysis of double mutant combinations revealed several mutations that affect early bulge formation and tip growth in *Arabidopsis*, expanding the genetic framework for hair morphogenesis [27•]. To determine the levels at which the different genes act, molecular analysis and characterization of the mutant alleles with cellular markers can now be carried out.

A new player in swelling formation and tip growth is the *LEUCINE-RICH REPEAT/EXTENSIN1* (*LRX1*) gene, as evidenced in *lrx1* mutants by the bloating of hairs at their base and defects in hair elongation [28••]. LRX1 protein localizes to the cell walls of root hairs at all stages from the onset of bulge formation onward. LRX1 harbors a putative extracellular leucine-rich repeat and an intracellular proline-rich extensin domain and is therefore the first extensin-like protein with an associated mutant phenotype [28••]. Thus, LRX1 is a good candidate for mediating communication between the cell wall and the cytoplasm during polarity establishment and maintenance.

Taken together, studies on cell axiality and polarity in the *Arabidopsis* root present challenges similar to those on embryo development. First, the pieces of genetic, molecular, and cell biological information from individual cell types need to be connected to present a mechanistic understanding of axiality and polarity. Second, manifestations of polarity in the different cell types should be connected and understood at the multicellular level.

Cell polarity in pollen tube and root hair tip growth

Pollen tubes (Figure 1e) and root hairs are tip-growing cells whose polar growth has many parallels to hyphal growth in fungi and neuronal-axon guidance in animals [29•]. Like their animal counterparts, pollen and root hair tips have been successfully used to study plant cell polarity. Tip growth requires the specification of a cortical site in the cell to which secretory vesicles are delivered and fused [30]. Inhibitor studies have implicated the actin cytoskeleton in the establishment of this site [31]. Using a green fluorescent protein (GFP)-tagged actin-binding domain of mouse talin [32,33], a dynamic form of tip-localized F-actin (i.e. short actin bundles [SABs]) has been discovered in tobacco pollen tubes [34••]. Similarly, studies employing modulators of ion fluxes, such as ion channel blockers and ionophores, have established a link between elevation of cytosolic calcium ion concentration [Ca^{2+}] at the pollen tube tip and its growth [35].

In both yeast and animal cells, signaling pathways involving members of the family of small Rho GTPases regulate polarized organization of the actin cytoskeleton. Rho-type proteins are recruited to cortical sites by polar cues, and induce remodeling of the actin cytoskeleton to designate a 'target patch' for subsequent elaboration of asymmetry [36,37]. Recently, roles for a plant-specific Rho GTPase subfamily, the Rop GTPases [38–40], have been established in both the control of the actin cytoskeleton and the regulation of tip-focused [Ca^{2+}] gradients in pollen tubes [34••,41,42,43••]. The identification of regulators and downstream effectors of Rop GTPases from *Arabidopsis* by yeast two-hybrid screening [44,45] and the subsequent use of these proteins to modify Rop GTPase activity *in vivo* [34••,43••] have helped to elucidate Rop function in cell polarity.

Rop regulators include Rho guanine-nucleotide dissociation inhibitor (GDI)-like proteins [44] and Rho GTPase-activating protein (GAP) homologs [45]. Rho GDIs inhibit the release of GDP from Rop proteins and solubilize membrane-associated Rho proteins. Rho GAPs stimulate the transition of Rop from a GTP-bound active state to a GDP-bound inactive state. A combination of overexpression experiments utilizing Rop proteins with modulated activity and coexpression of Rho GAP-like and Rho GDI-like proteins has successfully been applied [34••]. These studies revealed that ectopic localization of a pollen-specific Rop protein Rop1At to the apical region of the plasma membrane transforms the tip SAB into a network of fine filaments and induces a transverse actin band behind the tip. These cytoskeletal changes are accompanied by depolarized growth and can be suppressed by Rho GDI overexpression [34••,44]. Conversely, overexpression of RopGAP1 recovers normal actin organization and tip growth in Rop1At-overexpressing tubes [34••]. Together, these results indicate that the dynamics of tip actin are regulated by Rop signaling and that SABs are critical for polar growth in pollen tubes.

The tip-focused [Ca^{2+}] gradient also appears to be regulated by Rops [23•,41,42,43••]. Dominant negative *Rop1At* mutants or antisense *Rop1At* RNA inhibit pollen tube growth at low extracellular [Ca^{2+}] but this inhibition can be reversed by high extracellular [Ca^{2+}] [42]. Microinjection of anti-Rop antibodies disrupts the tip-focused intracellular [Ca^{2+}] gradient [41]. Moreover, expression of constitutively activated Rop GTPases in *Arabidopsis* root hairs abolishes polarized growth and delocalizes the tip-focused [Ca^{2+}] gradient [23•]. These data suggest that the interaction of Rops with [Ca^{2+}] gradients is important for the regulation of cell polarity in diverse tip-growing plant cells. Components connecting Rop GTPase function to [Ca^{2+}] gradient formation and other ion dynamics are unknown at present. However, the *TINY ROOT HAIR1* (*TRH1*) gene, which is required for the restriction of hair initiation to a single site and for tip growth, has recently been reported to encode a K^+ transporter [46•]. It will be interesting to see whether TRH1 may comprise an intermediary component linking Rop function to the modulation of ion dynamics in tip growing cells.

Taken together, the results of recent studies on Rop GTPases and their regulators provide mounting evidence for a Rop-GTPase-dependent signaling network that controls polar growth in pollen tube and root hair cells. What remains to be established is how the Rop GTPases connect to the actin cytoskeleton and to the regulation of ion influx and gradient formation. Further characterization and identification of downstream effectors of Rop GTPases should help to define distinct steps in the generation and maintenance of cell polarity in plants.

Trichomes and subsidiary mother cells: more handles on the cytoskeleton

As the cytoskeleton has multiple roles, it is of crucial importance to differentiate its specific functions in cell polarity from those in basic cellular processes. An accurate description of the behavior of the cytoskeleton during polarizing events in the cell has become available for *Arabidopsis* trichomes that form branches with a new polar axis. Using immunological staining methods or talin::GFP fusions, two groups have described the distribution of filamentous actin in trichomes [47,48]. Pharmacological actin disruption does not interfere with the establishment of new branches, that is new polar axes, but compromises cell outgrowth. Interestingly, actin disruption phenocopies the 'distorted' group of trichome mutants. Moreover, actin distribution is perturbed in this class of mutants [47,48], suggesting that they are affected in genes that directly or indirectly contribute to actin organization. Inhibition of the microtubular cytoskeleton, however, severely inhibits cell polarization, implicating a role for microtubules in setting up a new axis of polarity [49•]. GFP fusions to microtubule-associated protein 4 (MAP4) allow the visualization of dense knots of cortical microtubules at trichome branch-points (Figure 1f). Moreover, microtubule-stabilizing drugs can elicit new branch points in *stichel* trichome mutants, which typically do not branch at all, and in *zwichel* mutants, which carry fewer branches than do wild-type plants [49•]. Consistent with these observations, *ZWI* is homologous to genes encoding kinesin-like microtubule motor proteins [50], and the ZWI or kinesin-like calmodulin-binding protein (KCBP) colocalizes with microtubules during cell division in other cell types [51].

How the establishment of different axes of polarity is connected to the cell division machinery is an interesting question arising from the analysis of the *siamese* (*sim*) mutant. Wild-type *Arabidopsis* trichomes endoreduplicate [4], whereas mutation in the *SIM* gene leads to the conversion of endoreduplication cycles into cell divisions that give rise to multicellular trichomes [52•]. Two of the challenging questions emerging from work on the *sim* mutant are: do division planes correlate with branching in *sim* trichomes, and is the involvement of the tubulin cytoskeleton in

unicellular trichome branching evolutionarily related to control of the orientation of cell division in ancestral multicellular trichomes [53]? In the near future, cloning and analysis of the genes corresponding to the many available trichome mutants, in combination with cell biological analyses of these mutants, should add substantially to the understanding of polar axis formation in a single cell.

Mutations in the *Arabidopsis SPIRAL* genes also affect the microtubular cytoskeleton [54•]. *spiral* mutants, which exhibit helical growth patterns, are affected in axial expansion of cortical and (to a lesser extent) epidermal cells. Their phenotype can be reverted to wildtype by microtubule-interacting drugs. Visualization of cortical microtubules in the mutants reveals a defective organization, which apparently has dramatic consequences for the growth characteristics of entire organs.

A thorough description of cytoskeletal elements is also available for maize subsidiary mother cells, which become polarized before undergoing an asymmetric cell division. Recently, specific mutations have been described that interfere with this asymmetric cell division at different levels [55••]. The hypothesis emerging from this work is that an F-actin patch at the guard cell contact site transiently interacts with only the closest nucleus to specify fate by an as yet unknown mechanism, thus translating cell polarity into asymmetric cell fates. Whereas these data suggest that the actin patch is instrumental in asymmetric cell division, the guidance mechanism that directs the polarized cell division plane depends on cortical microtubules (Figure 1g). Therefore, as in trichomes, both the actin and tubulin cytoskeletons have distinct and unique roles in the guidance of a cellular polarization event. The availability of specific mutants provides a starting point for further analysis of this asymmetric cell division, which coordinates information within a multicellular context.

Conclusions

Evidence for the involvement of ion channels, the cytoskeleton, localized secretion, and specialized plasma membrane and cell wall regions in the establishment and maintenance of plant cell polarity has been available for some years. Recent genetic and molecular approaches have provided specific mutants that are defective in the axial and polar specialization of plant cells and have uncovered the molecular nature of some of the affected genes. Marker proteins that allow visualization of or directly participate in axialization and polarization events are now at hand for the molecular and cell biological dissection of these processes. Despite these substantial advances, key questions concerning mechanisms for the establishment of cell axiality and polarity remain unaddressed. What are the different cues that polarize the cells of higher plants and how do they operate? What is the order of intracellular molecular interactions in axialization/polarization in different cell types? And, at the multicellular level, by what mechanisms does polarization at the cellular level influence organ development? These and other unanswered questions may act as polarizing cues for the attention of plant scientists in the near future.

Acknowledgements

We thank Niko Geldner, Martin Hülskamp, and David Welch for discussion and suggestions on the manuscript, and Malcolm Bennett for suggestions and making available data before publication.

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. Cove DJ: The generation and modification of cell polarity. *J Exp Bot* 2000, 51:831-838.

2. Schnepf E: Cellular polarity. *Annu Rev Plant Physiol* 1986, 37:23-47.

3. Sachs T: Cell polarity and tissue patterning in plants. *Development* 1991, 1:83-93.

4. Hülskamp M, Misera S, Jürgens G: Genetic dissection of trichome cell development in ***Arabidopsis***. *Cell* 1994, 76:555-566.

5. Kropf DL: Induction of polarity in fucoid zygotes. *Plant Cell* 1997, 9:1011-1020.

6. Jürgens G, Grebe M, Steinmann T: Establishment of cell polarity during early plant development. *Curr Opin Cell Biol* 1997, 9:849-852.

7. Scheres B, Benfey PN: Asymmetric cell division in plants. *Annu Rev Plant Physiol Plant Mol Biol* 1999, 50:505-537.

8. Roberts S, Brownlee C: Calcium influx, fertilization potential and egg activation in ***Fucus serratus***. *Zygote* 1995, 3:191-197.

9. •• Hable WE, Kropf DL: Sperm entry induces polarity in fucoid zygotes. *Development* 2000, 127:493-501.
Monitoring of polarization events in *Pelvetia* following localized sperm delivery demonstrates that sperm entry provides a cue for zygote polarization. Polyspermy causes initiation of multiple rhizoids at the respective entry sites. As an early polarity marker, an actin patch colocalizes with the sperm pronucleus at the cell cortex towards which secretion is later directed.

10. Shaw SL, Quatrano RS: The role of targeted secretion in the establishment of cell polarity and the orientation of the division plane in ***Fucus*** zygotes. *Development* 1996, 122:2623-2630.

11. Donaldson JG, Jackson CL: Regulators and effectors of the ARF GTPases. *Curr Opin Cell Biol* 2000, 12:475-482.

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

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

14. • Chen JG, Ullah H, Young JC, Sussman MR, Jones AM: ABP1 is required for organized cell elongation and division in ***Arabidopsis*** embryogenesis. *Genes Dev* 2001, 15:902-911.
Loss of function mutants of *Arabidopsis AUXIN-BINDING PROTEIN1* (*ABP1*) are embryo lethal, and *abp1* mostly interferes with cell expansion. These findings are supported by antisense experiments in tobacco cell suspension cultures. In *Arabidopsis*, *abp1* mutants also display defects in oriented cell division including asymmetric hypophysis division.

15. Hamann T, Mayer U, Jürgens G: The auxin-insensitive ***bodenlos*** mutation affects primary root formation and apical–basal patterning in the ***Arabidopsis*** embryo. *Development* 1999, 126:1387-1395.

16. • Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY, Bandyopadhyay A, Estelle M: The ***axr6*** mutants of ***Arabidopsis thaliana*** define a gene involved in auxin response and early development. *Development* 2000, 127:23-32.
Homozygous *auxin resistant 6* (*axr6*) mutants display aberrant patterns of cell division in the embryo, leading to defects in cells of the suspensor, root and hypocotyl precursors, and provasculature. Defects in hypophysis formation in *axr6* mutant embryos appear to cause strong abnormalities in root formation.

17. Shevell DE, Kunkel T, Chua NH: Cell wall alterations in the • ***Arabidopsis emb30*** mutant. *Plant Cell* 2000, 12:2047-2060.
Immunogold labeling of plant cell wall components reveals specific changes in the secretion of lectin and pectin in *emb30* seedlings, which can be visualized using electron microscopy. The authors suggest a role for *EMB30* in cell wall establishment that may account for *emb30* defects in cell polarity and adhesion.

18. Grebe M, Gadea J, Steinmann T, Kientz M, Rahfeld JU, Salchert K, • Koncz C, Jürgens G: A conserved domain of the ***Arabidopsis*** GNOM protein mediates subunit interaction and cyclophilin 5 binding. *Plant Cell* 2000, 12:343-356.
A new conserved protein domain required for GNOM dimerization and for binding of a novel interactor cyclophilin 5 (Cyp5) is characterized in yeast and *in vitro*. Earlier interallelic complementation studies of *gnom*/*emb30* alleles support a functional role for GNOM dimerization *in vivo*.

19. Pimpl P, Movafeghi A, Coughlan S, Denecke J, Hillmer S, • Robinson DG: ***In situ*** localization and ***in vitro*** induction of plant COPI-coated vesicles. *Plant Cell* 2000, 12:2219-2236.
The authors describe their thorough immuno electron microscopic *in situ* localization of plant coat protein I (COPI)-complex components, and their characterization of *in vitro* recruitment of COPI components, including ARF1, to donor membranes.

20. Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, •• Bennett MJ: Localisation of the auxin permease AUX1 in the ***Arabidopsis*** root apex reveals two novel functionally distinct hormone transport pathways. *Genes Dev* 2001, in press.
The subcellular localization of the auxin influx carrier AUX1 protein, including its polar apical localization in protophloem and axial apical–basal distribution in epidermal cells, is described. AUX1 localization, auxin-response reporter studies, and direct auxin measurements suggest an AUX1 function in acropetal and basipetal auxin transport. AUX1 function in basipetal transport is supported by the restoration of auxin responses in lateral root cap and epidermal cells, and by the rescue of *aux1* gravitropism defects by membrane permeable but not influx-carrier-dependent auxins.

21. Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B: An auxin-dependent distal organizer of pattern and polarity in the ***Arabidopsis*** root. *Cell* 1999, 99:463-472.

22. Schindelman G, Morikami A, Jung J, Baskin TI, Carpita NC, •• Derbyshire P, McCann MC, Benfey PM: COBRA encodes a putative GPI-anchored protein, which is polarly localized and necessary for oriented cell expansion in ***Arabidopsis***. *Genes Dev* 2001, 15:1115-1127.
The authors describe the positional cloning of the *COBRA* gene, which encodes the first putative GPI-anchored protein with an associated phenotype in plants. Defects in longitudinal cell expansion in *cobra* mutants, together with axial longitudinal localization of the COBRA protein, demonstrate a role for COBRA in the regulation of longitudinal cell expansion. *cobra* defects in cellulose accumulation and the potential plasma-membrane–cell-wall localization of COBRA protein suggest an interaction of COBRA with cell wall components.

23. 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.
Localized Rop-type G proteins mark the incipient site of polar root hair initiation before morphologically observable changes become apparent. Later, Rop-type proteins localize to the hair bulge and tip. Early localization is sensitive to BFA treatment. Expression of constitutively activated Rops interferes with epidermal cell elongation and localization of actin and tubulin, as well as with root hair tip growth and associated calcium gradients.

24. Masucci JD, Schiefelbein JW: The ***rhd6*** mutation of ***Arabidopsis thaliana*** alters root-hair initiation through an auxin- and ethylene-associated process. *Plant Physiol* 1994, 106:1335-1346.

25. 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.
The authors describe their immunolocalization studies of actin, profilin, myosin VIII, expansin and an ER network that are found in the bulge during root hair formation. Inhibitors of the actin cytoskeleton disturb profilin mRNA and ER localization in the bulge, indicating that the assembly of these components is actin dependent.

26. Braun M, Baluska F, von Witsch M, Menzel D: Redistribution of actin, profilin and phosphatidylinositol-4,5-bisphosphate in growing and maturing root hairs. *Planta* 1999, 209:435-443.

27. Parker JS, Cavell AC, Dolan L, Roberts K, Grierson CS: Genetic • interactions during root hair morphogenesis in ***Arabidopsis***. *Plant Cell* 2000, 12:1961-1974.
Mutations in eight new genes and double mutant analysis elaborate the genetic framework for root hair morphogenesis. A combination of single and double mutant phenotypes suggests that the *SUPERCENTIPEDE1* and *CENTIPEDE2* genes are already active during hair initiation, and that *SHAVEN1* (*SHV1*) acts from early bulge formation onwards, whereas mutations in the *BRISTLED1*, *SHV2, SHV3*, *CENTIPEDE1* and *CENTIPEDE3* genes affect the transition to tip growth.

28. Baumberger N, Ringli C, Keller B: The chimeric leucine-rich •• repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in ***Arabidopsis thaliana***. *Genes Dev* 2001, 15:1128-1139.
LRX1 encodes a chimeric leucine-rich repeat/extensin protein that localizes to the root hair cell wall, where it becomes insolubilized during development. *lrx1* null mutants develop aborted, swollen or branched root hairs, indicating that LRX1 regulates root hair morphogenesis and elongation by controlling either polarized growth or cell wall formation and assembly.

29. Palanivelu R, Preuss D: Pollen tube targeting and axon guidance: • parallels in tip growth mechanisms. *Trends Cell Biol* 2000, 10:517-523.
A comprehensive review of the molecules and pathways involved in pollen tube polar growth and axon guidance. Similar intracellular molecules are recruited to control tip growth, whereas different extracellular molecules mediate guidance through the distinct plant and animal extracellular matrices.

30. Yang Z: Signaling tip growth in plants. *Curr Opin Plant Biol* 1998, 1:525-530.

31. Gibbon BC, Kovar DR, Staiger CJ: Latrunculin B has different effects on pollen germination and tube growth. *Plant Cell* 1999, 11:2349-2363.

32. Kost B, Spielhofer P, Chua NH: A GFP-mouse talin fusion protein labels plant actin filaments ***in vivo*** and visualizes the actin cytoskeleton in growing pollen tube. *Plant J* 1998, 16:393-401.

33. Kost B, Lemichez E, Spielhofer P, Hong Y, Tolias K, Carpenter C, Chua NH: Rac homologues and compartmentalized phosphatidylinositol 4,5-bisphosphate act in a common pathway to regulate polar pollen tube growth. *J Cell Biol* 1999, 145:317-330.

34. Fu Y, Wu G, Yang Z: Rop GTPase-dependent dynamics of tip- •• localized F-actin controls tip growth in pollen tube. *J Cell Biol* 2001, 152:1019-1032.
An elegant demonstration of how Rho GTPases and actin dynamics may be coupled to specify cell polarity in plants.

35. Pierson ES, Miller DD, Callaham DA, Shipley AM, Rivers BA, Cresti M, Hepler PK: Pollen tube growth is coupled to the extracellular calcium ion flux and the intracellular calcium gradient: effect of BAPTA-type buffers and hypertonic media. *Plant Cell* 1994, 6:1815-1828.

36. Drubin DG, Nelson WJ: Origins of cell polarity. *Cell* 1996, 84:335-344.

37. Johnson DI: Cdc42: an essential Rho-type GTPase controlling eukaryotic cell polarity. *Microbiol Mol Biol Rev* 1999, 63:54-105.

38. Yang Z, Watson JC: Molecular cloning and characterization of Rho, a Ras-related small GTP-binding protein from the garden pea. *Proc Natl Acad Sci USA* 1993, 90:8732-8736.

39. Winge P, Brembu T, Bones AM: Cloning and characterization of rac-like cDNAs from ***Arabidopsis thaliana***. *Plant Mol Biol* 1997, 35:483-495.

40. Li H, Wu G, Ware D, Davis KR, Yang Z: ***Arabidopsis*** Rop GTPases: differential gene expression in pollen and polar localization in fission yeast. *Plant Physiol* 1998, 118:407-417.

41. Lin Y, Yang Z: Inhibition of pollen tube elongation by microinjected anti-Rop1Ps antibodies suggests a crucial role for Rho-type GTPases in the control of tip growth. *Plant Cell* 1997, 9:1647-1659.

42. Li H, Lin Y, Heath RM, Zhu MX, Yang Z: Control of pollen tube tip growth by a Rop GTPase-dependent pathway that leads to tip-localized calcium influx. *Plant Cell* 1999, 11:1731-1742.

43. Zheng ZL, Yang Z: The Rop GTPase switch turns on polar growth •• in pollen. *Trends Plant Sci* 2000, 5:298-303.
An excellent review containing compelling data on a Rop-dependent signaling network that controls polar growth in the pollen tube. The authors suggest that two Rop-dependent feedback loops could be central to the behavior of the growing pollen tube. The positive-feedback loop might enable the rapid activation of Ca^{2+}-dependent localized exocytosis, whereas the negative-feedback loop might allow the coordination of exocytosis with cell wall remodeling.

44. Bischoff F, Vahlkamp L, Molendijk A, Palme K: Localization of AtROP4 and AtROP6 and interaction with the guanine nucleotide dissociation inhibitor AtRhoGDI1 from ***Arabidopsis***. *Plant Mol Biol* 2000, 42:515-530.

45. Wu G, Li H, Yang Z: ***Arabidopsis*** RopGAPs are a novel family of Rho GTPase-activating proteins that require the Cdc42/Rac-interactive binding motif for Rop-specific GTPase stimulation. *Plant Physiol* 2000, 124:1625-1636.

46. • Rigas S, Debrosses G, Haralampidis K, Vicente-Agullo F, Feldmann K, Grabov A, Dolan L, Hatzopoulos P: ***TRH1*** encodes a potassium transporter required for tip growth in ***Arabidopsis*** root hairs. *Plant Cell* 2001, 13:139-151.
Plants homozygous for loss-of-function *trh1* mutations initiate multiple hairs from trichoblasts and initiate hairs that abort growth soon after bulging. Cloning and functional analyses of *TRH1* reveal that it encodes a functional potassium transporter.

47. Mathur J, Spielhofer P, Kost B, Chua NH: The actin cytoskeleton is required to elaborate and maintain spatial patterning during trichome cell morphogenesis in ***Arabidopsis thaliana***. *Development* 1999, 126:5559-5568.

48. Szymanski DB, Marks MD, Wick SM: Organized F-actin is essential for normal trichome morphogenesis in ***Arabidopsis***. *Plant Cell* 1999, 11:2331-2348.

49. • Mathur J, Chua NH: Microtubule stabilization leads to growth reorientation in ***Arabidopsis*** trichomes. *Plant Cell* 2000, 12:465-477.
The trichome microtubular cytoskeleton, as visualized with a GFP::MAP4 fusion, involves microtubule criss-crossing into oryzalin-resistant (i.e. relatively stable) knots at branchpoints. Taxol-induced microtubule stabilization initiates new branchpoints in *stichel* and *zwichel* mutants, which typically have less branchpoints than do wild-type plants.

50. Oppenheimer DG, Pollock MA, Vacik J, Szymanski DB, Ericson B, Feldmann K, Marks MD: Essential role of a kinesin-like protein in ***Arabidopsis*** trichome morphogenesis. *Proc Natl Acad Sci USA* 1997, 94:6261-6266.

51. Bowser J, Reddy AS: Localization of a kinesin-like calmodulin-binding protein in dividing cells of ***Arabidopsis*** and tobacco. *Plant J* 1997, 12:1429-1437.

52. • Walker JD, Oppenheimer DG, Concienne J, Larkin JC: ***SIAMESE***, a gene controlling the endoreduplication cell cycle in ***Arabidopsis thaliana*** trichomes. *Development* 2000, 127:3931-3940.
Trichomes in the *siamese* mutant appear in clonally related clusters, become multicellular and display reduced endoreduplication, suggesting that the *SIM* gene is required for the regulation of the endoreduplication cycles in trichomes.

53. Hülskamp M: How plants split hairs. *Curr Biol* 2000, 10:R308-R310.

54. • Furutani I, Watanabe Y, Prieto R, Masukawa M, Suzuki K, Naoi K, Thitamadee S, Shikanai T, Hashimoto T: The ***SPIRAL*** genes are required for directional control of cell elongation in ***Arabidopsis thaliana***. *Development* 2000, 127:4443-4453.
spiral mutants have reduced anisotropic growth of etiolated hypocotyls, right-handed helical growth and irregular microtubule arrays. Microtubule-interacting drugs suppress *spiral* mutations, but also reduce anisotropic growth and induce left-handed twisting. Thus, *SPIRAL* genes may control axial expansion by preventing twisting through their action on microtubules.

55. •• Gallagher K, Smith LG: Roles for polarity and nuclear determinants in specifying daughter cell fates after an asymmetric cell division in the maize leaf. *Curr Biol* 2000, 10:1229-1232.
Four recessive epidermal-patterning mutants in maize – *dcd1, dcd2, brk1* and *pan1* – disrupt the asymmetric division of subsidiary mother cells (SMCs) next to guard mother cells (GMCs). The fate of an SMC daughter nucleus appears to be determined by its proximity to the F-actin patch at the boundary with the GMC.