Vascular development: tracing signals along veins

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The plant hormone auxin has been implicated in vascular development, but the molecular details of patterned vascular differentiation have remained elusive. Research in the past year has identified new genes that control vascular patterning, and auxin transport and perception. New experimental strategies have been employed to study vascular development. Together, these findings have generated a conceptual framework and experimental tools for the exploration of vascular-tissue patterning at the molecular level.

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Abbreviations

ARF	AUXIN RESPONSE FACTOR
AXR6	*AUXIN RESISTANT 6*
BDL	*BODENLOS*
EMB30	EMBRYO-DEFECTIVE 30
GEA1	GUANINE EXCHANGE FACTOR 1
GN	GNOM
mp	*monopteros*
PID	*PINOID*
PIN1	*PIN-FORMED 1*

Introduction

The formation of continuous vascular strands in response to local auxin sources is an amazing phenomenon that has stimulated conceptual discussions in the past and has now become tractable at the molecular level. In cell biological terms, auxins are a special class of plant hormones. Auxins are transported through an elaborate cellular machinery, and their distribution seems to be highly regulated. The control of auxin distribution is sufficiently precise to mediate differential cell behavior even within small groups of cells, as, for example, in the gravitropic responses of *Arabidopsis* roots. Therefore, precisely distributed auxin could also provide positional information in developmentally programmed patterning events.

Research in the past year has identified further genes that are involved in auxin perception and has provided new insights into the molecular biology of auxin transport and the mechanisms underlying its apical–basal directionality. Furthermore, new evidence implicates auxin distribution in cell-patterning events. In the *Arabidopsis* root meristem, a local maximum of perceived auxin seems to be instrumental in specifying patterned cell fates in the distal root [1••]. In shoot meristems, auxin signals determine the phyllotactic position of lateral organs within a peripheral zone [2•] and patterns of vascular networks can be dramatically and predictably altered by modifications of auxin transport during early organ development [3•,4•]. As a recurrent theme in these observations, developmentally regulated auxin distribution profiles may set up coarse patterns to serve as coordinate systems for further fine-tuning of gene activities. In vascular development, for example, auxins have been implicated as pathfinding signals in vascular-strand formation, but further local cell interactions are certainly required to generate functional vascular strands. In this review, we discuss recent findings in auxin signaling and vascular research in the context of general concepts of auxin-mediated vascular patterning.

Auxin transport and vascular-strand formation

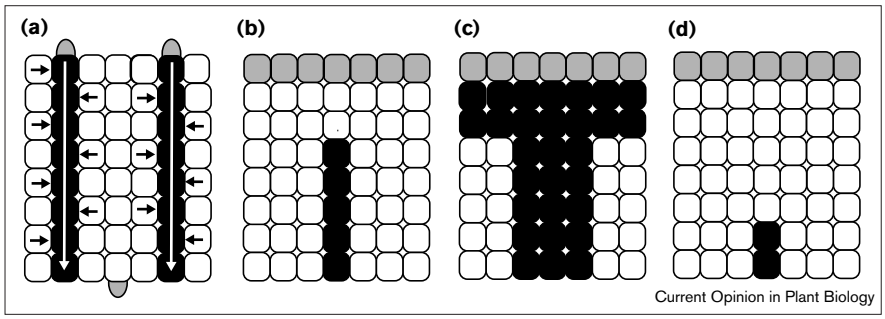
The role of local auxin signals in cell patterning is particularly tractable in vascular-strand formation because organized vascular strands develop in fixed spatial positions relative to the locality of auxin application (Figure 1). In suitable organs, vascular strands can be induced in a variety of positions, indicating that cells within a larger field can equivalently respond to the signal (summarized in [5], Figure 1a). Upon auxin application, however, vascular differentiation is sharply restricted to a narrow zone, which is basal to the auxin source. Thus, the local application of auxin does not merely trigger vascular differentiation, but initiates a sequence of oriented cellular events that result in the formation of a highly organized, functional cellular pattern. The process is dependent on polar auxin transport, but the patterning function of auxin may restricted to the most basic level: the definition of the route of a vascular strand (Figure 1).

Formation of functional vascular strands obviously involves further patterned cell differentiation within the developing strand as well as in non-vascular tissues [6]; the molecular nature of these fine-tuning mechanisms is still entirely elusive. The influence of local auxin sources on the positions of newly formed vascular strands suggests that a positive feedback mechanism stimulates auxin conductivity within preferred routes of auxin transport (referred to as ‘auxin canalization’, summarized in [5], Figure 1b–d). Debate remains, however, as to the cellular mechanisms by which preferred auxin transport routes are translated into vascular differentiation zones (alternative models are summarized in [6]) and the general relevance of auxin-transport-dependent pathfinding processes in normal organ development. The emerging molecular understanding of auxin transport may provide the tools to resolve some of these open questions.

Cell biology of auxin transport

Plant tissue polarity is stably maintained and is intimately associated with an apical–basal transport of auxin. Polar

Figure 1



Signaling in vascular-strand formation. **(a)** A vascular strand can develop in response to locally applied auxin (gray drops). Two apical auxin application sites are shown to indicate that all cells can equivalently respond to the signal. The area of cell differentiation is non-isotropic (indicated by white and black arrows, respectively), suggesting the presence of cellular mechanisms that constrain signaling in certain directions. First, vascular differentiation (black cells) will selectively occur basal to the auxin source; there is no vascular response to the basal application of auxin (gray drop). Second, vascular differentiation will remain restricted to narrow strips of cells. The apical–basal directionality of the auxin signal corresponds to the overall flow of auxin in the plant. Restriction to narrow cell zones could be controlled by feedback mechanisms that further enhance auxin conductivity along preferred routes of auxin transport (i.e. in adherence to the 'auxin canalization' hypothesis [5]). **(b-d)** Expected alterations in vascular differentiation patterns in response to (c) reduced auxin transport and (d) reduced auxin perception under conditions of auxin canalization from a dispersed auxin source (gray cells; normal conditions illustrated in [b]). Feedback stimulation of auxin conductivity promotes convergence of auxin flow towards preferred routes. Vascular differentiation occurs at above-threshold concentrations of auxin along these routes. (c) Auxin accumulation associated with reduced auxin transport should result in increased vascular differentiation closer to the auxin source, whereas (d) impaired auxin perception should restrict vascular differentiation to fewer sites that are further from the source.

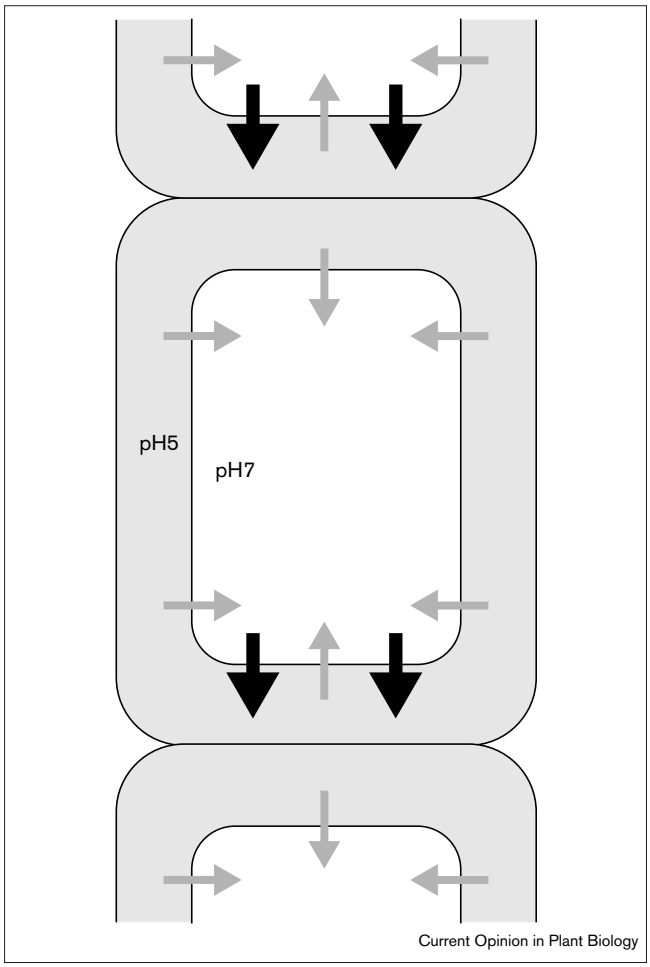
auxin transport proceeds in a cell-to-cell fashion and the existence of saturable sites in membrane vesicles indicates the presence of specific auxin import and export proteins (reviewed in [7]). The directionality of auxin transport has been attributed to the polar localization of auxin efflux-carrier molecules in plasma membranes (Figure 2). This hypothesis implies that polar auxin transport in intact plants involves the maintenance of the coordinated polarity of individual cells. Two lines of research have generated complementary insights into the molecular machinery underlying the regulation of auxin transport and the establishment of its polarity.

First, cell biological studies reveal possible modes of regulating and redirecting auxin transport. The use of specific types of auxins — such as α–naphthalene-acetic acid (NAA) and dichlorophenoxy-acetic acid (2,4-D), which do not interact with either the auxin influx or the efflux machinery, respectively — allows auxin import and export mechanisms to be distinguished from each other. In this experimental setup, the selective inhibition of protein synthesis and of vesicle transport reveals that auxin efflux, but not influx, depends on permanent protein synthesis and on proper vesicle transport from the Golgi to the plasma membrane [8,9]. Thus, at least one protein that is crucial for auxin efflux seems to be permanently resynthesized and reshuffled to the plasma membrane. Such a mechanism would allow auxin to be drained along flexible routes. Orientation of auxin flow could occur through regulated targeting of efflux carrier vesicles, and auxin-flow intensity could be modulated by the regulation of efflux-carrier genes. Alternatively, increased auxin conductivity in response to auxin exposure, as required for 'auxin canalization', could simply be a consequence of the increased efficiency of vesicle transport. In an (auxin) ligand-dependent vesicle trafficking mechanism, the amount of properly localized efflux carriers would be directly linked to the amount of available auxin, consistent with the observation that sustained auxin transport in isolated plant tissues requires an auxin source [9].

Second, genetic approaches seem to have identified *Arabidopsis* genes for targeted vesicle transport and auxin efflux. The *(At)PIN-FORMED 1* (*PIN1*) gene is a prime candidate for encoding an auxin efflux-carrier protein that is involved in vascular development. Auxin transport is reduced in the stems of *pin1* mutants relative to that in wild-type stems [10], and excess vascular tissue, probably reflecting auxin accumulation, is found in mutant stems and leaves (Figure 3) [3•,11]. The PIN1 protein contains characteristic membrane-spanning domains and, most intriguingly, seems to be localized in the basal side of xylem parenchyma cells [11]. Surprisingly, *pin1* null-mutants are viable plants, suggesting that auxin transport is partially maintained by redundantly acting genes. These may be found within the large family of membrane proteins to which PIN1 belongs [12].

While members of the PIN protein family may directly mediate auxin efflux, another *Arabidopsis* gene product, EMBRYO-DEFECTIVE 30 (EMB30)/GNOM (GN), seems to be required for their proper localization. Mutations in *EMB30/GN* lead to variably non-polar embryos, and mutant cells are unable to undergo organized growth (Figure 3) [13]. Consistent with a fundamental distortion of polar signaling, mutant vascular cells are randomly oriented rather than being aligned in strands. The *EMB30/GN* gene encodes a protein with high sequence similarity to yeast GUANINE EXCHANGE FACTOR 1 (GEA1), and this *Arabidopsis* gene can

Figure 2



'Chemi-osmotic' model for polar auxin transport. The polarity of the overall flow of the auxin indoleacetic acid (IAA) is attributed to the polar (selectively basal) localization of auxin efflux carriers in the plasma membrane (black outward arrows). Auxin influx into the cell occurs either directly through the plasma membrane (as IAAH) or is mediated by proton-symport carriers (acting on IAAH plus H^+; gray inward arrows). IAAH dissociates at intracellular pH, and efflux of IAA^- is strictly dependent on basally localized efflux carriers.

complement *gea1* mutations in yeast [14••]. GEA1 and a family of related nucleotide exchange factors regulate the activity of adenosyl ribosylation factor 1, a small ras-like protein that is required for targeted vesicle transport from the Golgi to the plasma membrane. By analogy, EMB30/GN could have an essential function in vesicle transport to position auxin efflux carrier proteins. This interpretation is supported by the observation that the PIN1 product is not properly localized in *emb30/gn* mutant embryos [14••].

Vascular patterns in organ development

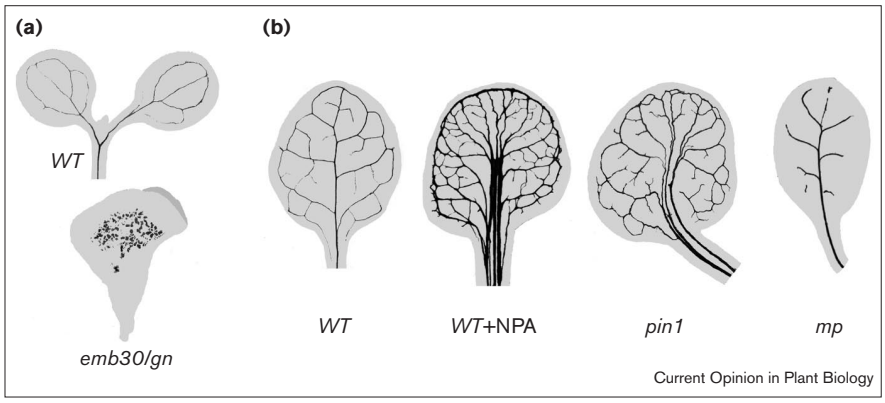
While mutations in *PIN* genes seem to provide long-sought-after entry points into the molecular biology of auxin transport, the chemical inhibition of auxin transport through the use of auxin efflux inhibitors enables the researcher to control the degree and stage of interference with auxin transport. Two recent studies have explored the effect of auxin transport (efflux) inhibition on vascular development in dicots [3•,4•]. First, in a variety of organs (predominantly tested in *Arabidopsis*), excess vascular tissue was formed and cell differentiation in vascular strands was less properly aligned when auxin efflux was inhibited. These observations are consistent with a function of polar auxin transport in draining auxin from apical sources and in aligning cell differentiation with the axis of auxin flow. Second, leaf vascular patterns (i.e. venation) were studied in detail. In *Arabidopsis*, and three other dicot species, the leaf venation patterns were dramatically altered, and the types of alterations were correlated to the development stage and dose at which auxin-efflux inhibitors were applied. Auxin efflux inhibition affects the width of an individual vascular strand only at early stages of strand development, and causes vascular differentiation to occur closer to the leaf margins only at early stages of leaf development (Figure 3). These findings suggest, first, that early differentiation stages are associated with changes in the expression of auxin carriers, which render the incipient strands less sensitive to transport inhibitors; and second, that major auxin sources are located near the margins of young leaf primordia. It should be possible to test both of these predictions in the near future.

Auxin perception

If auxin signaling is critically important in cell-patterning events, including vascular-strand formation, at least some classes of auxin-perception mutants should display characteristic vascular defects. Two genes identified in the past year, *AUXIN RESISTANT 6* (*AXR6*) and *BODENLOS* (*BDL*), mutate to produce phenotypes that are closely related to those of the previously identified *monopteros* (*mp*) mutants [15•,16•,17,18]. Common defects among these three mutants include impaired auxin perception, a generally reduced vascular system, defective embryo axis formation and a failure to produce an embryonic root. In early embryos of these mutants, cells already appear to be impaired in their capacity to orient division and expansion along the apical–basal axis. This defect is particularly evident in the hypocotyl primordia, and the amount of vascular tissues is generally reduced in these mutants (Figure 3). The failure to form a primary root at the base of the hypocotyl could be a consequence of the defects in embryo axis formation. It has recently been shown that the perturbed embryo development of *mp* mutants is associated with the absence of a basally located 'auxin maximum', which seems to be an essential prerequisite for the formation of a primary root [1••].

The simultaneous occurrence of defects in embryo-axis and vascular-strand formation in *axr6*, *bdl* and *mp* mutants suggests that these architectural features share an underlying molecular mechanism, probably involving the auxin-perception-dependent alignment of cellular events with the axis of auxin flow (i.e. 'cell axialization'). One may therefore expect

Figure 3



Vascular abnormalities resulting from presumed defects in polarity and the intensity of auxin transport, and in auxin sensitivity. **(a)** Disrupted vascular strands in the *emb30/gn* mutant seedling as opposed to the *Arabidopsis* wild-type (WT) seedling. EMB30/GN has been implicated in the proper polar positioning of auxin efflux carriers, and mutations in *emb30/gn* interfere with vascular-strand formation but not with vascular differentiation *per se*. **(b)** *Arabidopsis* leaf-venation patterns are altered (i.e. vascular differentiation close to the leaf margin is enhanced) in leaves treated with the auxin-efflux inhibitor napthylphthalamic acid (NPH) and in leaves defective for the presumed auxin efflux carrier PIN1. Leaf vascular patterns are characteristically reduced in a class of presumed auxin-insensitive mutants including *mp*. These cartoons illustrate the vascular defects described in [3•,13,18] .

that mutations in *AXR6*, *BDL* and *MP* affect auxin sensitivity and, in various non-overlapping tests, this has proven to be the case. Additional genetic evidence confirms that AXR6 and BDL function in auxin signal transduction. Mutations at the *AXR6* locus are semidominant and auxin insensitivity has been extensively documented in heterozygous mutants [15•]. Mutant defects in *bdl* embryos, in turn, are synergistically enhanced in double mutants with the *auxin resistant 1* mutant [16•], which itself does not have an embryo phenotype. Strong support for an involvement of MP in auxin signal transduction also comes from the previously reported identification of the MP gene product [19]. The *MP* gene encodes a nuclear protein with the signature domains of the AUXIN RESPONSE FACTOR (ARF) family: an amino-terminal DNA-binding domain, a central activation domain and two conserved putative protein-interaction domains near the carboxy terminus [20]. ARFs have been implicated in auxin signal transduction because of their capacity to bind to auxin response elements, which are short regulatory DNA sequences that mediate auxin-regulated gene expression [20]. The conserved carboxy-terminal protein domains, which are also found in the related family of auxin-inducible, short-lived, nuclear 'IAA/AUX' proteins, can mediate homo- and heterotypic interactions among members of both the ARF and the IAA/AUX protein families [20]. In *Arabidopsis*, both of these protein families comprise more than a dozen members, and it has been suggested that the specificity of individual auxin signaling pathways may be brought about by distinct protein combinations within transcriptional complexes. Some of these could be specific for cell 'axialization' and/or vascular differentiation. Both *AXR6* and *BDL* are currently being cloned, and it will be interesting to see whether their products act in the same pathway as MP.

For genes with locally restricted functions, it may be more difficult to assess their possible involvement in auxin transport or perception. Mutations in the *Arabidopsis* genes *ETTIN* and *PINOID* (*PID*) [21,22,23•] disrupt organ development and vascular patterning in flower organs in ways that are consistent with auxin-response defects. Moreover, *ETTIN* encodes an ARF-like protein (lacking the presumptive protein-interaction domains) [21] and *PID* a serine-threonine protein kinase, which upon overexpression antagonizes a number of auxin responses [23•]. It is therefore easily conceivable that both genes act as regulators of auxin response, but definition of their precise roles in auxin signaling and organ development may have to await new strategies to assess auxin-related parameters locally within the flower.

Eventually, direct screens for mutants with abnormal vascular development are required to identify genes irrespective of their relationship to auxin signaling pathways. Three new vascular-patterning genes have recently been identified in *Arabidopsis*. Mutations in the *SCARFACE* gene predominantly disrupt continuity in lower-order veins, but are associated with enhanced rather than reduced responses to external auxin [24•]. Mutations in two other genes, *COTYLEDON VASCULAR PATTERN 1* and *2*, result in specific vascular distortions throughout development, but apparently do not affect auxin perception or auxin transport [25•]. All three genes seem to be required at the early stages of provascular development, but further direct screens may also identify genes that regulate the spatial pattern of cell types within vascular strands.

Conclusions

Distortions in auxin signaling and transport have long been known to impinge on patterning processes in plants. It now appears that these effects may reflect genuine functions of auxin as a positional signal within pattern-specifying genetic networks. Auxin distribution as a coarse basic pattern refined by cascades of fine-tuning gene activities, would be consistent with the robust flexibility of meristem and vascular patterns. Vascular

networks, for example, though often highly reproducible retain remarkable flexibility to adapt to abnormal growth conditions or wounding. As has been suggested, auxins could serve as coordinating signals, adjusting local cell behavior to the overall growth requirements of the plant [5]. At present, most conclusions are still based on indirect evidence, largely because auxins remain basically invisible at cellular resolution. However, current rapid advances in the identification of relevant genes, mutants and markers have definitely opened the prospect of understanding signaling in vascular-pattern formation at the molecular level.

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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

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•• 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.

The paper demonstrates an instrumental role of a local maximum (i.e. concentration peak) of perceived auxin in patterning the distal root. This maximum is visualized through a synthetic auxin-response element. Its alteration in a number of auxin response and auxin transport mutants is associated with patterning defects. The generation of an ectopic auxin maximum by polar auxin transport inhibitors, auxin addition or laser ablation of cells in wild-type root meristems causes ectopic respecification of distal cell types.

2. Reinhard D, Mandel T, Kuhlemeier C: **Auxin regulates the initiation
• and radial position of plant lateral organs.** *Plant Cell* 2000, **12**:507-518.

The authors demonstrate that auxin is sufficient to induce lateral organ formation in certain zones of shoot meristems, thereby providing new insights into the role of auxin in defining the position of lateral organs along the circumference of the meristem. Auxin transport inhibition is used to block lateral organ formation in tomato vegetative meristems. In this test system, auxin induces leaf formation. Leaf initiation occurs at the circumference position of auxin application, suggesting an instrumental role for auxin distribution in positioning the lateral outgrowth. This response is, however, restricted to the meristem periphery, indicating that another, auxin-independent system regulates the response competence of cells as they are displaced from the center to the periphery of the meristem.

3. Mattsson J, Sung ZR, Berleth T: **Responses of plant vascular
• systems to auxin transport inhibition.** *Development* 1999, **126**:2979-2991.

The authors show that a variety of vascular patterns can be generated within a given genetic background under conditions of modulated auxin transport. Leaf-venation defects that are characteristic of abnormal auxin transport, as opposed to impaired auxin perception, are shown. The earliest stages of provascular development in leaf primordia are analyzed and the phenocritical stages of auxin transport for the formation of different vein classes are determined. From this information, it is predicted that major auxin sources are located near the margins of early leaf primordia and that early provascular differentiation is already associated with reduced sensitivity to auxin transport inhibitors.

4. Sieburth LE: **Auxin is required for leaf vein pattern in *Arabidopsis*.
• *Plant Physiol* 1999, **121**:1179-1190.

The paper shows the effects of auxin transport inhibitors on the venation pattern of *Arabidopsis* leaves. In addition, it demonstrates that severe effects are also caused by the application of a presumptive auxin antagonist. Analysis of the time course of leaf venation also suggested that no midvein is formed in the presence of the auxin antagonist and that, instead, the central region is occupied by a coalescence of secondary veins.

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

14. Steinmann T, Geldner N, Grebe M, Mangold M, Jackson C, 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.

The work described in this paper implicates the *Arabidopsis* gene *EMB30/GNOM* in vesicle transport during the establishment of polar auxin flow. A large body of literature has established the function of GEA1 and related yeast guanosine exchange factors in regulating the activity of the ras-like GTPase adenosyl ribosylation factor 1, which in turn, is required for vesicle trafficking from the Golgi to the plasma membrane. Selective inhibition of this function by brefeldin B obstructs vesicle trafficking and, in plant cells, polar auxin transport. Here, the authors show that the *Arabidopsis* gene *EMB30/GNOM* can act as a guanosine exchange factor and can substitute for GEA1 activity in yeast.

15. Hobbie L, McGovern M, Hurwitz LR, Pierro A, Liu NY,
• Bandyopadhyay A, Estelle M: **The *axr6* mutants of *Arabidopsis* define a gene involved in auxin response and early development.** *Development* 1999, **127**:23-32.

Homozygous *axr6* mutants fail to initiate both the hypocotyl and primary root in the embryo and form incomplete vascular systems. In heterozygous mutants, the same mutations confer measurable auxin insensitivity at various stages of development. Thus, *axr6* mutants provide additional evidence that defects in auxin signaling can severely perturb embryo and vascular development. Mutant alleles also confer auxin insensitivity to otherwise normal triploid plants, suggesting that the defects originate from an altered gene product, which has gained an abnormal function.

16. 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.

A new rootless embryo mutant, *bdl*, is described. Mutant seedlings have variably reduced hypocotyls and vascular systems, lack primary roots and are impaired in several responses to external auxin. Apical dominance is reduced in adult mutant plants. The seedling defect can be traced back to early embryo stages and is related to defects in the *Arabidopsis mp* mutant. A relationship between this mutation and auxin signaling defects is further suggested by a synergistic enhancement of the phenotype in *bdl* ; *axr1* double mutants.

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23. • Christensen SK, Dagenais N, Chory J, Weigel D: **Regulation of auxin response by the protein kinase PINOID.** *Cell* 2000, **100**:469-478.
The flowerless, pin-shaped inflorescence axes of *pid* mutants raised the possibility of an involvement of the *PID* gene in auxin transport or auxin perception. This manuscript describes the identification of the *PID* gene as a serine-threonine protein kinase, which is predominantly expressed at the flanks of lateral organ outgrowths during the embryonic and reproductive stages. Evidence for an involvement of PID in auxin signaling is based on the phenotype of *PID* overexpressing plants, in which auxin responses are reduced in a variety of assays.

24. • Deyholos M, Cordner G, Beebe D, Sieburth L: **The *SCARFACE* gene is required for cotyledon and leaf vein patterning.** *Development* 2000, **127**:3205-3213.
A screen for mutants with abnormal vascular patterns identified a new *Arabidopsis* gene, *SCARFACE*. The gene appears to be required in early provascular development, and mutations result in the fragmentation of vascular strands into short vascular 'islands'. Mutations affect vascular systems in cotyledons, vegetative leaves, and sepals and petals, and are associated with enhanced responses to exogenous auxin.

25. • Carland FM, Berg BL, FitzGerald JN, Jinamornphongs S, Nelson T, Keith B: **Genetic regulation of vascular tissue patterning in *Arabidopsis*.** *Plant Cell* 1999, **11**:2123-2137.
The simple, reproducible vascular pattern of *Arabidopsis* cotyledons was used to screen for venation-pattern mutants. Mutations in two newly identified genes, *COTYLEDON VASCULAR PATTERN 1* and *2* (*CVP1* and *2*), also affect the vascular patterns of vegetative leaves. The mutant phenotypes are distinct. Whereas vascular strands in *cvp1* mutants appear to be thickened, containing unusually large numbers of tracheary elements, *cvp2* mutations increase the number of secondary veins in the cotyledons. Both *cvp1* and *cvp2* mutations affect vascular-strand formation at early developmental stages. Nevertheless, *cvp1* and *cvp2* mutants are not visibly affected in auxin transport or auxin perception.