DEVELOPMENT OF SYMMETRY IN PLANTS

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Key Words asymmetry, axes, embryogenesis, lateral organs, flowers

Abstract Plant development involves specification and elaboration of axes of asymmetry. The apical-basal and inside-outside axes arise in embryogenesis, and are probably oriented maternally. They are maintained during growth post-germination and interact to establish novel axes of asymmetry in flowers and lateral organs (such as leaves). Whereas the genetic control of axis elaboration is now partially understood in embryos, floral meristems, and organs, the underlying mechanisms of axis specification remain largely obscure. Less functionally significant aspects of plant asymmetry (e.g. the handedness of spiral phyllotaxy) may originate in random events and therefore have no genetic control.

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WHAT IS SYMMETRY?

An object can be said to show symmetry if it appears the same after a transformation—for example, a rotation, a shift along a straight line to a new position or on reflection about a plane running through it (for more extensive, nonmathematical considerations of symmetry, see 21, 91). Symmetry therefore implies uniformity. The most symmetrical form possible for an organism is a sphere—a sphere remains the same on rotation or reflection in any axis passing through its midpoint and therefore shows infinite planes of rotational and reflectional symmetry. The early
stage embryos of higher plants and many animals approximate morphologically to spheres. In contrast, mature plant organs tend to show reduced symmetry. A leaf, for example, usually has only one vertical plane of reflectional symmetry passing through its midrib (a form of symmetry termed bilateral by biologists). Development of a plant therefore involves loss of uniformity as a consequence of the formation and elaboration of axes of asymmetry. Many of these axes can be traced back to asymmetry specified in early embryogenesis.

In many model animal species, development of asymmetry is understood well enough to allow it to be divided into discrete phases. First, an axis is specified—often by long-range signals or unequal partitioning of a determinant within a cell before division. Second, specification of different identities occurs in domains along the axis. This usually results from activation of different transcription factors that may interact to reinforce the distinctions between domains. Third, short-range interactions can then elaborate the pattern, a process that may be coupled with growth.

This review considers how axes of asymmetry might be formed during higher plant embryogenesis and how they can be maintained and elaborated during growth to maturity. Of particular interest is how the mechanisms that specify different axes are co-ordinated and interact to ensure a normally patterned, functional plant.

ASYMMETRY IN EMBRYOGENESIS

Apical-Basal (A-B) Asymmetry

The Fucus Paradigm  Plant A-B axis formation has been extensively characterized in brown algae, mainly members of the genus Fucus. Although they share no common multicellular ancestor with angiosperms and are therefore likely to have adopted different solutions to axis specification, fucoid algae provide insights into mechanisms that might operate in angiosperms. The subject has been reviewed recently (15, 29) and is summarized only briefly here. Fucoid algae produce spherical free-floating eggs lacking all signs of polarity. After fertilization, polar environmental cues (e.g. directional light or gravity) establish an axis of asymmetry that is initially labile and can reorient in response to a change in the direction of environmental cues. Polarity subsequently becomes fixed and organelles are asymmetrically distributed along the A-B axis. In the absence of polar environmental cues, zygotes are able to form A-B axes apparently at random, although the site of sperm entry may have an influence. After axis fixation, the cell wall at the basal pole subsequently bulges and a new cell wall is laid down perpendicular to the A-B axis, dividing the zygote asymmetrically into a larger apical cell, from which the shoot-like thallus forms, and a smaller basal cell, which gives rise to the root-like rhizoid. The cell wall is implicated in maintaining A-B asymmetry in the early embryo because a cell isolated with its walls intact can regenerate an embryo retaining the original polarity. In contrast, isolated protoplasts regain
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spherical symmetry and sensitivity to environmental cues. Ablation experiments suggest that signaling between apical and basal cells is not necessary to reinforce cell fates (e.g. an apical cell retains its identity when the rhizoid cell is ablated, and vice versa), but that cell walls may continue to have a role in this process.

A-B Asymmetry in Angiosperms

Asymmetry along the apical basal (A-B) axis is usually apparent in mature angiosperm embryos in the position of the shoot meristem apically and the root meristem basally separated by embryonic stem (hypocotyl) and root. The A-B axis of asymmetry is the first to become apparent in embryogenesis, and it forms the major axis of growth of roots and shoots after germination.

As in fucoid algae, initial division of the angiosperm zygote is usually asymmetric, frequently resulting in a large, vacuolated basal cell and a smaller, cytoplasmically dense apical cell (Figure 1, see color plate) (71). Subsequent embryonic development has been best characterized in *Arabidopsis* by observation and fate mapping of genetically marked cells (81), and involves a relatively invariant pattern of cell division. The basal cell of *Arabidopsis* divides horizontally to form a filamentous suspensor of 6 to 9 cells through which metabolites can pass to the developing embryo, and may be a source of factors necessary for normal embryonic growth (108, 109). The most apical derivative of the basal cell, the hypophysis, follows a different fate and divides asymmetrically to give a smaller, apical daughter cell that forms the quiescent center and a larger basal cell that gives rise to the central (columella) root cap initials (25, 80). The remainder of the embryo is derived from the original apical cell, which divides twice parallel to the A-B axis and subsequently perpendicular to it to form an embryo with two four-celled tiers. The upper tier gives rise to the most apical structure of the embryo—the shoot apical meristem and most of the cotyledons, whereas the lower tier contributes the basal part of the cotyledon, the hypocotyl, embryonic root, and the remaining initials of the root apical meristem.

Maternal Orientation of the A-B Axis

The mechanisms of A-B axis specification in angiosperm embryos remain enigmatic. In contrast to fucoid algae, the zygotes of most angiosperms are derived from egg cells that show A-B asymmetry before fertilization. They are usually elongated and show an asymmetrical distribution of organelles along the future A-B axis (71). They are also oriented relative to maternal tissues and the female gametophyte, so that the apical pole of the embryo faces the proximal part of the ovule. Polarity of the gametophyte can in turn be traced to early stages of ovule development when the archesporial cell (the diploid precursor of the female gametophyte) elongates along the future embryonic A-B axis (20, 55, 76), and this asymmetry is maintained through subsequent development of the gametophyte in the polarity of meiotic and mitotic divisions and the fate and migration of haploid nuclei (84).

The invariant orientation of the embryonic A-B axis implies that it is determined by asymmetry of the ovule. However, embryos can form an A-B axis in the absence
Figure 1  Axes of asymmetry in *Arabidopsis* embryogenesis. Embryos are shown in longitudinal sections along the apical-basal axis above, with transverse sections at the positions of arrowheads below. Cell walls formed since the previous stage are shown by thicker lines and derivative of the upper and lower tiers and hypophysis in blue, green, and yellow, respectively. [Redrawn from (79, 59).] Not to scale.
of ovules—for example, from cultured protoplasts (33, 51), zygotes (33, 38, 43), or zygotes formed by fertilization of spherical egg cell protoplasts in vitro (13). Ectopic embryos can also be formed from epidermal cells of Arabidopsis plants misexpressing the embryo-specific gene LEAFY COTYLEDONS1 (46) or naturally from the leaves of certain species (94). Therefore, A-B axis formation might be regarded as an intrinsic property of embryogenic cells, analogous to that of Fucus zygotes which can form an axis in the absence of asymmetric environmental cues. In the same way that directional light or gravity sets the direction of the Fucus axis, asymmetry of the ovule might influence the orientation of the embryonic A-B axis in angiosperms. Somatic embryos produced in culture often orient their A-B axis with reference to existing asymmetry of the embryonic tissue or isolated initial. For example, somatic embryos can be induced to form from pollen grains of Hyoscyamus niger, which first undergo an asymmetric division to produce a larger vegetative cell and a smaller generative cell, as in normal pollen development. Embryos develop from the vegetative cell with their basal pole toward the generative cell, which itself divides to form a suspensor-like structure (70). These observations favor a mechanism in which the axis is oriented by existing internal asymmetry of the embryogenic cell, rather than by signals from other tissues.

In genetically tractable animal species, specification of at least one primary axis of asymmetry involves maternally expressed gene products deposited in the egg [e.g. anterior-posterior axis formation in Drosophila embryos; summarized in (14)]. These genes and others needed for their expression and deposition of their products are identified by maternal-effect mutations disrupting embryonic pattern. Genetic analysis in Arabidopsis has identified the SHORT INTEGUMENT (SIN) gene as a potential component of the maternal contribution to embryonic asymmetry (72). Homozygous sin mutants produce defective embryos that often lack A-B asymmetry, even when fertilized with pollen from wild-type plants. This suggests that SIN is needed maternally for embryo polarity (72). In addition, SIN is required for at least one aspect of ovule asymmetry—cells of sin mutant integuments (maternal tissues around the gametophyte) show reduced elongation along the long axis of the ovule, corresponding to the embryonic A-B axis (74). Therefore, the embryonic effect of sin mutations might result from polarity defects in the ovule.

**The Role of Asymmetric Cell Division** The first asymmetric cell division of the angiosperm zygote appears to be necessary for elaboration of a normal A-B axis. Arabidopsis zygotes lacking GNOM/EMB30 (GN) gene activity show reduced elongation along the A-B axis, undergo a more symmetric first division, and form embryos with variable defects in A-B polarity (57, 59, 82). The most severely affected embryos develop into spherically symmetrical balls of tissue, whereas in others, A-B polarity is reversed with respect to maternal tissues (102). Similarity of the GN gene product to a yeast protein required for membrane vesicle formation suggested a role in export of material to growing cell walls (either existing walls that are growing or new walls being formed at cell division). This view is supported
by postembryonic expression of $GN$ in wild-type plants and the requirement for $GN$ activity in cell elongation, division, and adhesion in a variety of tissues (17, 82). Because the primary defect in $gn$ mutant zygotes appears to be failure of the first asymmetric division, this division appears necessary for correct A-B asymmetry.

**Genes Elaborating the A-B Axis**

Mutations in other *Arabidopsis* genes affect development of specific elements of the A-B pattern. For example, *gurke* (*gk*) mutant embryos often fail to form cotyledons and a shoot apical meristem (97); *hobbit* (*hbt*) mutant embryos lack root meristems because they failed to undergo a horizontal asymmetric division in the hypophysis at the eight-cell stage (Figure 1, see color plate) (80, 106), and *fackel* (*fk*) mutant embryos lack hypocotyl tissue between apical and basal structures (59). The $gn$ mutation is epistatic to $gk$, $fk$, and $hbt$ mutations, affects embryos earlier in development, and disrupts A-B patterning globally. Therefore, $GK$, $FK$, and $HBT$ may be expressed in domains along the A-B axis in response to $GN$-dependent polarity. The domains are proposed to correspond to apical or basal tiers of the eight-cell embryo ($GK$ and $FK$, respectively) or the hypophysis ($HBT$; Figure 1, see color plate). To explain why each mutation has an effect first in one domain, but then subsequently affects neighboring cells, interaction between domains is proposed to refine and elaborate the A-B pattern. For example, lower tier cells inhibit suspensor fate in the hypophysis and allow cells in both domains to interact at their boundary to organize a functional root meristem (reviewed in 39a, 58). Although the patterns of $GK$, $FK$, and $HBT$ expression have yet to be reported, other genes show early asymmetric expression along the A-B axis. These include *AtML1*, a homeobox gene of unknown developmental function, which is first expressed in the apical cell after the first embryonic division (47). This indicates that an A-B pre-pattern capable of directing asymmetric gene expression exists at this stage.

**A Role for Auxin in A-B Asymmetry?**

Analysis of another class of *Arabidopsis* mutants has suggested that the phytohormone auxin is involved in A-B axis development. Several lines of evidence suggest that the *MONOPTEROS* (*MP*) and *BODENLOS* (*BDL*) loci act in response to auxin signaling: *MP* encodes a transcription factor capable of binding auxin-responsive promoter elements (37) and *mp* mutant plants produced via tissue culture show phenotypes characteristic of disrupted auxin activity (69). Similarly, *bdl* mutants are insensitive to auxin and share other phenotypes with the auxin-insensitive mutant *axr1* (35). Mutations in either *MP* or *BDL* block formation of the central and basal regions of the embryo. The defects can be traced back to the two-cell stage, where the apical cell may divide horizontally, rather than vertically, resulting in an embryo with four cell tiers expressing apical characters (10, 35). Together, these findings implicate auxin signaling in specification of basal identity early in embryonic A-B axis formation. However, the expression of *MP* and *BDL* provides no clues as to where the potential auxin signal might come from. *MP* mRNA appears to accumulate uniformly along the A-B axis of early wild-type embryos (37), which suggests that
MP activity might confer sensitivity to auxin on all these cells. The observation that the bdl mutation affects development of apical as well as basal regions in an mp mutant background suggests that BDL is also expressed apically (35).

**Signals Reinforcing A-B Asymmetry**  Signaling between the products of the apical and basal daughter cells is also implicated in maintaining A-B asymmetry. Mutations in a number of different *Arabidopsis* genes, including two RASPBERRY loci (107), three ABNORMAL SUSPENSOR loci (85), and TWIN2 (TWN2) (110), lead to arrest of embryo development. Suspensor cells are then able to assume embryonic patterns of division and marker gene expression, suggesting that a viable embryo is needed to suppress embryo fate in the suspensor. At least one of these genes, TWN2, encodes a housekeeping function (valyl-tRNA synthetase) necessary for continued growth and division of the original embryo (110). In contrast, twn1 mutations allow formation of ectopic embryos without arresting development of the original embryo, suggesting that TWN1 might have a more specific role in the inhibition of embryo fate in the suspensor, from as early as the two-cell stage (101).

**Inside-Outside Asymmetry of the Embryo**  An histological distinction between inner and outer parts of the *Arabidopsis* embryo becomes apparent from the eight-cell stage (Figure 1, see color plate) (59). Previous divisions of the original apical cell have been anticlinal, producing new cell walls perpendicular to the surface of the embryo. The next round of cell division is periclinal and asymmetric, as new cell walls are formed approximately parallel to the surface. Larger daughter cells on the outside of the embryo form the protoderm. They subsequently divide only anticlinally to give rise to the epidermis of the embryo, including the outer cell layer of the shoot apical meristem. The behavior of the smaller, inner daughter cells depends on their position along the A-B axis. In the basal region, they are split by vertical, periclinal divisions to give an inner cell, which will give rise to vasculature and pericycle, and an external cell, which will form ground tissue (light-green and darker-green cells in Figure 1, see color plate). These cells are then subdivided by further vertical, periclinal divisions. Vertical anticlinal divisions increase the number of cells in the circumference of each layer, and horizontal divisions subdivide the basal tier of the embryo (79). The result is a hypocotyl and root consisting of concentric cell layers that appear radially symmetrical in transverse section (Transition Stage embryo in Figure 1, see color plate). Division of the hypophysis appears similarly ordered, whereas those of internal cells in the apical region are more randomly oriented.

**Origins of Inside-Out symmetry**  In addition to having A-B asymmetry, the zygote is also inherently asymmetric along an axis from its inside to its outside (or vice versa). Therefore, the first periclinal divisions in the eight-cell embryo might be oriented by signals from outside the embryo, or polarity within the cells...
themselves. Removal of the protoderm of lemon embryos at the globular stage has provided no evidence that external or internal signals play a part in specifying protoderm cell fate: Exposed underlying cells proliferated to form undifferentiated callus (16). One speculation is that inside-out polarity is set by the zygotic cell wall. Internal cell walls of the embryo are produced de novo, whereas all external walls are descended from that of the zygote and may therefore inherit a determinant of outside identity. After germination, the outer cell wall grows to cover the entire shoot and primary root (lateral roots organize a new epidermis from internal cells of the cortex; 54). This proposal is consistent with the fate of cells produced by aberrant periclinal divisions in the shoot protoderm of dicots. The inner daughter cell, which lacks derivatives of the external wall, is displaced internally and assumes a non-protoderm fate, while the outside daughter cell retains the external wall and protoderm identity. Similarly, inner daughter cells of the protoderm are able to contribute to internal tissue of monocot organs and dicot petals during normal development (discussed in 93).

**The Role of Asymmetric Cell Division**  The periclinal divisions in the eight-cell Arabidopsis embryo appear necessary for correct inside-out patterning. Mutations in a number of genes, including KNOLLE (KN), disrupt this division and thereby prevent formation of a normal protoderm (26, 48, 59, 96, 107). KN encodes a syntaxin required for membrane vesicle fusion during cell wall synthesis, and kn mutant embryos frequently form misoriented or incomplete cell walls that allow cytoplasmic continuity between daughter cells along the inside-outside axis (42). Defects in patterning along this axis are therefore consistent with a failure of cell division to partition a determinant of inner or outer cell fate. However, kn and other mutants including fass, which shows random orientation of cell divisions (59, 98), retain some aspects of inside-outside asymmetry. They produce vascular tissue centrally, surrounded by ground tissue, although the number and arrangement of cells in each layer may be disrupted. Cells toward the outside of kn mutant embryos also express the lipid transfer protein gene AtLTP1, which is normally restricted to the single layer of protoderm cells (102). Periclinal cell divisions therefore appear necessary to maintain or elaborate correct inside-outside polarity, rather than to form it.

**Genes Elaborating Inside-Outside Asymmetry**  Genes that may respond to radial asymmetry in the lower part of the embryo have been identified by mutations that affect formation of functional root meristems (79). These include SCARECROW (SCR), which encodes a transcription factor necessary for the asymmetric periclinal division that splits outer cortical initials from those of the endodermis internally (23). SCR is expressed in the common cortical-endodermal initial before division, which suggests that it responds to an inside-outside pre-pattern. Subsequent expression is confined to the endodermal daughter cell, which suggests that the periclinal division further refines the pattern along the inside-outside axis. In the absence of the periclinal division, progeny of the initial cell express both cortical
and endodermal characters, consistent with this view. In the apical region of the embryo, expression of \textit{ATML1} becomes confined to the protoderm following the first asymmetric division (47), while that of \textit{WUSCHEL} (\textit{WUS}), required for apical meristem function, is established in the internal daughters of the most apical cells (56). This indicates that a pre-pattern along the inside-outside axis can also direct gene expression in this region.

\textbf{Interaction Between Axes of Asymmetry} The observation that inside-outside symmetry has different consequences along the apical-basal axis (e.g. in patterns of gene expression and cell division) implies that the two axes interact to specify cell fate. Expression of \textit{WUS} in internal apical cells indicates that interaction occurs at least as early as the 16-cell stage. Responses to the two axes can be uncoupled genetically. For example, \textit{gn} mutants, which show defects in A-B polarity soon after fertilization, may produce a normal protoderm layer expressing the marker \textit{AtLTP1} (102), which implies that inside-out asymmetry is not dependent on A-B asymmetry. Similarly, \textit{kn} mutants show a disrupted inside-outside pattern but retain aspects of A-B asymmetry, including asymmetry of the first zygotic division.

\textbf{Bilateral Symmetry of the Embryo}

\textbf{Development of Bilateral Symmetry} The combination of A-B and inside-out axes of asymmetry results in a globular embryo that shows full rotational symmetry in the arrangement of tissues, i.e. the embryo appears the same after any rotation about its A-B axis (although the degrees of rotational symmetry are fewer if the radial walls separating cells in each layer are taken into account; Figure 1, see color plate). At this stage, a further axis of asymmetry becomes imposed on the embryo, which in most dicots results in formation of a pair of cotyledons. Viewed in transverse section, apical cells at the same distance from the center are now only equivalent if diametrically opposite each other. The embryo can therefore be said to show twofold rotational symmetry about its A-B axis. Viewed from the side, the heart-shaped embryo has only one plane of reflectional symmetry (along its A-B axis) and therefore is often said to show bilaterally symmetry—a term usually reserved for onefold rotational symmetry. For convenience, the term bilateral symmetry is used here.

Genes needed to impose the additional asymmetry might therefore be identified by mutants that block the globular to heart transition. However, this must be treated with caution. First, formation of cotyledons is an apical character and can be affected by mutations in A-B pattern genes [e.g. \textit{FK} (59)]. Second, a block in globular-heart transition is among the most common class of embryonic mutant phenotype in \textit{Arabidopsis}, which suggest that reduced activity of any one of over 200 genes can become limiting at this stage (19, 30). In at least two cases, the affected genes appear to be required for housekeeping functions (83, 100). Similarly, mutations that block bilateral symmetry of maize and rice embryos (which first becomes apparent in flattening of the scutellum) are equally frequent (40, 88).
Identification of genes that specify bilateral symmetry before cotyledon formation will therefore require detailed genetic and phenotypic characterization of a large number of mutants.

**Auxin and Bilateral Symmetry**  A class of more informative *Arabidopsis* mutations allows formation of cotyledons with full rotational symmetry. The result is an embryo with a single, cup-shaped cotyledon that resembles a golf tee. Such cotyledons are produced occasionally by *gn* mutants with apical defects (82), which suggests that asymmetry might be dependent on correct specification of apical identity. However, mutations in two additional genes, *PIN-FORMED1 (PIN1)* and *PIDOID (PID)*, give similar phenotypes and implicate polar auxin transport in imposition of bilateral symmetry (6, 44, 66). *PIN1* encodes a component of the auxin efflux carrier that functions in directional transport of auxin (32, 67). *PID* has a similar mutant phenotype to *PIN1*, is required for normal activity of polar auxin transport in mature tissues, and acts redundantly with other genes involved in auxin responses, which suggests that it has a similar role (6). Involvement of auxin transport is further supported by the finding that globular embryos of black mustard, *Brassica juncea*, form cup-shaped cotyledons when cultured in the presence of auxin transport inhibitors (34, 44). The question remains as to how movement of auxin is involved in specifying cell fate at the apex. One proposal is that auxin is removed from cells adjacent to cotyledon initials and low concentrations are necessary for non-cotyledon fates (34). The view is supported by the finding that embryos of *B. juncea* also produce cup-shaped cotyledons when cultured in the presence of high concentrations of exogenous auxin (34). However, involvement of auxin transport in another direction, e.g. along the A-B axis, cannot be ruled out.

**Genes Elaborating Bilateral Symmetry**  Formation of cup-shaped cotyledons may result from failure to specify non-cotyledon fate in cells which would normally lie in the sinus between cotyledons (termed the *embryonic peripheral region*, EPR, by Long & Barton (45)). Therefore cotyledon identity might be a default state and its inhibition in the EPR necessary for bilateral symmetry. This hypothesis is supported by the expression pattern of the transcription factor gene *AINTEGUMENTA (ANT)*. In postembryonic growth, *ANT* expression is confined to lateral organ initials and primordia (27) and acts redundantly in formation of floral organs (27, 41). It is expressed in developing cotyledons, and might therefore have a similar redundant role in their formation. However, at an earlier stage of embryo development (late globular), *ANT* is expressed in a radially symmetric ring of cells around the apex, which includes the EPR and only becomes confined to cotyledon initials before the transition to the heart stage (45). This shift from radial to bilateral expression of *ANT* occurs after two other genes have begun to show bilateral expression. The *CUP-SHAPED COTYLEDON1 (CUC1)* gene, encoding a NAC protein of unknown biochemical function, is expressed in a stripe of cells running across the apex of the late globular embryo from which the EPR and SAM will form (2). Embryos lacking activity of *CUC1* and a second gene, *CUC2*, often
produce cup-shaped cotyledons and lack SAMs, which suggests that CUC activity is required to repress cotyledon fate (1, 2). The homeobox gene SHOOT MERISTEMLESS, STM, is expressed in a similar domain to CUC1 in the late globular embryo (45). Strong stm mutations prevent formation of a functional SAM, but also allow production of cotyledons that are united at their bases (5). Therefore, STM also acts to inhibit cotyledon fate in the EPR, although it does not appear to do this by repressing ANT expression (45). CUC activity is required for STM expression in the late globular embryo, which suggests that bilateral CUC expression is responsible for the bilateral expression of STM. However, STM expression can be detected in embryos at earlier developmental stages than those reported to express CUC1, which suggests that interactions between these two genes might be more complex, or that earlier CUC1 expression has yet to be detected.

Although STM is not necessary for bilateral symmetry, its early pattern of expression reveals how this symmetry might arise. It is first detected in one or two cells on the flank of the midglobular embryo and subsequently becomes established in a second domain diametrically opposite. Intermediate cells then express STM to form the stripe seen at the late globular stage (45). If the midglobular embryo possesses only A-B and radial symmetry, cells at the same position along the A-B axis and at the same horizontal distance from it should be equivalent and express the same complement of genes (as observed for the ring of ANT expression). The finding that a group of asymmetrically placed cells express STM suggests that transcription is not activated in response to existing polarity, but is determined at random. Because it is unlikely that a random event would also establish the second domain of STM expression exactly opposite the first, the first domain is likely to determine the position of the second by interaction (e.g. by inhibiting EPR identity locally and therefore promoting cotyledon fate).

Unlike the A-B and inside-out axes of the embryo, orientation of the axis of bilateral symmetry appears to have no functional consequences for the plant, and might therefore be left to chance. Alternatively, it may be specified by maternal tissues. The ovule itself is bilaterally symmetrical. It imposes additional asymmetry on the late-stage embryo, which has to fold its cotyledons back along its A-B axis as it grows to fill the ovule. The axis of bilateral symmetry in the mature embryo coincides with that of the ovule and, in addition, one cotyledon becomes longer than the other (although both contain the same number of cells). Because heart-stage embryos appear randomly oriented, the ovule would appear to position the late-stage embryo by forcing it to rotate about its A-B axis to fit the seed coat, rather than by specifying its axis of bilateral symmetry earlier in development.

Although reduction in rotational symmetry has been discussed in the context of the apical region, it also becomes apparent in the central parts of the dicot embryo. In Arabidopsis, vascular tissue of the root and hypocotyl shows a bilateral arrangement, with two opposite xylem poles extending radially from the stele (31). The positions of these poles correspond to those of the cotyledons, but it is not yet known whether cotyledons might induce xylem formation, xylem induce cotyledons, or whether both respond independently to bilateral symmetry. A
reduction in the degree of rotational symmetry is also apparent in the epidermal cells of the mature embryo in the alternate arrangement of two cell types around the circumference [e.g. hair-cell and non-hair-cell progenitors in the embryonic root, protruding and nonprotruding cells in the hypocotyl (9, 25)] Signaling from underlying cells or the radial walls separating them is implicated in specifying the fates of different cells, and interaction between epidermal cells in reinforcing them (8).

ELABORATION OF ASYMMETRY AFTER EMBRYOGENESIS

Phyllotaxy and Translational Symmetry in the Shoot

By maturation, most dicots show A-B asymmetry, with root meristem and SAM separated by hypocotyl and embryonic root tissues. After germination, the SAM gives rise to shoot tissue in the A-B axis and lateral organs (e.g. leaves), which elaborate their own axes of asymmetry (see below). The relative positioning of organ and stem components (termed phyllotaxy) is usually regular, and can therefore be described in terms of symmetry. For example, leaves at successive positions (nodes) along the A-B stem axis may occur immediately above each other and therefore one node can be made to resemble the node above or below by a shift along the A-B axis (referred to mathematically as a translation). Alternatively, a node may need to be both translated and rotated to resemble an adjacent node, in which case the stem shows spiral symmetry. However, the term spiral phyllotaxy is traditionally reserved for one specific example of spiral symmetry in which plants form a single organ at each node and successive organs are offset by less than 180° [often approximately 137° (73)].

Phyllotaxy results from specification of organ and stem fate in cells of the SAM. Although the mechanisms involved remain poorly characterized, most available evidence suggests that organ initials specify the position of future organs by a process involving local inhibition of organ fate. For example, surgical ablation of one group of leaf initials in the fern, Dryopteris, allowed subsequent leaves to be initiated closer to the site of ablation (105). Similarly, premature leaf initiation from the tomato SAM, induced by localized application of expansin protein, could disrupt the positions at which subsequent leaves were formed (28). However, the positions of future leaves in Arabidopsis are marked by expression of the PINHEAD/ZWILLE gene in vascular initials below future organ initials. This occurs before changes in expression of other genes that mark organ fate become apparent (e.g. down-regulation of STM in the SAM), which suggests the possible involvement of inductive signals originating basally (53).

Spiral phyllotaxy involves an increase in asymmetry. Shoots with spiral phyllotaxy usually develop from a seedling with an opposite pair of cotyledons (i.e. two-fold rotational symmetry). Maintenance of the phyllotaxy shown in cotyledons
would result in a shoot with an opposite pair of leaves at each node, and, because leaves inhibit leaf formation locally, with alternate nodes rotated by 90°. This form of decussate phyllotaxy is seen in many species, including *Antirrhinum*. However, in most plants that show spiral phyllotaxy, symmetry is reduced at nodes with a single leaf (they now have only one plane of reflectional symmetry) and also by the inherent chirality of a spiral (it is either left- or right-handed). Species with spiral phyllotaxy tend to produce equal proportions of left- and right-handed forms, and no genetic basis for the handedness of the spiral has been detected, which suggests that it is specified at random (3). In *Arabidopsis*, the first two leaves are initiated together, opposite each other and at 90° to the cotyledons. A single-leaf primordium is then formed nearer to one or other of the initial leaves, to either its left or right side. If it is closer to one side, the resulting spiral phyllotaxy is left-handed; if it is closer to the other, the spiral is right-handed. Therefore, the handedness of the spiral seems likely to represent random positioning of the third-leaf primordium.

Many other randomly determined inequalities may operate to reduce symmetry in plant development. For example, ovules in one chamber of an *Arabidopsis* carpel develop later than in the other, and the pattern of vascular tissue or lobes on one side of a leaf is not an exact reflection of the other side. Inequality between cotyledons has been suggested to result in the unorthodox, asymmetric morphology of *Monophylea horsfieldii*. This species, like other members of the Gesneriaceae, produces embryos with a pair of equal-sized cotyledons but no SAM. One of the cotyledons grows to produce a large photosynthetic organ that then forms reproductive inflorescences from its midrib. The other cotyledon appears to be capable of growth but is repressed by its partner, because all embryos in which one cotyledon has been removed at random can produce a normal plant (99).

**Formation of New Axes of Asymmetry in Organs**

In contrast to the stem, which maintains the original A-B and radial axes established early in embryogenesis, formation of lateral organs involves initiation and elaboration of new axes of asymmetry. Organ initiation first becomes apparent as a group of cells grow out from the shoot apical meristem to form the proximal-distal (P-D) organ axis. Further asymmetry along the P-D axis may become apparent later, for example, in monocot leaves between proximal sheath and distal blade tissue, or in simple dicot leaves between the proximal petiole and distal blade. A mature organ usually also shows asymmetry along its dorsal-ventral (D-V, or adaxial-abaxial) axis. The dorsal epidermal cells of leaves often differ from ventral, and asymmetry is often seen in the arrangement of internal cell layers. Leaves and other lateral organs also appear to have a medial-lateral axis (M-L, from midline to edge). Leaf primordia are either flattened along this axis at emergence from the SAM or become flattened by growth of the primordium soon after initiation. Further M-L asymmetry is reflected in the position of the midrib medially and blade tissue laterally.
**Proximal-Distal Asymmetry**  Because initiation of an organ primordium from the SAM involves growth along the P-D axis, this axis is the first to become apparent (52). Mutations that prevent specification of the P-D axis are therefore likely to block organ initiation, which makes them difficult to distinguish from mutations in genes needed to specify organ fate. The mechanisms of P-D asymmetry are, as a consequence, poorly understood.

Inflorescence meristems of *Arabidopsis* plants mutant for either the *PIN1* or *PID* genes produce stem-like tissue without lateral organs. However, the inflorescence meristems of both *pid* and *pin1* mutants produce lateral bulges that resemble organ primordia but fail to elaborate a P-D axis (6, 66), which suggests that the two genes are needed for P-D outgrowth. *PIN1* and *PID* are necessary for normal polar auxin transport, which suggests that auxin is involved in P-D axis formation. Homeobox genes of the *knotted1*-like family (*knox* genes) are also implicated in formation or elaboration of the P-D axis. Expression of these genes is normally confined to the SAM and excluded from organ initials from before primordium initiation (reviewed in 90). Ectopic *knox* gene expression in organs causes them to develop proximal identities (e.g. sheath) at a more distal position. Although these results indicate that *knox* gene activity can influence the P-D axis of leaves, their implications for the role of *knox* genes in wild-type organs are not clear (discussed in 31, 39). In the *Arabidopsis* carpel, the *ETTIN (ET)* gene is required to prevent proximal development of distal stylar tissue (87). At least part of *ET* function appears to be repression of TSL2, a nuclear protein kinase needed for formation of the distal carpel (75, 86). *ET* encodes a transcription factor similar to the *MP* gene product and is therefore likely to act in auxin-mediated responses. It is transcribed uniformly along the P-D axis of carpel initials and primordia, but only in a ventral domain. Again, the significance of these findings for P-D axis elaboration is not yet clear (discussed in 65).

**Dorsal-Ventral Asymmetry**  In contrast, mutations in a number of different genes disrupt D-V asymmetry of lateral organs. For example, loss of *PHANTASTICA (PHAN)* activity of *Antirrhinum* allows production of leaves and petal lobes consisting only of ventral cell types (104), which suggests that the MYB transcription factor encoded by *PHAN* is needed for specification of dorsal identity and that ventral identity occurs by default (Figure 2) (103, 104). The semidominant *phabulosa-1d (phb-1d)* mutation in *Arabidopsis* has the opposite effect of dorsalizing all lateral organs in a dose-dependent manner, although semidominance does not clarify whether the mutation involves loss of a ventralizing activity, or a gain-of-function of a dorsalising gene (Figure 2) (60).

Although *PHAN* is required for dorsal cell identity, it is expressed in both dorsal and ventral organ initials; this suggests that other, spatially restricted factors are also needed for asymmetry. A number of candidates have been identified in *Arabidopsis*. Loss-of-function mutations in the *ARGONAUTE (AGO)* gene, which encodes a potential translation initiation factor, cause partial ventralization of organs, whereas constitutive *AGO* overexpression partially dorsalizes them (11, 53).
Although this suggests that AGO specifies dorsal identity, it is expressed ubiquitously in organ initials and primordia of wild-type plants. A structurally similar gene, PINHEAD/ZWILLE (PNH/ZLL), is expressed in an apical region of the SAM including organ initials but confined to a dorsal domain of organs at the time of primordium initiation (53, 63). Whereas pnh mutations allow formation of organs with normal D-V asymmetry, ago; pnh double mutants (or ago mutants carrying a single wild-type copy of PNH) show more severely ventralized leaves, which suggests that PNH/ZLL and AGO act redundantly to specify dorsal fate (53).

Conversely, several members of the YABBY transcription factor gene family, FILAMENTOUS FLOWER (FIL), YABBY2 (YAB2), and YAB3, are involved in specifying ventral identity in Arabidopsis organs (77, 89). Expression of FIL, YAB2, and YAB3 is uniform in organ initials within the SAM but becomes limited to ventral regions of primordia (including cotyledons) at initiation. FIL acts redundantly with YAB3 to specify ventral fate. Constitutive expression of either YAB3 or FIL appears sufficient to confer ventral characters on the dorsal regions of leaves (89).

Dorsal expression of ZLL/PNH and ventral expression of YAB2, YAB3, and FIL presumably occurs in response to D-V asymmetry present within the SAM (where patterned expression in organ initials is first seen). The three YABBY genes are expressed independently of each other, but regulated by PHB (89). Isolation of PHB should therefore provide a clue to the mechanisms acting upstream of ventral fate specification. However, because the D-V axis of the organ corresponds to the A-B axis of the stem, it seems likely that the D-V asymmetric pre-pattern is established in organs in response to the A-B axis of the SAM. This view is supported by surgical experiments in which incisions were made above leaf initials in potato and sesame, isolating them from contact with more apical SAM cells. This resulted in formation of ventralized organs (36, 92), and suggests that signals originating apically are necessary for dorsal fate. The origin of the P-D organ axis is less clear. At primordium initiation it corresponds to the inside-outside axis of the meristem and may therefore be derived from this. However, the inside-outside axis of organs appears to have another role, discussed below.

Interaction Between Axes The two major axes of organ growth (P-D and M-L) lie at right angles to each other and to the D-V axis, which implies that they are not
specified independently (otherwise at least one of them should be random). They are also oriented with respect to the remainder of the plant, suggesting that at least two of them are derived from asymmetry present in the SAM. This connection is a functional necessity—shoots, for example, tend to grow toward light and therefore leaves, which are flattened in two axes perpendicular to the stem, expose their largest photosynthetic area to incident light.

*Antirrhinum phan* and *Arabidopsis phb-1d* mutant phenotypes suggest a connection between M-L growth and D-V asymmetry. Extreme *phan* or *phb-1d* mutant leaves consist of only ventral or dorsal cell types, respectively, and have no lateral blade; this suggests that both dorsal and ventral identities are required for M-L growth (60, 104). The mutants can also produce leaves that are mosaics of normal tissue and either ventralized tissue (in *phan*) or dorsalized tissue (in *phb-1d*). In both cases, juxtaposition of dorsal and ventral identities results in an ectopic leaf blade. Therefore, interaction between dorsal and ventral cells might direct M-L growth of the wild-type blade. The extreme *phan* or *phb-1d* mutant leaves also suggest that the M-L axis, although recognizable morphologically, might not be specified directly. In the absence of D-V asymmetry, mutant leaves are needle-like and show full rotational symmetry in the arrangement of tissue layers; this indicates that inside-out and P-D axes of asymmetry within the organ can be specified independently of the D-V axis. The fate of a cell along the M-L axis of a wild-type leaf might therefore be specified by its coordinates along the inside-outside and D-V axes (a polar system), rather than along D-V and M-L axes (a Cartesian system). Organs are formed from all cell layers of the meristem. Therefore a mechanism of inside-outside asymmetry that involved cell layering could be inherited by organs from the SAM.

Dicot leaves tend to have few markers for M-L asymmetry (often only the distinctions between midrib, blade, and leaf edge). In contrast, the presence of a distinct marginal domain in the maize leaf is suggested by the *narrow sheath* (*ns*) mutant phenotype. In *ns* mutants, SAM cells that would normally become initials of the leaf margins fail to participate in primordium initiation, which results in narrower leaves lacking marginal characters (78). Maize leaves differ from those of dicots in showing marked flattening in the M-L axis at primordium emergence, whereas in dicots flattening usually occurs later. However, the *maize leafbladeless1* (*lb1*) mutation, which conditions a phenotype similar to *phan* in *Antirrhinum*, suggests that M-L flattening results from D-V interactions (95). Severely affected *lb1* mutant leaves lack dorsal cell types and are radially symmetrical, whereas other leaves produce ectopic M-L axes where dorsal and ectopic ventral identities are juxtaposed.

### Asymmetry of the Flower

**Apical-Basal (Radial) Asymmetry**  The flowers of most species, including *Antirrhinum* and *Arabidopsis*, show asymmetry in the identity of organs produced in concentric whorls. The axis of asymmetry corresponds to the A-B axis of the flower. Because the axis is usually highly compressed, it is more usually termed radial. The function and regulation of floral homeotic genes that specify the distinctions
between organs in different whorls have been characterized extensively and are the subject of many recent reviews (e.g., 61). However, the mechanisms that specify A-B/radial asymmetry in the floral meristem, leading to activation of floral homeotic genes in specific domains, are less well understood [see, for example, (68)].

**Dorsal-Ventral Asymmetry**

Flowers of many species, including *Antirrhinum*, show an additional, dorsal-ventral, axis of asymmetry coincident with the A-B axis of the inflorescence stem. Such flowers are termed zygomorphic or bilaterally symmetrical [the ecological significance and evolution of this character are discussed in (64)]. The *Antirrhinum* corolla consists of five distal petal lobes (two dorsal, two lateral, and one ventral) that differ in identity along the D-V axis and, with the exception of the ventral petal, also show internal D-V asymmetry. D-V asymmetry is also seen in the adjacent whorl, in which development of the dorsal stamen arrests and the remaining pair of lateral stamens develop differently from the ventral pair. Three genes, *CYCLOIDEA (CYC)*, *DICHOTOMA (DICH)*, and *RADIALIS (RAD)*, are required for dorsal identity (18, 50). In *cyc* mutants, lateral petals adopt ventral identities and dorsal petals become partly lateralized. The remaining dorsal/lateral characters in *cyc* mutants can be attributed to *DICH* activity, because all petals are ventralized in *cyc; dich* double mutants. In wild-type flowers, *CYC* expression occurs in dorsal petal initials and in the dorsal-most part of lateral petals. However, *CYC* is required for normal development of lateral petals as a whole, which suggests that it acts non-cell-autonomously in their more ventral regions (18). *RAD* is likely to be involved in this process, because homozygous *rad* or *cyc* mutations condition similar phenotypes, and *rad/+; cyc/+* trans-heterozygotes show slight ventralization of lateral petals, which suggests that the two genes act sequentially in the same developmental pathway.

In contrast, loss of *DIVARICA (DIV)* activity allows the ventral petal to assume lateral identity, which suggests that *DIV* acts to specify ventral fate (4). *DIV* activity is limited to the ventral petal by the action of the dorsalizing genes *CYC* and *DICH*, with *CYC* alone repressing it in lateral petals. Further evidence for antagonism of *DIV* and *CYC* is provided by the observation that three wild-type doses of *DIV* can partially overcome the dorsalizing effect of *CYC* in lateral petals. However, the range over which *CYC* acts non-cell-autonomously is increased by *DIV* activity; more complex interactions may thus be possible. Because the ventralizing effects of *DIV* are dose-dependent, one model for D-V axis specification is that a decreasing gradient of *DIV* activity is formed from the ventral to the dorsal part of the flower, as a result of *CYC* and *DICH* repressing *DIV* dorsally. Different levels of *DIV* activity along the D-V axis could then specify regional identities (4).

One effect of *CYC* expression is to retard growth of the dorsal part of the floral meristem. A related gene, *teosinte branched*, has a similar effect on growth of axillary meristems in maize (24), which suggests a common role for members of this gene family in growth control (22). To what extent growth-repression is necessary for the dorsalizing effects of *CYC* in the *Antirrhinum* flower is not yet known.
CYC expression is established in a dorsal domain of the early floral meristem, which suggests that it responds to A-B asymmetry of the inflorescence axis. Two observations support this view. First, centroradialis mutants of Antirrhinum produce ectopic flowers from the inflorescence apex (12). The flowers lack D-V asymmetry, which indicates that A-B asymmetry in the flanks of the inflorescence meristem may be needed. Second, ablation of parts of the inflorescence meristem dorsal to developing floral meristems or localized application of auxin can result in the formation of flowers with radial asymmetry (7).

PERSPECTIVES

Most of the genes known to influence asymmetry in plants appear either to act indirectly in the process (e.g. in cell division) or, because they have a restricted domain of expression or action, to be required for axis elaboration. The mechanisms of axis specification, which establish the fundamental asymmetry, remain poorly understood. A distinction between axis specification and elaboration may be partly artificial because new axes can be elaborated from existing ones in postembryonic development. However, the question of what regulates asymmetry of gene expression or action remains. Identification of upstream regulators of asymmetrically expressed genes like the YABBIES, may provide part of the answer. Axis specification in animals can result from a chemical gradient, which specifies position along the axis in a concentration-dependent manner. The finding that auxin transport and response mutations affect plant asymmetry has provided a hint that auxin might be involved in such a process, and its role should quickly become clearer. Further insights should also come from molecular analysis of PHB and DIV genes, which act dose-dependently and might therefore form gradients of activity which specify asymmetry along the D-V axis of lateral organs and flowers.

ACKNOWLEDGMENTS

I thank John Bowman for making results available before publication, and John Golz for his comments on the manuscript.

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