Networks in leaf development
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Shoots are characterized by indeterminate growth resulting from divisions of undifferentiated cells in the central region of the shoot apical meristem. These cells give rise to peripheral derivatives from which lateral organ initials are recruited. During initial stages of cell recruitment, the three-dimensional form of lateral organs is specified. Lateral organs such as leaves develop and differentiate along proximodistal (base-to-tip), dorsoventral (top-to-bottom) and mediolateral (middle-to-margin) planes. Current findings are refining our knowledge of the genes and genetic interactions that regulate these early processes and are providing a picture of how these pathways may contribute to variation in leaf form.

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Abbreviations
AGO1 ARGONAUTE1
AS1 ASYMMETRIC LEAVES1
CIN CINCINNATA
FIL FILAMENTOUS FLOWER
GA gibberellic acid
GRAM GRAMINAFOLIA
JAG JAGGED
KAN KANADI
ns narrow sheath
PHAN PHANTASTICA
PHB PHABULOSA
PHV PHAVOLUTA
PRS PRESSED FLOWER
REV REVOLUTA
rs2 rough sheath2
SAM shoot apical meristem
yab3 yabby3

Introduction
Leaves are the fundamental lateral appendage of land plants. The initiation and ground-plan patterning of leaves is a multistage process of interdependent events. Leaf primordia are initiated by recruitment of founder cells from the peripheral region of the shoot apical meristem (SAM). The extent of founder-cell recruitment from the flanks of the SAM varies between species, with Arabidopsis and maize representing two developmental extremes (Figure 1). Development along adaxial–abaxial (dorsoventral), proximal–distal, and midvein–margin (mediolateral) planes establishes leaf polarity (Figures 1 and 2). After this ground plan is set, continued cell division and expansion further contribute to leaf shape and form. At the same time, there is differentiation of specific cell and tissue types, such as outer layer epidermal cells, inner photosynthetic mesophyll and vasculature. Together, these processes establish the specialized function of the leaf as the main light-harvesting organ of the plant.

Leaf initiation and patterning has long been the subject of experimental and theoretical work. Recent studies are building on results of classic work and are now defining molecular networks that are involved in many different aspects of leaf development. This review aims only to spotlight several of the more recent studies that have contributed to our understanding of the mechanisms that regulate leaf initiation, ground-plan patterning and specification of final form. Several recent reviews provide further reading [1–3].

Cell recruitment
Leaf development initiates by recruitment of cells from the peripheral region of the SAM. Although the extent of founder-cell recruitment from the flanks of the SAM varies between species (Figure 1; [4–6]), genetic programs that control this early stage of leaf development appear to be conserved. For example, founder-cell recruitment in both Arabidopsis and maize is intimately linked with downregulation of KNOX homeobox transcription factors [3]. Auxin could be one regulator of this early event as chemical inhibition of polar auxin transport results in failure to downregulate KNOX expression in founder cells [7]. However, in Arabidopsis, PIN-FORMED1, a gene necessary for polar auxin transport in the SAM, is not required for downregulation of KNOX genes in founder cells [8**]. Furthermore, in maize, ectopic expression of KNOX genes disrupts polar auxin transport and inhibition of polar auxin transport mimics the effects of KNOX gene overexpression [7,10]. Potentially, there are mutual inhibitory interactions between KNOX genes and auxin transport in the SAM.

Recent work on the maize narrow sheath (ns) mutants has added to this picture. ns1 and ns2 are duplicate genes that are required for KNOX downregulation specifically in
founder cells that specify the lateral domain of the leaf [11]. Leaves that are mutant for ns1 and ns2 are narrower than wildtype leaves but only in the proximal region of the leaf. The ns genes encode WUSCHEL-related homeobox transcription factors and are related to PRESSED FLOWER (PRS) in Arabidopsis [12*]. prs mutations primarily affect floral organ development, but prs mutants also lack stipules that are normally found at the margins and base of the Arabidopsis leaf (Figure 1a; [12*,13]). Therefore, like ns mutants in maize, prs mutants are disrupted in the specification of lateral and proximal features of the leaf. Consistent with a requirement for regional specific founder cell recruitment, ns and PRS are both expressed in two lateral foci in the peripheral region of the SAM (Figure 3).

Dorsoventrality and lamina outgrowth

It is now well established that a dorsoventrally flattened lamina requires adjacent adaxial and abaxial leaf domains [1,14]. Loss of either the adaxial or abaxial domain results in partial or complete radialisation of the leaf.

Adaxial identity in Arabidopsis is specified by the class III HD-ZIP family genes PHABULOSA (PHB), PHAVOLUTA (PHV) and REVOLUTA (REV) [15*,16,17,18,19*]. Dominant gain-of-function mutants have adaxial leaves. Loss of function of any one gene in this family has little effect on leaf development. When combined, however, mutations in all three genes results in radial abaxial leaves, a phenotype that is complementary to the dominant mutant phenotypes [15*]. Conservation of the function of this gene family is relected in mutations in other species. Thus, dominant mutations in rolled leaf1, the maize orthologue of REV, and in NSPHAVOLUTA, a tobacco orthologue of PHV, also result in leaf polarity defects with gain of adaxial features on the abaxial side of the leaf [20*,21*]. Consistent with a role in determining leaf polarity, the expression of this gene family is confined
Adaxial–abaxial polarity. Gene expression patterns in the shoot apex of (a) Arabidopsis and (b) maize. PHB/PHV/REV genes (red) are expressed in the SAM. In Arabidopsis, these genes are expressed throughout the initiating primordium and their expression becomes confined to the adaxial domain upon further development of the primordium. In maize, the expression of the REV-related gene rolled leaf1 is adaxial in initiating and young primordia, and becomes confined to the margins later in development. Regulatory microRNAs, miRNA165/166 (yellow), are expressed on the abaxial side of the leaf in a domain that is complementary to the expression zone of target PHB/PHV/REV genes. The expression pattern of PRS in Arabidopsis and ns in maize is confined to a lateral region of the SAM (purple) and to the margins of leaf primordia (not indicated). The expression domain of the KNOX gene SHOOT MERISTEMLESS (blue) in Arabidopsis is also indicated. P1 to P5 indicate successive leaf primordia. M, meristem.

Another class of leaf patterning genes are members of the YABBY family. Of the five YABBY genes in Arabidopsis, three are expressed in leaves [28,30,31]. Deletion analysis of the promoter of one YABBY gene, FILAMENTOUS FLOWER (FIL), has revealed independent cis-acting sequences that promote the expression of FIL throughout the leaf primordium and repress FIL expression on the adaxial side of the leaf primordium. This demonstrates that FIL is actively excluded from the adaxial domain [32]. The role of YABBY genes in abaxial specification is not clear, however, because loss-of-function mutants do not have obvious dorsoventral defects. This may be due, in part, to redundancy within the YABBY gene family and also with KAN genes. For instance, there are no obvious polarity defects in the fil and yab3 (yab3) double mutant, but abaxial fate is compromised in this double mutant when KAN activity is reduced [26,30]. These two YABBY genes have further interactions with KAN genes. Double kan1 kan2 mutants have ectopic lamina outgrowths on the abaxial side of the leaf. It is not clear what preconditions lamina outgrowth as this phenotype is not always evident in backgrounds in which abaxial fate is disrupted. However, ectopic outgrowths are dependent on FIL and YAB3, supporting a role for YABBY genes in lamina outgrowth. GRAZINAFOLIA (GRAM) in Antirrhinum is
closely related to FIL and YAB3 [33*]. As with YABBY genes in Arabidopsis, GRAM is expressed in the abaxial domain of the leaf. However, genetic interactions indicate that GRAM may regulate leaf polarity by repressing adaxial fate in the abaxial domain of the leaf. Furthermore, GRAM appears to promote adaxial fate in a non-cell-autonomous manner. This suggests that the role of YABBY genes in dorsoventral polarity differs between species, although it is also possible that differences in loss-of-function phenotypes of different species reflect divergent levels of redundancy.

**PHANTASTICA genes and leaf patterning**

Leaf patterning can be altered by the ectopic expression of class I KNOX genes (reviewed in [3,34]). Typically, these genes are expressed in the SAM and downregulated in leaf primordia. The myb domain transcription factors PHANTASTICA (PHAN) in Antirrhinum, rough sheath2 (rs2) in maize and ASYMMETRIC LEAVES1 (AS1) in Arabidopsis are key negative regulators of KNOX gene expression in leaves [35–38]. The sequence identities and expression patterns of these genes clearly indicate that they form an orthologous gene family. The mutant phenotype in each species is not immediately comparable, however, leading to disparate interpretations of the defect. In phan mutants, early leaves have patches of abaxial tissue associated with ectopic lamina outgrowth on their adaxial leaf surface. The lamina of later leaves becomes progressively restricted to the distal tip of the leaf. In the most extreme form, the leaf is radial and abaxial [14]. PHAN is therefore required for leaf dorsoventral patterning. In comparison, as1 leaves are shorter and rounder than wildtype leaves, have occasional lobes and show no obvious dorsoventral defects [35,39,40], although under some conditions the base of the petiole may be radial [41,42]. Despite these differences, the maize gene rs2 rescues as1, indicating that function is conserved within this gene family.

Overexpression of either AS1 or rs2 in Arabidopsis does not result in dorsoventral defects [42,43]. This is in contrast to overexpression of the LOB domain gene AS2, which is a protein partner of AS1 [42,44]. Mutations in AS2 result in leaf patterning defects that are comparable to those of as1, and in growth-condition-dependant radialisation of the petiole base [39,40,42,45]. However, overexpression of AS2 results in dorsoventral defects that are consistent with adaxialisation of the leaf [42,46*]. Lamina outgrowths, similar to those of kan1 kan2 mutants, occur on the abaxial side of leaf of AS2-over-expressing lines. This phenotype is dependent on AS1. Together, the data indicate that AS1 and AS2 have at least retained the potential to pattern dorsoventral development and act by repression of abaxial fate in the adaxial domain of the leaf [46*]. If this is the case, adaxial specification in Arabidopsis is determined by pathways that are redundant with AS1.

The phenotype of the maize rs2 mutant sheds a different light on the role of PHAN genes in leaf development. In rs2 mutants, proximal features of the sheath, ligule and auricle (Figure 2) are displaced distally into the leaf blade [47]. rs2, therefore, is defective in proximodistal patterning. On the basis of the maize phenotype, an alternative interpretation of the phan phenotype proposed that radialisation of the Antirrhinum leaf is due to transformation of distal lamina into tissue that has petiole or stem features [37]. A recent report describing the effects of reduced NSPHAN in tobacco revisits this interpretation [48*]. The upper leaves of plants with reduced NSPHAN expression are similar to those of phan mutants, with leaf lamina being confined to the distal tip of the leaf. More proximal regions develop dorsoventrality but, as in the petiole, there is no leaf lamina. The tobacco leaves are radial only at the base of the leaf. Surprisingly, the radial region has phloem surrounding xylem, a morphology that is indicative of abaxialisation, but the expression of NSPHB, a marker for adaxial fate, remains on the adaxial side of the leaf. This suggests that the radial leaves maintain some adaxial features. Thus, reducing NSPHAN levels potentially disrupts proximodistal development, with proximal features of stem and petiole being displaced distally along the leaf base-to-tip axis.

The lower leaves of antisense NSPHAN plants have adaxial ectopic lamina outgrowths. Unlike those of phan mutants, these outgrowths are not associated with obvious dorsoventral defects. Instead, the lamina outgrowths of antisense NSPHAN plants may be associated with delayed maturation or indeterminacy in the leaf caused by misexpression of KNOX genes. Consistent with this idea, ectopic lamina is suppressed by application of gibberellic acid (GA), a hormone whose function is negatively regulated by KNOX genes [48*,49,50]. Misregulation of KNOX genes in simple leaved species is typically associated with ectopic shoots. However, reducing GA levels in a background in which KNOX genes are ectopically expressed can generate ectopic callus, lamina or shoots on the leaf [49]. Thus, the formation of shoot as opposed to leaf may be determined by the relative temporal and spatial levels of KNOX genes and GA.

Interestingly the tomato PHAN orthologue, LePHAN, is expressed in the SAM and on the adaxial side of leaf primordia [51**,52]. Decreased LePHAN expression results in leaves that have various degrees of reduced lamina development. The proximal region may be radialised and the distal lamina can develop as a trumpet or as leaflets at the tip of the radial petiole. Leaflets may be confined to the abaxial side of the petiole or be arranged around the entire petiole circumference, phenotypes that are comparable to non-peltate and peltate palmate leaves, respectively. LePHAN antisense phenotypes are correlated with the expression of LePHAN being progressively confined to the distal tip of the leaf. Furthermore, the
expression domain of PHAN in a range of species that have compound leaves correlates with the type of compound leaf, whether pinnate, palmate or peltate-palmate. Convergent evolutionary changes in the expression of PHAN may be responsible for phenotypic variation in compound leaf species. Equally likely, PHAN expression could be a consequence of developmental variation mediated by one or more alternative regulators.

Elaboration of the leaf lamina

Subsequent to events that direct leaf polarity, the control of final leaf shape and size continues by coordinated regulation of cell division and expansion along the length and width of the leaf [53]. Cessation of cell division and differentiation proceeds along the proximodistal plane from leaf tip to base (Figure 2). In the dorsoventral plane, continued divisions in the adaxial mesophyll differentiate the adaxial pallisade from the abaxial spongy mesophyll [54,55**-56]. Another dimension of control is provided by the relative rates of cell division across the mediolateral plane of the developing leaf. Cell divisions cease in the mid-region of the leaf slightly ahead of divisions at the margins. Maintaining this pattern of cell division is crucial for the development of a flat leaf surface, as highlighted by mutations in CINNAMATA (CIN) in Antirrhinum [55**,57]. In cin mutants, the leaf margin is ruffled as a result of the slower arrest of cell divisions at the leaf margins relative to the middle of the leaf. A comparable phenotype is seen in jaw-D mutants in Arabidopsis [58**]. In this case, overexpression of a miRNA results in the downregulation of target genes belonging to the TCP family of transcription factors, which are closely related to CIN in Antirrhinum [58**,59]. Ubiquitous expression of TCPs partially suppresses the jaw-D mutant phenotype, and the degree of rescue is dependent on the level of target gene expression. This suggests a dose-dependent interaction between the miRNA and its direct target. Interactions such as these may serve to fine-tune development.

Another gene that is involved in specifying final leaf shape is JAGGED (JAG) [60*,61*]. In jag mutants, the leaves are more predominantly serrated than those in the wildtype and the distal tips of floral organs are jagged, absent or reduced. Misexpression of JAG results in ectopic leaf-like lamina outgrowths in proximal leaf regions and on the stem. The development of blade on the petiole is reminiscent of the phenotypes of recessive blade on petiole (bop) mutants and of dominant mutations that affect the LEAFY PETIOLE gene [62-64]. In one sense, these mutations may represent the disruption of proximodistal specification, with transformation of proximal features to a more distal fate. The expression of a cell division marker is decreased in jag mutant leaves, however, indicating that JAG is required to prevent premature cell differentiation. In this respect, JAG and JAW may specify opposing but complementary functions in lamina outgrowth.

Conclusions

Early surgical experiments demonstrated that the separation of an initiating primordium from the SAM resulted in a radial leaf that has abaxial features [65,66]. Subsequently, the notion of interdependence of SAM function and leaf patterning has been reinforced by studies defining genes and genetic interactions that are essential to these processes. In simple leaves, this involves turning off the KNOX genes that maintain meristem indeterminacy. KNOX repression is maintained during leaf development by several different gene classes, including PHAN, BOP, and YABBY genes, all apparently acting in distinct pathways. As cells are recruited into the initiating leaf primordia, adaxial PHB family class III HD-ZIP genes repress abaxial KAN genes. Abaxial fate is either established or reinforced by KAN and miRNA-mediated repression of PHB family genes. Layered onto this information, YABBY genes contribute to abaxial fate as well as to mediolateral outgrowth of the leaf lamina. In addition to specification of dorsoventral fate, PHB and KAN family genes are required for correct patterning of polar vascular development. Furthermore, PHB family genes and regulation of the corresponding miRNA are required for SAM function, potentially reflecting polar central-peripheral development along the main axis of the shoot (65,66); see del Mar Castellano and Sablowski, this issue). Thus, this shared genetic pathway can be seen as a reflection of a common developmental theme.

Although positional information in the developing primordium depends on contact with or signalling from the SAM, the nature of this signal is still to be determined. Suggested candidates are regulators of adaxial fate and may be either a miRNA or a sterol ligand that targets the PHB gene family [15*,16,20**,24**]. Although some genes that specify dosoroventrality also affect other planes of leaf development, our understanding of the mechanisms that regulate proximodistal outgrowth and bilateral symmetry is limited. Flexibility in these pathways is likely to contribute to the diversity of leaf form.

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References and recommended reading

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

- of special interest
- - of outstanding interest


9. Vernoux T, Kronenberger J, Grandjean O, Laufs P, Traas J: A fate map of the PIN-FORMED1 and PIONOID genes, that are involved in polar auxin transport in the shoot apical meristem. Evidence is presented to support a role for these genes and for polar auxin transport in establishing the site of organ initiation and maintaining the regular phyllotactic pattern of organ initiation.


13. Matsumoto N, Okada K: A homeobox gene, /prs/ mutants are duplicate genes that are required for the recruitment of a specific group of founder cells comprising those in the lateral regions of the initiating leaf primordium. The cloning of these genes demonstrates their close relationship to PRR in Arabidopsis. Mutations in pri principally affect floral organ development but this work shows that stipules at the base of the leaf are absent from the pri mutant. Two points of interest result from this work. First, the expression patterns of pri/nos and the phenotypes of nos/prs mutants correlate with domain-specific founder-cell recruitment. Second, a comparison of the maize and Arabidopsis phenotypes can be interpreted in terms of classical ideas on dicot and monocot leaf morphology.


49. Kidner CA, Martienssen RA: Spatially restricted microRNA directs leaf polarity through ARGOUANT
The authors report the overexpression phenotype of AS2
35S::GUS to be a member of the YABBY gene class, closely related to FUS3 and YAB3. The phenotype of gram mutants, their expression pattern, their genetic interactions with phan and clonal analysis are used to derive a model for GRAM function. The authors conclude that GRAM has two functions that are specific to adaxial fate: repression of adaxial fate in the abaxial domain and non-cell-autonomous positive regulation of adaxial fate in the adaxial domain.


This paper describes the effects of reducing the expression of the tobacco orthologue of PHAN. Some of these phenotypic effects are comparable to the phan mutant phenotype in Antirrhinum whereas others, such as bladeless dorsoventrally patterned regions of the leaf and defects in upgrowth of leaf lamina from the adaxial leaf surface, are novel. The role of the tobacco NSPHAN in leaf patterning is addressed in terms of these phenotypes. The authors conclude that NSPHAN patterns dorsoventral and proximodistal development.


52. Comprehensive analysis of the expression pattern of PHAN in divergent compound-leaf species. The authors report a correlation between the expression domain of PHAN and the type of compound leaf. Potentially, regulation of the domain of PHAN expression represents a point of selection for evolutionary divergence in leaf patterning.


56. Novel and insightful interpretation of a mutation in Antirrhinum that affects leaf shape by disruption of growth along the margin of the leaf relative to that in the central region of the leaf.


60. Gene expression profiling is used to identify genes that are misexpressed in the jaw-D dominant activation-tagged mutant. Genes that are down-regulated in jaw-D are TCPs related to CIN in Antirrhinum. The jaw-D mutant phenotype is shown to correspond to misregulation of a miRNA that directly targets several TCP genes. This work correlates the function of a miRNA with target gene expression and addresses the phenotypic effects caused by misexpression of the miRNA as well as mutated versions of the target that are no longer sensitive to miRNA-mediated cleavage.


The authors of this paper and of [61] describe the phenotype and cloning of a gene, JAGGED (JAG), that is required for elaboration of final leaf shape. This gene appears to function by inhibiting differentiation in lateral
organs. Furthermore, ectopic expression of JAG results in ectopic leaf lamina and outgrowth of the cryptic bract at the base of flowers. This evidence demonstrates that JAG is sufficient to induce lamina proliferation. Also, the formation of bracts confirms that these are suppressed features of the Arabidopsis inflorescence.


See annotation for [60*].


