

Signalling in Plant Lateral Organ Development

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INTRODUCTION

A plant lateral organ, defined here as either a leaf or a leaf-like organ of the shoot or flower, arises from a group of initial cells within the flanks of the shoot apical meristem (SAM) or floral meristem. For example, the tobacco leaf is formed from a group of ~100 initial cells in all three histogenic layers of the SAM (Poethig and Sussex, 1985). Lateral organ initial cells follow fates that are very different from the more central SAM or neighboring cells that will form tissues of the shoot or floral axis. They change their patterns of expansion and division and elaborate new axes of growth—away from the SAM and laterally. As a lateral organ matures, cell division ceases and cells differentiate. The mature leaf typically consists of several million cells and ~20 different cell types. However, it shows a characteristic shape, size and pattern of tissues. For example, the *Antirrhinum* leaf, which is typical of many species, shows a patterned arrangement of tissues that is particularly apparent along the dorsiventral (adaxial–abaxial) organ axis and in the distribution of veins. Patterning is also seen in the nonrandom distribution of epidermal cell types, including trichomes and stomatal guard cells (Figure 1).

Two contrasting mechanisms are commonly invoked to explain such patterned growth and cell identity: either that it is an intrinsic function of initial cells, in which case daughter cells must inherit information about their identities from their parents; or that cells sense their position and respond to it, which implies that fate specification involves cell–cell signaling.

The fate of a cell in a developing lateral organ involves a characteristic pattern of growth and division and finally, differentiation of its progeny into specific cell types. If inherited cell identity has a role in this process, we would expect cells that share a common ancestry to follow similar fates. Such a relationship between cell lineage and fate is inherent in some aspects of lateral organ development. For example, the majority of cell divisions in a developing leaf lamina involve the formation of new anticlinal cell walls (i.e., parallel

to the dorsiventral axis). Therefore, daughter cells are likely to remain within the same cell layer and to differentiate as cells of the same type (e.g., as palisade mesophyll; Figure 1). Restricted orientation of division could therefore provide the basis for heritable cell identity within a layer. It also raises the possibility that cell identity might itself affect cell division patterns, maintaining the clonal distinction of layers and effectively creating developmental compartments within which cells inherit identity as they proliferate. Similar lineage restrictions may create different developmental compartments earlier in lateral organ development. They occur, for example, between the initials of *Antirrhinum* floral organs at about the same time that each begins to express the unique combination of homeotic genes that specifies its identity (Vincent et al., 1995). Therefore, cells that have been specified as initials of one floral organ type (e.g., petal) are likely to remain within the developing primordium of that organ.

Lineage, however, is clearly incapable of explaining all aspects of lateral organ development. Early experiments with periclinal chimeras, in which the clonally distinct layers of the SAM were marked genetically, allowed the origins of cells in the mature leaf to be identified (Stewart and Derman, 1975). This revealed that a single tissue layer within the leaf is derived from multiple cells of the early leaf primordium. In the case of the leaf mesophyll layers, these initials usually involve two distinct cell lineages (the L2 and L3 layers of the SAM; Figure 2), suggesting that cell–cell interactions are involved in specifying their fate as mesophyll initials. Similarly, rare periclinal division within the L1 layer of a leaf consisting of a wild-type outer layer (L1) overlying an L2 carrying a chlorophyll mutation (Figure 2) displaces a wild-type daughter cell into the underlying mutant L2. The displaced cell shows the growth and division appropriate to its new internal position, ultimately giving rise to a clone of green cells with mesophyll identity rather than the epidermal identity expected from its ancestry (e.g., Stewart and Derman, 1975; Figure 2B). This implies that interactions between cells can override lineage in controlling cell fate.

While these and many other observations reveal the importance of cell–cell signals in re-specifying the fate of displaced cells, they do not preclude the involvement of inherited cell identity in cells that are not displaced. For

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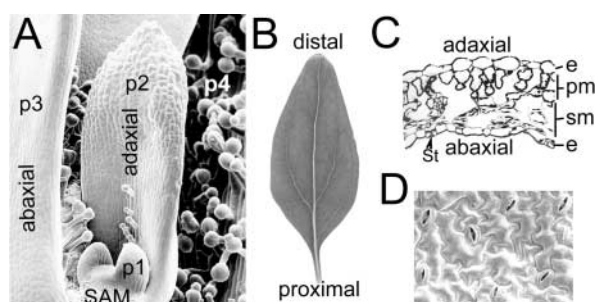


Figure 1. Growth and Patterning of a Dicot Leaf.

(A) Leaves are initiated in opposite pairs from the periphery of the *Antirrhinum* shoot apical meristem (SAM). Progressively older primordia are denoted (P1–P4). The leaves begin to flatten by lateral growth soon after primordium initiation. Trichomes (leaf hairs) are formed from the adaxial leaf surface in a nonrandom arrangement.

(B) The mature *Antirrhinum* leaf has undergone considerable growth along its proximal–distal axis and shows a characteristic pattern of veins.

(C) A transverse section through its blade shows an asymmetric arrangement of cell types along the adaxial–abaxial axis. e, epidermis; pm, palisade mesophyll; sm, spongy mesophyll; st, stoma.

(D) In the abaxial leaf epidermis, stomata show a nonrandom arrangement and are separated by at least one jigsaw piece–shaped pavement cell.

example, lineage might have a significant role in maintaining cell identity but can be over-ridden to prevent occasionally displaced cells retaining inappropriate identities. In contrast, a purely signaling-based mechanism would involve each daughter cell defaulting to a developmental ground state and having to reinterpret its identity *de novo* after each normal cell division. Re-specification of displaced cells also has a major experimental consequence in largely preventing the use of cell displacement to test effects of lineage-dependent fate. More telling, though experimentally more difficult tests would involve following the fates of cells isolated from sources of signals or defective in signal sensing.

One exception to the general observation that displaced cells assume fates appropriate to their new positions has been provided by the *tangled1* (*tan1*) mutant of maize. *tan1* mutants are affected in the polarity and timing of cell divisions (Smith et al., 1996). Aberrant divisions in the single layer of bundle-sheath (bs) cells surrounding each leaf vein can displace cells into the surrounding mesophyll cells, and the displaced cells retain bs identity rather than assuming mesophyll fate (Jankovsky et al., 2001). These divisions occur after expression of C4 photosynthetic enzymes characteristic of bs cells and after normal bs cells have ceased division. It is therefore possible that bs cells are displaced after the re-specification mechanism is able to operate. Normal bs cells are more closely related to each other and to other vein cells than they are to mesophyll (Langdale et al., 1989), raising the possibility that lineage has a role in their specification.

A further and more specialized case of lineage-determined fate involves daughter cells that assume two different fates in response to the intrinsic asymmetry of their parental cell or of the process of DNA replication or mitosis that gives rise to them. Asymmetric cell fate is found in several aspects of lateral organ development, including the divisions that give rise to stomatal initials and less-specialized epidermal cells, discussed below.

A number of mechanisms might account for inheritance of the same or different cell identities. DNA methylation status or chromatin modification have been proposed to represent the inherited factor in transmission of stable states of gene expression or of asymmetric daughter cell fates in fission yeast (e.g., Dalgaard and Klar, 2001). Preliminary analyses of genes required for these processes in plants, including the Chromatin Assembly Factor–like proteins encoded by *Arabidopsis* *FASCIATA* genes (Kaya et al., 2001), hint at roles in maintaining stable states of regulatory gene expression (reviewed by Finnegan and Kovac, 2000; Habu et al., 2001). An alternate explanation for lineage-dependent identity is that it involves inheritance of intracellular molecules or extracellular factors associated with the cell wall. Such

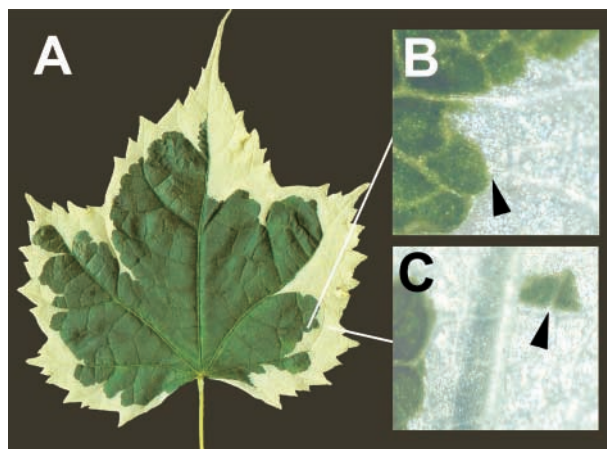


Figure 2. Cell Lineage in a Periclinal Chimera.

This shoot apical meristem of this *Abutilon* × hybridum cultivar has a mutant sub-epidermal layer (L2) that is unable to produce chlorophyll.

In the mature leaf **(A)**, the contribution of cells derived from the L2 (yellow) and from the L3 is variable. Compare, for example, the outline of the yellow-green boundary in the two halves of the leaf. Although many boundaries between L2- and L3-derived cells correspond to veins, others pass through groups of cells that have assumed the same mesophyll fate, regardless of their ancestry (arrowhead in **[B]**). The wild-type L1 layer mostly gives rise to the epidermis, which does not synthesize chlorophyll, except in guard cells. Rare periclinal division, however, can displace an L1 cell internally, where it gives rise to mesophyll cells appropriate to this internal position (arrowhead in **[C]**).

inheritance of cell wall components is implicated in the maintenance of fate during early development of the brown alga *Fucus* (Berger et al., 1994), but has yet to be demonstrated for higher plants. Inheritance of intracellular molecules is implicated in asymmetric cell fate in the maize leaf epidermis. Here, a subsidiary cell adjacent to a stomatal guard cell and a larger epidermal cell are formed by asymmetric division of a precursor cell. The location of an internal patch of actin in the precursor cell correlates with subsidiary cell fate, even in mutants with defects in orientation of cell divisions. Similarly, mutations that disrupt formation of the patch also disrupt asymmetric division and fate (Gallagher and Smith, 2000).

While ability of position to override effects of cell lineage largely obscures any role of inherited cell identity, there is overwhelming evidence for cell–cell interactions in re-specifying displaced cell fate. Cell–cell interactions also appear likely to operate routinely in other aspects of lateral organ development, including organ initial specification within the SAM. The development of plant lateral organs, particularly leaves, is also highly plastic and responds to environmental cues. For example, the frequency of stomata is influenced by the availability of water, light, and CO₂ (e.g., Woodward and Kelly, 1995), and therefore environmental signals must be integrated with intrinsic developmental processes.

Cell–cell interactions in animal development can be broadly classified according to the distances over which the signals act. Morphogens, which are involved in specifying major axes of asymmetry, such as the body and appendage axes of *Drosophila*, lie at one extreme (Vincent and Briscoe, 2001). These are secreted proteins or transcription factors that form concentration gradients extending over many cells (or nuclei in the embryo). Cells respond according to the concentration of morphogen they experience, effectively converting a gradient into discontinuous domains of gene expression and cell identity. At the other extreme, signaling may involve interactions only between adjacent cells. In *Drosophila* neurogenesis, for example, the membrane-bound ligand Delta binds its receptor, Notch, at the surface of adjacent cells to prevent their assuming the same identity—a case of lateral inhibition (Artavanis-Tsakonas et al., 1995).

Here we compare three aspects of plant lateral organ development in which cell–cell interactions occur over different distances: (1) specification of lateral organ fate at the shoot apex, (2) asymmetry within lateral organs, and (3) stomatal cell fate in the leaf epidermis. We do not consider several equally important and well-studied aspects of lateral organ development that are also likely to involve signaling, notably leaf venation (which has been reviewed recently by Dengler and Kang, 2001) and control of trichome fate in the lateral organ epidermis, which in *Arabidopsis* appears partly homologous to specification of hair cells and non-hair cells in the root epidermis (reviewed by Schiefelbein, 2000). Despite the need to invoke cell–cell interactions in all these processes, the mechanisms of interaction remain poorly under-

stood, perhaps because redundancy, lethality, or sheer complexity has hindered identification of the relevant genes by mutation. An alternate explanation is that plants employ less-conventional mechanisms than the ligand–receptor signaling prevalent in animals, which makes them harder to identify genetically. We consider one potential alternative, physical force, in the context of lateral organ fate specification.

Specification of Lateral Organ Fate at the SAM

Lateral organ initiation occurs at regular positions in the periphery of the meristem in a pattern termed phyllotaxy. When each node has one organ, and those at adjacent nodes are opposite, as in maize, the phyllotaxy is termed distichous or alternate. Spiral phyllotaxy also involves a single organ at each node, but with organs at adjacent nodes typically offset by $\sim 137^\circ$. Organs therefore occur in one or more spirals along the shoot axis, as in *Arabidopsis* vegetative and inflorescence shoots, and the spiral can be either left- or right-handed. Two opposite organs at each node that are offset by 90° from those at adjacent nodes is usually termed decussate, and the term whorled is often used for more than two organs at each node, as in the flowers of many species.

One way to address the question of how lateral organ and non-lateral organ fates are specified at the shoot apex is through analysis of genes involved early in lateral organ development. Lateral organ initials within the *Arabidopsis* SAM are marked by expression of a number of genes that control early stages of lateral organ development including *AIN-TEGUMENTA* (*ANT*; Elliott et al., 1996; Klucher et al., 1996), *LEAFY* (*LFY*; Blazquez et al., 1997) and *ASYMMETRIC LEAVES1* (*AS1*; Byrne et al., 2000), and members of the *YABBY* family (Sawa et al., 1999; Siegfried et al., 1999). They are also characterized by lack of expression of the *knotted1*-like homeobox (*knox*) gene, *SHOOT MERISTEMLESS* (*STM*), which is found throughout the remainder of the SAM (Long et al., 1996). Patterned expression of these genes therefore predicts lateral organ fate from at least one plastochron before morphological signs of primordium initiation. (*Plastochron* is used here to denote the time between initiation of successive primordia.) In the case of the *Arabidopsis* *PIN-HEAD/ZWILLE* gene, expression in basal vascular initials is proposed to reflect the positions at which the next five primordia will initiate (Lynn et al., 1999), suggesting that leaf primordium identity may respond to a genetic prepattern acting several plastochrons before primordium formation, when leaf initials become clonally distinct (Poethig and Sussex, 1985).

Although analysis of genes acting early in lateral organs has suggested when leaf fate might first be specified, it has revealed little of the underlying mechanisms of specification. *STM*, for example, is needed to repress *AS1* expression in the SAM and, although *AS1* is not required for lateral organ

formation, its ectopic expression leads to SAM cells of *stm* mutants assuming lateral organ-like fates (Byrne et al., 2000). Repression of *AS1* by *STM* can therefore be considered part of the mechanism responsible for elaborating lateral organ and non-lateral organ fates in shoots, but does not identify the underlying prepattern to which *STM* expression responds.

An additional category of genes overlaps in expression with *STM* in cells that will form non-lateral organ cells of the shoot axis. These include the Arabidopsis *CUP-SHAPED COTYLEDON1* and *2* (*CUC1* and *2*) that encode members of the NAC family of potential transcriptional regulators (Aida et al., 1997; Takada et al., 2001). *CUC1* and *CUC2* are proposed to limit growth of non-lateral organ regions because *cuc1 cuc2* double mutants can form united organs. Similarly, loss of activity of a related Petunia gene, *NO APICAL MERISTEM*, leads to initiation of additional, ectopic organs in flowers (Souer et al., 1996). Limited epistasis experiments suggest that *CUC* activity promotes *STM* expression (Aida et al., 1999; Takada et al., 2001). Because *stm* mutants have partially united cotyledons similar to those of *cuc1 cuc2* mutants, *CUC* genes might act partly via *STM* to repress lateral organ fate and growth. Analysis of *CUC* gene function has therefore revealed a potentially earlier step in elaboration of lateral organ and non-lateral organ fates, although it has not revealed the prepattern regulating *CUC* gene expression.

The nature of the prepattern that determines lateral organ and non-lateral organ fates has long fascinated biologists and mathematicians. Early observations of phyllotactic patterns recognized that primordia tend to initiate in the greatest space available in the periphery of the SAM at positions most distant from existing primordia and the SAM apex. Further, spontaneous or induced changes in the position of one primordium could be maintained in subsequent development, for example as a shift in the handedness of spiral phyllotaxy. Such observations suggested that existing primordia are able to determine the position at which lateral organs subsequently form. Surgical and pharmacological experiments conducted early in the last century further supported this view and led to the field theory, which proposed that the central zone (cz) of the meristem and pre-existing primordia produce a diffusible inhibitory signal (Schoute, 1913; Wardlaw, 1949). In this early example of a lateral inhibition model, new primordia arise in the greatest space because this corresponds to the position of least inhibition. An alteration in meristem size without a corresponding change in primordium size is therefore predicted to alter the pattern of leaf initiation, as is seen in a number of mutants that have enlarged meristems, including the Arabidopsis *clavata* and *fasciata* mutants (Leyser and Furner, 1992; Clarke et al., 1993) and the maize *abphyl1* (*abph1*) mutant (Greyson et al., 1978). In *abph1* plants, an enlarged meristem produces a decussate pattern of leaves, but subsequent reversion to normal meristem size results in a distichous phyllotaxy being re-established (Greyson et al., 1978; Jackson and Hake, 1999). Similarly, reducing the size of lateral organ primordia

allows more organs to form from a meristem of the same size, as seen in the whorled arrangement of reduced, needle-like leaves in Antirrhinum *phan* mutants (Waites and Hudson, 1995).

The inhibitory field model can be most conveniently explained in terms of a single hypothetical inhibitor, originating from existing lateral organs and the cz of the meristem. More recent evidence, however, suggests that lateral organ fate involves at least two specification steps, and therefore, involvement of a single inhibitor is likely to be an oversimplification. Historically, the cz was recognized as a histologically distinct region with lower cell division rates than the surrounding peripheral zone, from which lateral organs initiate (Lyndon, 1998). More recently, the cz has been equated with a population of naive stem cells that, in Arabidopsis, express the signaling ligand *CLAVATA3* (*CLV3*). While the equivalence of these different definitions of cz identity awaits testing, one obvious feature of the cz is that no lateral organs are formed from it. In Arabidopsis, the size of the cz is regulated by the *CLV* signaling pathway (Nakajima and Benfey, 2002). *CLV* signaling, involving perception of the *CLV3* ligand from the cz by the more widely expressed *CLV1-CLV2* receptor, limits expression of the stem cell-promoting transcription factor *WUSCHEL* (*WUS*) to more basal and internal cells of the SAM (Brand et al., 2000). *WUS*, in turn, acts to promote more apical *CLV3* expression via an additional, unknown signaling pathway (Schoof et al., 2000). These interactions are therefore sufficient to explain the control of the cz size. If the cz is considered to be the region of the SAM from which lateral organs are not produced, then these interactions can also account for the signaling proposed to inhibit organ formation at the apex of the SAM. How this signaling might regulate genes involved more directly in lateral organ fate is, however, currently unknown.

One candidate for a second signal involved in phyllotaxy is the phytohormone, auxin. It is synthesized at the shoot apex and actively transported between cells. When polar auxin transport (PAT) is inhibited chemically, phyllotactic patterns are altered or lateral organs and floral meristems fail to initiate, resulting in a pin-like inflorescence axis (Okada et al., 1991; Reinhardt et al., 2000). Several Arabidopsis mutants mimic these effects, including *pin-formed1* (*pin1*; Okada et al., 1991), *pinoid* (*pid*; Bennett et al., 1995), and *monopterous* (*mp*; Berleth and Jürgens, 1993; Przemeck et al., 1996), which are all implicated in auxin transport or responses to auxin signaling.

PIN1 encodes an auxin efflux carrier that is expressed in developing primordia and vascular tissue (Gälweiler et al., 1998; Vernoux et al., 2000). The failure of *pin1* mutants to form primordia can therefore be interpreted as a need to either remove auxin from lateral organ initials or to accumulate auxin, depending on whether the hormone inhibits or promotes primordium formation. Alternatively, differences in auxin concentrations between lateral organ and non-lateral organ initials, rather than absolute auxin levels, might affect organ formation. Several lines of evidence support the role

of auxin as a lateral organ promoter. First, the *pin1* mutant phenotype can be rescued by exogenous auxin (Reinhardt et al., 2000). Local application of auxin can also induce primordium formation at the point of application in wild-type tomato and in wild-type or *pin1* mutant *Arabidopsis*. The size of primordia was further found to be proportional to the amount of auxin applied. Locally applied auxin is, however, unable to induce primordia from the cz, suggesting that auxin signaling acts in lateral organ formation independently of the CLV–WUS interaction. Loss of PID protein kinase activity results in an inflorescence phenotype similar to *pin* (Bennett et al., 1995). *PID* has been interpreted as a negative regulator of auxin responses (e.g., its overexpression renders plants less sensitive to auxin and reduces expression of auxin-induced genes; Christensen et al., 2000). Because *PID* is expressed in lateral organ primordia, loss of primordia in *pid* mutants would be inconsistent with a role for auxin in promoting lateral organ formation. Other experiments, however, have suggested that *PID* promotes auxin responses, more consistent with *PID* expression in lateral organs mediating their formation in response to auxin (Benjamins et al., 2001).

Current evidence therefore favors a model in which existing primordia accumulate auxin and prevent neighboring cells from forming lateral organ primordia by depleting their auxin concentration, as originally proposed by Sachs (see Sachs, 1991). Higher auxin concentrations at more distant positions should therefore allow primordium formation (and perhaps promote increased auxin accumulation).

Recent investigations have also addressed the relationship between auxin signaling and the expression of genes involved early in lateral organ development. Although both *pin* and *pid* mutants fail to initiate lateral organ primordia and floral meristems in the inflorescence, they show patterned expression of *UNUSUAL FLORAL ORGANS*, *LFY*, and *ANT* that marks the differences between lateral organs or floral meristems and the apical inflorescence meristem (Christensen et al., 2000; Vernoux et al., 2000). These observations suggest that auxin signaling is required for lateral organ outgrowth but not for the earlier step of organ fate specification. Other studies have shown that lateral organ fate is necessary for the promotion of the activity of the apical meristem (e.g., Waites et al., 1998). This is consistent with *pin1* and *pid* mutants, which retain a functional SAM, presumably as a result of continued specification of lateral organ fate.

Analysis of the PAT mutant *pin1* has suggested a more fundamental role for auxin signaling in lateral organ fate specification. It showed that *LFY* promoter activity, characteristic of lateral organs, occurred in concentric rings around the periphery of the *pin1* meristem (Vernoux et al., 2000). This observation is consistent with a model for phyllotaxy involving two signaling processes. One, which does not involve PAT, defines evenly spaced rings of cells that are competent to assume lateral organ fate. And a second, auxin-dependent mechanism partitions each ring into lateral

organ and non-lateral organ initials. Such multistep models for phyllotaxy have been proposed previously (e.g., Loiseau, 1969) and are supported by the observation of unpartitioned primordia in an *Antirrhinum* mutant (Carpenter et al., 1995).

Although phyllotaxy can be explained by the action of signals (e.g., auxin, *CLV3*, and at least one additional molecule that promotes *CLV3* expression) an alternative, but not necessarily exclusive, mechanism has been proposed to involve physical forces. Green et al. (1996) described the meristem as a growing surface (the L1 and L2 layers, or tunica) that is constrained at its margins and supported by an elastic foundation (the L3, or corpus). If the surface has an inherent wavelength, it will tend to deform with this periodicity into a regular series of bumps and hollows, which Green et al. (1996) proposed would represent lateral organ primordia and the creases between them. Zones of inhibition in this model can be viewed as the reluctance of the tunica to bend sharply close to where it has already bent to form an existing primordium. Deformation would therefore represent the prepattern to which expression of lateral organ- and meristem-specific genes respond. Modelling has shown that such a process is theoretically capable of accounting for many aspects of phyllotaxy, including its propagation and self-adjustment after perturbation (Green, 1996). Although such a biophysical mechanism is unlikely to require the action of specific genes, and is difficult to test genetically, several investigations have provided supporting evidence. The sunflower inflorescence (capitulum) is formed from a large, flat meristem that initiates primordia in multiple spirals. Each primordium then gives rise to a bract subtending a flower. Experimental compression of the developing capitulum caused the formation of ridges running along phyllotactic spirals, rather than distinct primordia, and these developed into extended (united) bracts that lacked flowers (Hernandez and Green, 1993). Therefore, physical force could both alter phyllotaxy in a predictable way and change primordium fate.

The protein expansin promotes cell expansion by loosening cell walls. In some species, isoforms of expansin appear to be upregulated in lateral organ primordia, consistent with roles in primordium initiation and growth (Fleming et al., 1997; Reinhardt et al., 1998). When expansin gene expression was induced in the tobacco apex at a position representing initials of the next-but-one primordium, the primordium initiated prematurely and caused reversal of the phyllotactic spiral. Because the resulting leaf developed normally, loosening of the cell wall was proposed to be sufficient to activate all subsequent steps in leaf development (Pien et al., 2001). Earlier experiments, in which outgrowth was induced by application of expansin protein to the SAM, led to outgrowths with only limited leaf-like characteristics (Fleming et al., 1997), presumably because the protein was unable to affect more internal cells. The effects of altered expansin activity provide support for Green's model, in which distortion of the meristem is causal in lateral organ fate. However, expansin was unable to induce primordium

formation in positions at which organs would not usually form, suggesting that lateral organ initials are specified before primordium outgrowth, and that expansin merely causes precocious outgrowth of existing initials. Comparing the effects of expansin with expression of genes that act as early markers for lateral organ and non-lateral organ fates, as well as inducing expansin activity in auxin mutants, should help to resolve this question.

A further level of organization within the apex that might be relevant to fate specification is the arrangement of plasmodesmatal connections that potentially allow communication between cells. Plasmodesmata interconnect symplastic fields that may include cells from the same or different lineages. They are also able to regulate the passage of potential signaling molecules, including developmentally important transcription factors or their RNA precursors (e.g., *KNOTTED1*; Lucas et al., 1995; Haywood et al., 2002), and may be involved in determining the polarity of movement. For example, the *Antirrhinum* MADS-domain transcription factor *DEFICIENS* can move outwards to the L1 but not inwards from it (Perbal et al., 1996). Studies of the birch and *Arabidopsis* meristems reveal that symplastic fields predict some aspects of meristem fate and are highly dynamic (Rinne and van der Schoot, 1998; Gisel et al., 1999). Whether symplastic signaling is important in maintaining fate within a field or for specifying different fates remains to be determined.

SPECIFICATION OF POLARITY IN LATERAL ORGANS

Elaboration of Dorsiventral Asymmetry

Most lateral organs are considered dorsiventral because they are flattened perpendicular to their adaxial-abaxial (or dorsiventral) axis, along which they also show an asymmetric distribution of cell types. The flattened shape of leaves or petals has obvious functional significance, providing a large area and arrangement of cell types specialized for photosynthesis or attraction of pollinators. Because lateral organs form in the periphery of apical meristems, their dorsiventral axis corresponds to a radial axis of the meristem (i.e., their adaxial surface is formed toward the center of the meristem). A connection between dorsiventrality of lateral organs and polarity of the meristem was suggested by early surgical experiments (Sussex, 1955; Snow and Snow, 1959; Hanawa, 1961). When an incision or impermeable barrier was inserted adaxial to a group of leaf initials, the resulting primordium was needle-like and consisted only of abaxial cells. This suggested (i) that signals originating from the center of the SAM are required for adaxial fate, (ii) that abaxial fate occurs in the absence of adaxial, and (iii) that adaxial fate is required for lateral growth of the leaf. Primordia isolated at a later stage of development were able to develop normally, implying that they no longer needed signals from

outside the leaf to maintain their dorsiventral asymmetry. Subsequent analysis of the *Antirrhinum phantastica* (*phan*) gene, which is required for adaxial cell identity, suggested that interaction between organ cells with adaxial and abaxial identities was responsible for the lateral growth that flattened the organ (Waites and Hudson, 1995). Because *phan* functions redundantly with cold-sensitive factors, its role in promoting adaxial organ fate could be separated by temperature treatment from an additional function in promoting primordium formation. Temperature shift experiments were unable to separate these two roles in time, suggesting that dorsiventral organ asymmetry is specified before primordium initiation, consistent with the involvement of a signaling mechanism in the SAM (Waites et al., 1998).

Recent analysis in *Arabidopsis* has provided support for the involvement of an adaxial morphogen within the SAM leading directly to asymmetric gene expression within lateral organ primordia. This involved the identification of a small gene family, encoding likely transcription factors with homeodomain, bZIP, and START motifs that promote adaxial cell types in *Arabidopsis* lateral organs. These show similar expression patterns in the SAM and lateral organ initials and become restricted to an adaxial domain about the time of primordium initiation. Gain-of-function mutations in two members, *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*), cause dose-dependent adaxialisation of organs, which at their most extreme develop only adaxial cell types and fail to grow laterally (McConnell and Barton, 1998; McConnell et al., 2001). Independent gain-of-function mutations involve mis-sense mutations that alter the START domain, a motif that is implicated in binding sterol lipid ligands in a number of functionally diverse proteins (McConnell et al., 2001). This suggests that *PHB* and *PHV* proteins are activated by a lipid ligand that is present only in adaxial cells. (An alternative interpretation is that they are repressed by an abaxial ligand.) The gain-of-function mutations are proposed to cause ligand-independent activity in cells positioned more abaxially, resulting in ectopic adaxial fates.

Because the domains of *PHB* and *PHV* RNA expression extend abaxially in gain-of-function mutants, the activated proteins are likely to promote accumulation of their own RNA (McConnell et al., 2001). *PHB* and *PHV* show graded RNA accumulation in lateral organ primordia, with the highest levels adaxially. This therefore suggests a dorsiventral gradient of protein activity resulting from a gradient of the activating sterol ligand. *PHB* and *PHV* expression also extends from lateral organ primordia and initials to the center of the SAM, suggesting that the ligand is also present in the SAM. It is attractive to speculate that this ligand corresponds to the signaling molecule predicted from the surgical experiments, which is produced centrally in the apex, forms a concentration gradient toward the periphery, and specifies adaxial organ fate in a concentration-dependent manner (i.e., is a morphogen). This raises a number of exciting questions about the identity of the ligand or ligands of *PHB* and *PHV* and whether they can move between cells.

The ability of *PHB* and *PHV* to promote their own expression might also provide the basis for the leaf primordium to maintain cell identity without further signals from the SAM, consistent with the ability of isolated primordia to complete normal development.

PHB and *PHV* act to restrict expression of two families of regulatory genes involved in abaxial cell fate specification, the *KANADIs* and *YABBYs*, which are initially expressed throughout lateral organ initials and become restricted to the abaxial domain about the time that *PHB* and *PHV* are localized to adaxial cells (Eshed et al., 1999, 2001; Sawa et al., 1999; Siegfried et al., 1999; Kerstetter et al., 2001). Ectopic adaxial expression of *KANADI (KAN)* genes causes adaxial cells to assume abaxial fates, suggesting that much of the role of *PHB* and *PHV* in promoting adaxial fate might result from repression of *KAN*. Expression of all three gene families, however, overlaps in lateral organ initials within the SAM, suggesting that other factors, specific to organ primordia, are required for the activation of *PHB/PHV* or repression of *KAN*.

Interaction between primordium cells with adaxial and abaxial identities is proposed to be necessary for lateral growth of the leaf (Waites and Hudson, 1995). The time at which genes promoting adaxial or abaxial fate become restricted to their respective domains broadly corresponds with the changes in patterns of cell division that accompany the start of leaf blade growth (Sawa et al., 1999; Siegfried et al., 1999; Eshed et al., 2001; Kerstetter et al., 2001; McConnell et al., 2001). Early divisions are highest in two lateral domains of the primordium, termed blastozones, that probably act as lamina initials. Expression of organ polarity genes persists in the growing organ, suggesting that adaxial–abaxial interaction might be required for oriented cell divisions until relatively late in development. While there is increasing evidence to support the role of adaxial–abaxial interaction in controlling lateral organ growth, the signaling mechanisms remain unknown. The leaf lamina, however, is formed from as few as four layers of cells along its dorsiventral axis, and therefore the proposed interactions between dorsal and ventral cells need control cell division over only two cell diameters.

Elaboration of Proximal–Distal Asymmetry in Lateral Organs

The dorsiventral organ axis typically shows an asymmetric distribution of tissues but undergoes little growth. In leaves, more growth typically occurs along the proximal–distal (P–D) axis (representing the base to tip of the mature leaf). However, limited P–D asymmetry of tissues in dicot leaves hinders testing of the developmental relevance of this axis and the mechanisms that might be involved in its specification and elaboration. Monocot leaves, in contrast, often show obvious P–D patterning. The maize leaf consists of a proximal sheath and distal blade with ligule and auricle structures

between. Disruptions to this pattern result from ectopic expression of Class I *knox* genes that are normally restricted to cells of the SAM and internode initials. Although such gain-of-function phenotypes are inherently difficult to interpret, one explanation for the effect of ectopic *knox* gene expression is that it alters the developmental age of cells, allowing them to respond to a P–D prepattern in a way that is inappropriate for their position (Freeling, 1992). The basis for the prepattern, however, remains elusive.

The dicot gynoecium, in contrast to the dicot leaf, shows marked asymmetry of tissues along its P–D axis, which often also represents its predominant axis of growth. The Arabidopsis gynoecium is a complex organ—it has a pollen-receptive stigma distally, followed by the style, ovary, and a short internode (gynophore) separating it from the pedicel at its proximal end. The ovary consists of two lateral valves and is divided into two locules by a septum that is formed by postgenital fusion of outgrowths from the ovary walls. This distinct arrangement of cell types has allowed identification of mutations that disrupt P–D patterning of the gynoecium.

Disruptions to the arrangement of gynoecium tissues occur in mutants defective in auxin transport and perception (e.g., *pin1*, *pid*, and *mp*) or following application of inhibitors of PAT (Bennett et al., 1995; Przemeck et al., 1996; Nemhauser et al., 2000). This has led to the proposal that auxin acts as a morphogen in carpel development. In this model, auxin is produced distally and is actively transported proximally forming a concentration gradient to which cells respond; stigma and style, valve, and gynophore identities are promoted by high, medium, and low auxin concentrations, respectively (Figure 3). The apical boundaries between style and valve initials and the basal boundary between valve and internode therefore represent the points at which two different threshold concentrations are reached within the gradient. PAT inhibitors shift both of these boundaries distally (i.e., cells adopt more basal fates than normally appropriate for their positions) but also reduce the spacing between boundaries, so that the gynoecium shows an increase in stigma/style and gynophore identities at the expense of ovary (Nemhauser et al., 2000). This is consistent with PAT inhibitors causing a steeper auxin concentration gradient.

The effect of PAT inhibitors is similar in several respects to that of Arabidopsis *ettin (ett)* mutations. Decreasing activity in a series of *ett* mutants leads to progressive replacement of proximal valve tissue with tissue resembling both abaxial style and gynophore and tissues between the valves of the ovary with adaxial style tissue (Sessions and Zambryski, 1995; Sessions, 1997). The most severe *ett* mutants lack all valve tissue and form only a style-like structure. Application of PAT inhibitors to weak *ett* mutants causes a more severe mutant phenotype, suggesting that ETT perceives the auxin concentration gradient (Nemhauser et al., 2000). Changes to ETT activity (mutations) alter the threshold levels that give rise to boundaries (Figure 4).

Consistent with this view is the finding that *ETT* encodes a

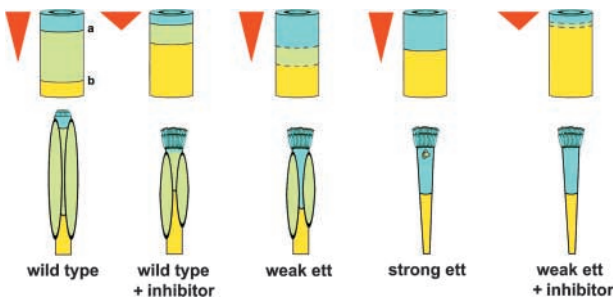


Figure 3. Development of the Arabidopsis Gynoecium.

The gynoecium primordium is depicted as a cylinder in which the apical boundary (a) separates the stigma/style (blue) from the ovary valve (green), and the basal boundary (b) separates valve from gynoepithelium (yellow). Auxin is produced apically and transported basally, forming a concentration gradient within the developing gynoecium (red arrow). The predicted effects of reduced polar auxin transport and ETT activity on the concentration gradient and positioning of boundaries are shown above the resulting gynoecium phenotypes. Dashed lines in *ett* mutant primordia indicate boundaries that are disrupted through a loss of ETT activity. When polar auxin transport (PAT) is inhibited, the apical and basal boundaries are shifted apically and the distance separating them reduced. The effects on gynoecium development are proposed to reflect a steeper morphogen gradient. Gynoecia of weak *ett* mutants display similar characteristics, which are suggested to involve both boundaries moving toward each other because sensitivity to auxin has been altered. Application of PAT inhibitor to the weak *ett* mutant causes a further reduction in valve region. This, together with the disrupted boundaries, results in a loss of valve identity and a phenotype that is similar to strong *ett* mutants. Strong *ett* mutants are not affected by PAT inhibitors. (Figure adapted from Nemhauser et al., 2000).

member of the auxin response factor family of transcriptional regulators that show auxin-dependent binding to auxin response promoter elements (Sessions et al., 1997; Kepinsky and Leyser, 2002). Therefore, the ETT protein could potentially act to transduce an auxin concentration gradient into patterned gene expression (Sessions, 1997; Nemhauser et al., 2000). ETT activity, however, appears unlikely to set the apical and basal boundaries in response to auxin, because its domain of RNA expression corresponds to the region between the boundaries and is unaffected by auxin treatment (Nemhauser et al., 2000).

Further doubt about the proposed role of *ETT* has been raised by the finding that it represses the basic helix-loop-helix transcription factor gene, *SPATULA* (*SPT*; Heisler et al., 2001). *SPT* is expressed distally, outside the *ETT* domain, and promotes development of distal gynoecium structure (*spt* mutant gynoecia have reduced stigmatic tissues and lack internal distal cell types; Alvarez and Smyth, 1998, 1999). This repression might involve direct binding of ETT protein to auxin response promoter elements in the *SPT* promoter. Mis-expression of *SPT* can account for most of the carpel defects of *ett* mutants, including the aberrant positions

of the apical and basal boundaries, because these are restored in *spt ett* double mutants (Alvarez and Smyth, 1998; Heisler et al., 2001). Thus, it appears that the effects of *ett* mutants on the carpel boundaries result indirectly from *SPT* expression and not from an inability to respond to an auxin gradient. This could be tested further by examining the effects of PAT inhibitors on boundary placement in *ett spt* mutant gynoecia.

CONTROL OF STOMATAL CELL FATE

Stomata are pores formed between a pair of specialized epidermal guard cells that allow gas exchange to be regulated. Arabidopsis is typical of many dicots in which the guard cells of one stoma are separated from others by at least one less-specialized epidermal pavement cell (e.g., Figure 1D; Kagan et al., 1992; Korn, 1993). The pair of guard cells is formed by symmetric division of a guard mother cell (GMC). The GMC results from asymmetric divisions of a meristemoid or meristemoid mother cell (MMC; Figure 4). A number of mechanisms have been proposed to explain the pattern of stomatal and non-stomatal cells in the epidermis. At one extreme, an invariant pattern of cell divisions is proposed to result in each GMC being completely surrounded by non-GMCs derived from the same lineage (Figure 4; Larkin et al., 1996). Such a pattern could be an intrinsic property of the MMC and need not involve communication between cells. This view has received support from the observation that the boundaries of clones marked early in development rarely pass between guard cells and their neighboring pavement cells, consistent with the surrounding cells and GMC arising from the same lineage (Larkin et al., 1997; Serna and Fenoll, 2000). Alternatively, spacing might involve cell-cell interactions that prevent cells close to MMCs, meristemoids, or GMCs from assuming similar fates (a lateral inhibition model) or that influence cell division polarity.

Such questions were addressed by carefully analyzing the

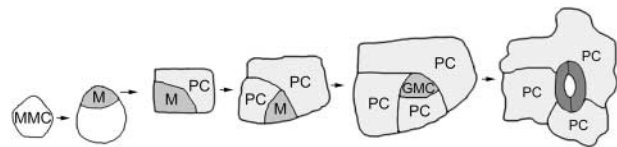


Figure 4. The Stereotypic Division Model for Stomatal Spacing.

A meristemoid mother cell (MMC) divides asymmetrically to form a smaller meristemoid (M). The meristemoid then undergoes three rounds of asymmetric cell divisions, in which the larger daughters assume fates as less-specialized pavement cells (PC). The meristemoid then becomes a guard mother cell (GMC) that divides symmetrically to form two guard cells surrounding a central pore. This pattern of division would result in each stoma being surrounded by at least one pavement cell derived from the same meristemoid. (Redrawn from Geisler et al., 2000).

origins of epidermal cells through daily observations of Arabidopsis leaves or cotyledons (Geisler et al., 2000). This study revealed that the cell divisions giving rise to a GMC were far from stereotypic (as in Figure 4) and involved a variable number of meristemoid divisions. The majority of guard cell pairs therefore had at least one adjacent pavement cell that was derived from a different MMC, so purely lineage-based mechanisms were unable to explain stomatal spacing. MMCs could form adjacent to existing guard cells or their precursors (MMCs, meristemoids, and GMCs), although they did so less frequently and never formed from a cell adjacent to two existing guard cell precursors. This suggested that cell–cell interactions were involved in specification of MMC fate but that these interactions were insufficient to account for spacing of all stomata. While MMCs located next to each other showed random orientation of cell division, those adjacent to existing meristemoids, GMCs, or guard cells always divided to form a new satellite meristemoid opposite the existing guard cell precursor. This asymmetric division did not appear to reflect an inherited cellular polarity, because it could occur in cells derived from a lineage different from that of the guard cell precursor. This again suggested the involvement of signaling, rather than cell intrinsic polarity. Because three quarters of stomata arose from satellite meristemoids, such oriented cell division constituted a major spacing mechanism. When adjacent meristemoids were formed, they very rarely gave rise to adjacent stomata, either because they were separated by an oriented division or because one of them assumed pavement cell fate. Adjacent meristemoids are derived from different lineages, implying the involvement of cell–cell interaction in these processes.

Several important aspects of stomatal patterning can therefore be explained by signaling that acts within one cell diameter of guard cell precursors to inhibit cell fate and orient cell divisions (although it does not rule out a role for inherited asymmetry in some cells). This mechanism is also compatible with the effects of environmental and developmental cues that alter the frequency of stomata (Gray et al., 2000; Berardini et al., 2001; Brownlee, 2001), and it will be interesting to determine which spacing mechanisms these cues affect (e.g., MMC frequency, the number of meristemoid divisions, etc). Because MMC fate involves lateral inhibition, it can also explain the relative insensitivity of stomatal frequency to differences in organ size.

All these aspects of stomatal spacing are affected by loss-of-function mutations in the Arabidopsis *TOO MANY MOUTHS (TMM)* gene (Yang and Sack, 1995). *tmm* mutants show several defects consistent with compromised signaling; for example, they form MMCs adjacent to existing stomatal lineages, and the divisions that form satellite meristemoids are oriented randomly (Geisler et al., 2000). This suggests that *TMM* is involved in signaling and that a common *TMM*-dependent signaling process may be involved in all the mechanisms contributing to stomatal spacing. The role of *TMM* and other genes with related

phenotypes (Yang and Sack, 1995), should cast more light on this process.

CONCLUSIONS

We have considered several aspects of plant lateral organ development that appear to be dependent on cell–cell signaling, although the signaling mechanisms involved are largely obscure. Auxin, sterol lipids, and physical forces have been invoked as signals acting over distances of several cells and even acting as morphogens in specifying different fates according to concentration. The involvement of auxin (which can have pleiotropic effects) and of physical force (which is unlikely to depend directly on gene activity) is difficult to test genetically. Other signaling mechanisms, however, lend themselves to genetic characterisation. Identification of *PHB*-like transcription factors provides a potential route to identifying their activating ligands and so testing the roles of the ligands in cell–cell signaling. In the case of stomatal cell placement, much of the observed patterning can be explained by signals acting between adjacent cells, for example, a mechanism analogous to Delta-Notch signaling in *Drosophila*. Further insights into the identity of these signals may come when the genes altered in the stomatal spacing mutants have been identified. Perhaps the strongest evidence for the nature of a cell–cell signaling mechanism is provided by the CLV pathway, in which the secreted CLV3 peptide probably acts as a ligand for the CLV1 receptor kinase. The Arabidopsis genome contains numerous CLV3-like genes and potential CLV1-like receptors (Cock and McCormick, 2001), raising the possibility that homologous signaling mechanisms operate in many aspects of plant development.

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REFERENCES

- Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. (1997). Genes involved in organ separation in *Arabidopsis*: an analysis of the cup-shaped cotyledon mutant. *Plant Cell* **9**, 841–857.
- Aida, M., Ishida, T., and Tasaka, M. (1999). Shoot apical meristem and cotyledon formation during *Arabidopsis* embryogenesis: Interaction among the *CUP-SHAPED COTYLEDON* and *SHOOT MERISTEMLESS* genes. *Development* **119**, 823–831.

- Alvarez, J., and Smyth, D.R.** (1998). Genetic pathways controlling carpel development in *Arabidopsis thaliana*. *J. Plant Res.* **111**, 295–298.
- Alvarez, J., and Smyth, D.R.** (1999). *CRABS CLAW* and *SPATULA*, two *Arabidopsis* genes that control carpel development in parallel with *AGAMOUS*. *Development* **126**, 2377–2386.
- Artavanis-Tsakonas, S., Matsuno, K., and Fortini, M.** (1995). Notch signalling. *Science* **268**, 225–230.
- Benjamins, R., Quint, A., Weijers, D., Hooykaas, P., and Offringa, R.** (2001). The PINOID protein kinase regulates organ development in *Arabidopsis* by enhancing polar auxin transport. *Development* **128**, 4057–4067.
- Bennett, S.R.M., Alvarez, J., Bossinger, G., and Smyth, D.R.** (1995). Morphogenesis in *pinoid* mutants of *Arabidopsis thaliana*. *Plant J.* **8**, 505–520.
- Berardini, T.Z., Bollman, K., Sun, H., and Poethig, R.S.** (2001). Regulation of vegetative phase change in *Arabidopsis thaliana* by cyclophilin 40. *Science* **291**, 2405–2407.
- Berger, F., Taylor, A., and Brownlee, C.** (1994). Cell fate determination by the cell wall in early *Fucus* development. *Science* **263**, 1421–1423.
- Berleth, T., and Jürgens, G.** (1993). The role of the *monopteros* gene in organising the basal body region of *Arabidopsis* embryo. *Development* **118**, 575–587.
- Blazquez, M.A., Soowal, L.N., Lee, I., and Weigel, D.** (1997). *LEAFY* expression and flower initiation in *Arabidopsis*. *Development* **124**, 3835–3844.
- Brand, U., Fletcher, J., Hobe, M., Meyerowitz, E., and Simon, R.** (2000). Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity. *Science* **289**, 617–619.
- Brownlee, C.** (2001). The long and the short of stomatal density signals. *Trend. Plant Sci.* **6**, 441–442.
- Byrne, M.E., Barley, R., Curtis, M., Arroyo, J.M., Dunham, M., Hudson, A., and Martienssen, R.A.** (2000). *Asymmetric leaves1* mediates leaf patterning and stem cell function in *Arabidopsis*. *Nature* **408**, 967–971.
- Carpenter, R., Copesey, L., Vincent, C., Doyle, S., Magrath, R., and Coen, E.** (1995). Control of flower development and phyllotaxy by meristem identity genes in *Antirrhinum*. *Plant Cell* **7**, 2001–2011.
- Christensen, S.K., Dagenais, N., Chory, J., and Weigel, D.** (2000). Regulation of auxin response by the protein kinase PINOID. *Cell* **100**, 469–478.
- Clarke, S.E., Running, M.P., and Meyerowitz, E.M.** (1993). *CLAVATA1*, a regulator of meristem and flower development in *Arabidopsis*. *Development* **119**, 397–418.
- Cock, J.M., and McCormick, S.** (2001). A large family of genes that share homology with *CLAVATA3*. *Plant Physiol.* **126**, 939–942.
- Dalgaard, J.Z., and Klar, A.J.** (2001). Does *S. pombe* exploit the intrinsic asymmetry of DNA synthesis to imprint daughter cells for mating-type switching? *Trend. Genet.* **17**, 153–157.
- Dengler, N., and Kang, J.** (2001). Vascular patterning and leaf shape. *Curr. Opin. Plant Biol.* **4**, 50–56.
- Elliott, R.C., Betzner, A.S., Huttner, E., Oakes, M.P., Tucker, W.Q.J., Gerentes, D., Perez, P., and Smyth, D.R.** (1996). *AIN-TEGUMENTA*, an *APETELA2*-like gene of *Arabidopsis* with pleiotropic roles in ovule development and floral organ growth. *Plant Cell* **8**, 155–168.
- Eshed, Y., Baum, S.F., and Bowman, J.L.** (1999). Distinct mechanisms promote polarity establishment in carpels of *Arabidopsis*. *Cell* **99**, 199–209.
- Eshed, Y., Baum, S.F., Perea, J.V., and Bowman, J.L.** (2001). Establishment of polarity in lateral organs of plants. *Curr. Biol.* **11**, 1251–1260.
- Finnegan, E.J., and Kovac, K.A.** (2000). Plant DNA methyltransferases. *Plant Mol. Biol.* **43**, 189–201.
- Fleming, A.J., McQueen-Mason, S., Mandel, T., and Kuhlemeier, C.** (1997). Induction of leaf primordia by the cell wall protein expansin. *Science* **276**, 1415–1418.
- Freeling, M.** (1992). A conceptual framework for leaf development. *Dev. Biol.* **153**, 44–58.
- Gallagher, K., and Smith, L.G.** (2000). Roles for polarity and nuclear determinants in specifying daughter cell fates after an asymmetric cell division in the maize leaf. *Curr. Biol.* **10**, 1229–1232.
- Gälweiler, L., Guan, C., Müller, A., Wisman, E., Mendgen, K., Yephremov, A., and Palme, K.** (1998). Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* **282**, 2226–2230.
- Geisler, M., Nadeau, J., and Sack, F.D.** (2000). Oriented asymmetric divisions that generate the stomatal spacing pattern in *Arabidopsis* are disrupted by the *too many mouths* mutation. *Plant Cell* **12**, 2075–2086.
- Gisel, A., Barella, S., Hempel, F.D., and Zambryski, P.C.** (1999). Temporal and spatial regulation of symplastic trafficking during development in *Arabidopsis thaliana* apices. *Development* **126**, 1879–1889.
- Gray, J.E., Holroyd, G.H., vander Lee, F.M., Bahrami, A.R., and Sijmons, P.C.** (2000). The HIC signalling pathway links CO₂ perception to stomatal development. *Nature* **408**, 713–716.
- Green, P.B., Steele, C.S., and Rennich, S.C.** (1996). Phyllotactic patterns: a biophysical mechanism for their origin. *Ann. Bot.* **77**, 515–527.
- Green, P.B.** (1996). Expression of form and pattern in plants—a role for biophysical fields. *Semin. Cell Dev. Biol.* **7**, 903–911.
- Greyson, R.I., Walden, D.B., Humes, J.A., and Erickson, R.O.** (1978). The ABPHYL syndrome in *Zea mays*. II. Patterns of leaf initiation and the shape of the shoot meristem. *Can. J. Bot.* **56**, 1545–1550.
- Habu, Y., Kakutani, T., and Paszkowski, J.** (2001). Epigenetic developmental mechanisms in plants: Molecules and targets of plant epigenetic regulation. *Curr. Opin. Genet. Dev.* **11**, 215–220.
- Hanawa, J.** (1961). Experimental studies of leaf dorsiventrality in *Sesamum indicum* L. *Bot. Mag. Tokyo* **74**, 303–309.
- Haywood, V., Kragler, F., and Lucas, W.** (2002). Plasmodesmata: Pathways for protein and riboprotein signalling. *Plant Cell* **14** (suppl.), S303–S325.
- Heisler, M.G.B., Atkinson, A., Bylstra, Y.H., Walsh, R., and Smyth, D.R.** (2001). *SPATULA*, a gene that controls development of carpel margin tissues in *Arabidopsis*, encodes a bHLH protein. *Development* **128**, 1089–1098.
- Hernandez, L.F., and Green, P.B.** (1993). Transductions for the expression of structural pattern: Analysis in sunflower. *Plant Cell* **5**, 1725–1738.

- Jackson, D., and Hake, S.** (1999). Control of phyllotaxy in maize by the *abp1* gene. *Development* **126**, 315–323.
- Jankovsky, J.P., Smith, L.G., and Nelson, T.** (2001). Specification of bundle sheath cell fates during maize leaf development: Roles of lineage and positional information evaluated through analysis of the *tangled1* mutant. *Development* **128**, 2747–2753.
- Kagan, M.L., Novoplansky, N., and Sachs, T.** (1992). Variable cell lineages form the pea epidermis. *Ann. Bot.* **69**, 303–312.
- Kaya, H., Shibahara, K.I., Taoka, K.I., Iwabuchi, M., Stillman, B., and Araki, T.** (2001). *FASCIATA* genes for chromatin assembly factor-1 in *Arabidopsis* maintain the cellular organization of apical meristems. *Cell* **104**, 131–142.
- Kepinsky, S., and Leyser, O.** (2002). Ubiquitination and auxin signalling: A degrading story. *Plant Cell* **14** (suppl.), S81–S95.
- Kerstetter, R.A., Bollma, K., Taylor, R.A., Bomblied, K., and Poethig, R.S.** (2001). *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* **411**, 706–709.
- Klucher, K.M., Chow, H., Reiser, L., and Fischer, R.L.** (1996). The *AINTEGUMENTA* gene of *Arabidopsis* required for ovule and female gametophyte development is related to the floral homeotic gene *APETALA2*. *Plant Cell* **8**, 137–153.
- Korn, R.W.** (1993). Evidence in dicots for stomatal patterning by inhibition. *Int. J. Plant Sci.* **154**, 367–377.
- Langdale, J.A., Lane, B., Freeling, M., and Nelson, T.** (1989). Cell lineage analysis of maize bundle sheath and mesophyll cells. *Dev. Biol.* **133**, 128–139.
- Larkin, J.C., Marks, M.D., Nadeau, J., and Sack, F.** (1997). Epidermal cell fate and patterning in leaves. *Plant Cell* **9**, 1109–1120.
- Larkin, J.C., Young, N., Prigge, M., and Marks, M.D.** (1996). The control of trichome spacing and number in *Arabidopsis*. *Development* **122**, 997–1005.
- Leyser, H.M.O., and Furner, I.J.** (1992). Characterisation of three shoot apical meristem mutants of *Arabidopsis thaliana*. *Development* **116**, 397–403.
- Loiseau, J.E.** (1969). La Phyllotaxie. (Paris: Masson et Cie).
- Long, J.A., Moan, E.I., Medford, J.I., and Barton, M.K.** (1996). A member of the KNOTTED class of homeodomain proteins encoded by the SHOOTMERISTEMLESS gene of *Arabidopsis*. *Nature* **379**, 66–69.
- Lucas, W.J., Bouché-Pillon, S., Jackson, D.P., Nguyen, L., Baker, L., Ding, B., and Hake, S.** (1995). Selective trafficking of KNOTTED1 homeodomain protein and its mRNA through plasmodesmata. *Science* **270**, 1980–1983.
- Lyndon, R.F.** (1998). The Shoot Apical Meristem—Its Growth and Development. (Cambridge, UK: Cambridge University Press).
- Lynn, K., Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P., and Barton, M.K.** (1999). The *PINHEAD/ZWILLE* gene acts pleiotropically in *Arabidopsis* development and has overlapping functions with the *ARGONAUTE1* gene. *Development* **126**, 469–481.
- McConnell, J.R., and Barton, M.K.** (1998). Leaf polarity and meristem formation in *Arabidopsis*. *Development* **125**, 2935–2942.
- McConnell, J.R., Emery, J.F., Eshed, Y., Bao, N., Bowman, J., and Barton, M.K.** (2001). Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. *Nature* **411**, 709–713.
- Nakajima, K., and Benfey, P.N.** (2002). Signalling in and out: Control of cell division and differentiation in the shoot and root. *Plant Cell* **14** (suppl.), S265–S276.
- Nemhauser, J.L., Feldman, L.J., and Zambryski, P.C.** (2000). Auxin and *ETTIN* in *Arabidopsis* gynoecium morphogenesis. *Development* **127**, 3877–3888.
- Okada, K., Ueda, J., Komaki, M.K., Bell, C.J., and Shimura, Y.** (1991). Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *Plant Cell* **3**, 677–684.
- Perbal, M.C., Haughn, G., Saedler, H., and Schwarz-Sommer, Z.** (1996). Non-cell-autonomous function of the *Antirrhinum* floral homeotic proteins DEFICIENS and GLOBOSA is exerted by their polar cell-to-cell trafficking. *Development* **122**, 3433–3441.
- Pien, S., Wyrzykowska, J., McQueen-Mason, S., Smart, C., and Fleming, A.** (2001). Local expression of expansin induces the entire process of leaf development and modifies leaf shape. *Proc. Natl. Acad. Sci. USA* **98**, 11812–11817.
- Poethig, S., and Sussex, I.M.** (1985). The cellular parameters of leaf development in tobacco: A clonal analysis. *Planta* **165**, 170–184.
- Przemeck, G.K.H., Mattsson, J., Hardtke, C.S., Sung, Z.R., and Berleth, T.** (1996). Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. *Planta* **200**, 229–237.
- Reinhardt, D., Mandel, T., and Kuhlemeier, C.** (2000). Auxin regulates the initiation and radial position of plant lateral organs. *Plant Cell* **12**, 507–518.
- Reinhardt, D., Wittwer, F., Mandel, T., and Kuhlemeier, C.** (1998). Localized upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem. *Plant Cell* **10**, 1427–1437.
- Rinne, P.L., and van der Schoot, C.** (1998). Symplasmic fields in the tunica of the shoot apical meristem coordinate morphogenetic events. *Development* **125**, 1477–1485.
- Sachs, T.** (1991). Pattern Formation in Plant Tissues. (Cambridge, UK: Cambridge University Press).
- Sawa, S., Watanabe, K., Goto, K., Kanaya, E., Morita, E.H., and Okada, K.** (1999). *FILAMENTOUS FLOWER*, a meristem and organ identity gene of *Arabidopsis*, encodes a protein with a zinc finger and HMG-related domains. *Genes Dev.* **13**, 1079–1088.
- Schiefelbein, J.W.** (2000). Constructing a plant cell. The genetic control of root hair development. *Plant Physiol.* **124**, 1525–1531.
- Schoof, H., Lenhard, M., Haecker, A., Mayer, K., Jürgens, G., and Laux, T.** (2000). The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes. *Cell* **100**, 635–644.
- Schoute, J.C.** (1913). Beiträge zur Blattstellungslehre. *Ré. Trav. Bot. Néerl.* **10**, 153–235.
- Serna, L., and Fenoll, C.** (2000). Stomatal development and patterning in *Arabidopsis* leaves. *Physiol. Plant* **109**, 351–358.
- Sessions, A., Nemhauser, J., McCall, A., Roe, J.L., Feldman, K.A., and Zambryski, P.C.** (1997). *ETTIN* patterns the *Arabidopsis* floral meristem and reproductive organs. *Development* **124**, 4481–4491.
- Sessions, R.A.** (1997). *Arabidopsis* (Brassicaceae) flower development and gynoecium patterning in wild type and *ettin* mutants. *Am. J. Bot.* **84**, 1179–1191.
- Sessions, R.A., and Zambryski, P.C.** (1995). *Arabidopsis* gynoecium structure in the wild type and *ettin* mutants. *Development* **121**, 1519–1532.

- Siegfried, K.R., Eshed, Y., Baum, S., Otsuga, D., Drews, G.N., and Bowman, J.L.** (1999). Members of the *YABBY* gene family specify abaxial cell fate in *Arabidopsis*. *Development* **126**, 4117–4128.
- Smith, L.G., Hake, S., and Sylvester, A.W.** (1996). The *tangled-1* mutation alters cell division orientations throughout maize leaf development without altering leaf shape. *Development* **122**, 481–489.
- Snow, M., and Snow, R.** (1959). The dorsiventrality of leaf primordia. *New Phytol.* **58**, 188–207.
- Souer, E., van Houwelingen, A., Kloos, D., Mol, J., and Koes, R.** (1996). The *No Apical Meristem* gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* **85**, 159–170.
- Stewart, R.N., and Derman, H.** (1975). Flexibility in ontogeny as shown by the contribution of the shoot apical layers to leaves of periclinal chimeras. *Am. J. Bot.* **62**, 935–947.
- Sussex, I.M.** (1955). Experiments on the cause of dorsiventrality in leaves. *Nature* **174**, 351–352.
- Takada, S., Hibara, K., Ishida, T., and Tasaka, M.** (2001). The *CUP-SHAPED COTYLEDON1* gene of *Arabidopsis* regulates shoot apical meristem formation. *Development* **128**, 1127–1135.
- Vernoux, T., Kronenberger, J., Grandjean, O., Laufs, P., and Traas, J.** (2000). *PIN-FORMED 1* regulates cell fate at the periphery of the shoot apical meristem. *Development* **127**, 5157–5165.
- Vincent, C.A., Carpenter, R., and Coen, E.S.** (1995). Cell lineage patterns and homeotic gene activity during *Arabidopsis* flower development. *Curr. Biol.* **5**, 1449–1458.
- Vincent, J.P., and Briscoe, J.** (2001). Morphogens. *Curr. Biol.* **11**, 852–854.
- Waites, R., and Hudson, A.** (1995). *phantastica*: A gene required for dorsoventrality of leaves in *Antirrhinum majus*. *Development* **121**, 2143–2153.
- Waites, R., Selvadurai, H., Oliver, I.R., and Hudson, A.** (1998). The *PHANTASTICA* gene encodes a MYB transcription factor involved in growth and dorsoventrality of lateral organs in *Antirrhinum*. *Cell* **93**, 779–789.
- Wardlaw, C.W.** (1949). Further experimental observations on the shoot apex of *Dryopteris aristata* Druce. *Philos. Trans. R. Soc. Lond. B.* **233**, 415–451.
- Woodward, F.I., and Kelly, C.K.** (1995). The influence of CO₂ concentration on stomatal density. *New Phytol.* **131**, 311–327.
- Yang, M., and Sack, F.D.** (1995). The *too many mouths* and *four lips* mutations affect stomatal production in *Arabidopsis*. *Plant Cell* **7**, 2227–2239.

NOTE ADDED IN PROOF

A recently published paper by Kessler et al. (2002) shows that cell identity in a maize leaf may be specified through lineage, as cells displaced from the L1 to the L2 layer in leaves of the *xc1* mutant retain their L1 identity.

Kessler, S., Seiki, S., and Sinha, N. (2002). *Xc1* causes delayed oblique periclinal cell divisions in developing maize leaves, leading to cellular differentiation by lineage instead of position. *Development* **129**, 1859–1869.