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Establishment of polarity in angiosperm lateral organs

John L. Bowman, Yuval Eshed and Stuart F. Baum

In seed plants, lateral organs such as leaves and floral organs are formed from the flanks of apical meristems. Therefore, they have an inherent positional relationship: organ primordia have an adaxial side next to the meristem, and an abaxial one away from the meristem. Surgical and genetic studies suggest that a morphogenetic gradient, which originates in the meristem, converts the inherent polarity into a functional one. Once an adaxial–abaxial axis of polarity is established within organ primordia, it provides cues for proper lamina growth and asymmetrical development. Several key participants in this process have been identified, and analyses of these genes support and refine our views of axis formation in plants. The complex relationships between and within various members of these plant-specific gene families (class III HD-ZIPs, YABBYs and KANADIs) might account for a substantial part of the morphological variation in lateral organs of seed plants.

The bodies of SEED PLANTS (see Glossary) are generally composed of two distinct classes of organ: roots and stems have overall radial symmetry, whereas LATERAL ORGANS, such as leaves and floral organs, display lateral growth and distinct asymmetrical development. The inherent relationship between the lateral organs and the apical meristem from which they develop is the basis for the definition of three primordial axes (Fig. 1). First, primordia have a proximal–distal axis that is clearly defined by their attached (proximal) and free (distal) ends. Asymmetry along the proximal–distal axis is inherent and can be seen in many leaves that have proximal petiole and distal blade. Second, primordia also have a left–right axis whose positional relationship to the meristem is unclear, but could be associated with PHYLLOTAXY. In some cases, for example the leaves of most *Eucalyptus* species, asymmetries are evident in the left–right axis. Third, primordia have an adaxial–abaxial axis (Fig. 1), with one side forming next to (*ad*, close to) and the other side away from (*ab*, away from) the meristem. In most angiosperm leaves, polarity in the adaxial–abaxial

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Glossary

Lateral organs: Leaves and putatively homologous organs, such as cotyledons, bracts, sepals, petals, stamens and carpels.
Phyllotaxy: The arrangement of lateral organs produced by the meristem.
Seed plants: Plants that propagate via seeds, namely the gymnosperms (conifers, cycads, ginkgo, gnetales, etc.) and angiosperms (flowering plants). It is thought that leaves in these taxa are homologous organs.
Zygomorphy: Asymmetry, as shown by flowers of *Antirrhinum* when compared with the actinomorphic arrangement of the components of *Arabidopsis* flowers.

axis is evident from differences in morphology and anatomy (Fig. 2). These asymmetries reflect the function of the two leaf surfaces: the adaxial, or top, surface is optimized for light capture and photosynthesis, and the abaxial, or bottom, surface for gas exchange. Differential and asymmetrical growth along these three axes results in the variety of morphological forms observed in the lateral organs, from the elaborate petals of orchids to the reduced leaves of acacias.

Lateral organs of angiosperms develop from cells recruited from the peripheral zone of the apical meristem [1]. It seems that lateral-organ anlagen are initially composed of a few cells with uniform histology and apparently homogeneous gene expression patterns. However, as lateral-organ primordia emerge from the flanks of the meristem, polarity is evident in terms of gene expression patterns and morphological differentiation. This suggests that adaxial–abaxial polarity is established in lateral organs during their transition from anlagen to morphologically distinct primordia. Although fully differentiated lateral organs have more than just two cell types, the establishment of polarity requires only the generation of two distinct cell populations. Subsequent interactions between the two populations could then generate a range of diverse of cell types.

Establishment of adaxial–abaxial polarity

In elegant experiments carried out nearly 50 years ago, Sussex [2,3] and others [4,5] made incisions to isolate incipient leaf primordia from the shoot apical meristem. Their observations provide a conceptual framework for the interactions between lateral-organ primordia and apical meristems. Where separation preceded primordium formation (e.g. at the stage of an anlage), the isolated primordia developed into radially symmetrical, apparently abaxialized organs. This developmental pattern suggests that the apical meristem could be the source of a signal required for proper abaxial/adaxial development of the leaf [2,3]. One interpretation of these data is that a signal(s) emanating from the apical meristem promotes adaxial identity in the cells of the lateral-organ primordium closest to the meristem. In the absence of such a signal (e.g. in cells of the lateral-organ primordium distant from the meristem), differentiation to an abaxial identity could be the default pattern. Sussex also showed that isolated leaf primordia that had already begun their morphological differentiation developed

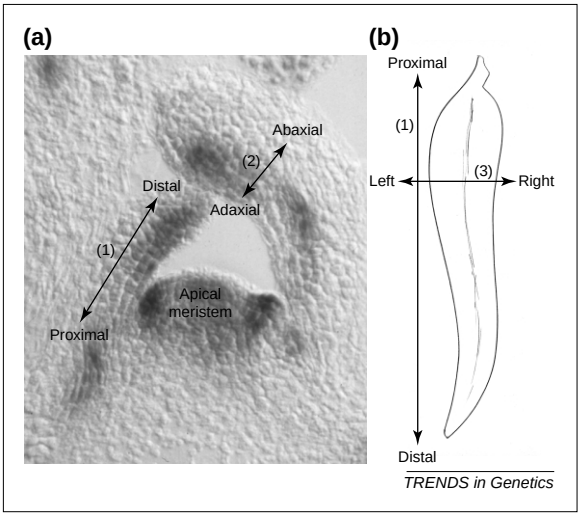

Fig. 1. The three axes of leaves and other lateral organs in seed plants, at the meristem (a) and in a leaf (b). On the proximal–distal axis (1), the distal end is the leaf tip and the proximal end is attached to the stem. On the adaxial–abaxial axis (2), the adaxial side is adjacent to, and the abaxial side distant from, the meristem. In most angiosperm leaves, polarity in the adaxial–abaxial axis is evident from differences in morphology and anatomy between upper and lower surfaces. Most leaves are symmetrical along the left–right axis (3), but some, such as this *Eucalyptus* leaf, display asymmetries along this axis.

autonomously into phenotypically normal leaves [2,3]. This indicates that abaxial and adaxial domains are established during the transition from leaf anlage to leaf primordium. Development of the isolated organs as radially symmetrical structures also implies that the establishment of adaxial–abaxial polarity is a requirement for proper laminar growth [2,3]. These classical experiments show that factors intrinsic and extrinsic to lateral-organ primordia contribute to the designation of cells as adaxial or abaxial.

Several recent studies in *Arabidopsis* and *Antirrhinum* have provided insight into the molecular genetic mechanisms by which polarity is established in lateral organs, and these are the focus of this paper.

The view from above: establishment of adaxial identity

A landmark study by Waites and Hudson [6] gave the first glimpse of the genetic machinery that controls adaxial–abaxial polarity. The radially symmetrical leaves of *Antirrhinum phantastica* (*phan*) mutants were interpreted as being abaxialized, on the basis of epidermal cell morphologies and the arrangement of vascular tissues (Fig. 3) [6]. When plants with temperature-sensitive alleles of *phan* are grown in intermediate conditions, outgrowths of tissue are produced along ectopic adaxial–abaxial boundaries, leading Waites and Hudson [6] to propose that juxtaposition of cells destined to be abaxial and adaxial is required for normal lamina outgrowth. This implies that signalling between the two distinct cell types is necessary to induce growth, in line with the proposal of Sussex [2,3] that proper establishment of polarity is required for lamina development. In the absence of any adaxial tissue, the abaxialized organs of *phan* mutants develop as filamentous, radially

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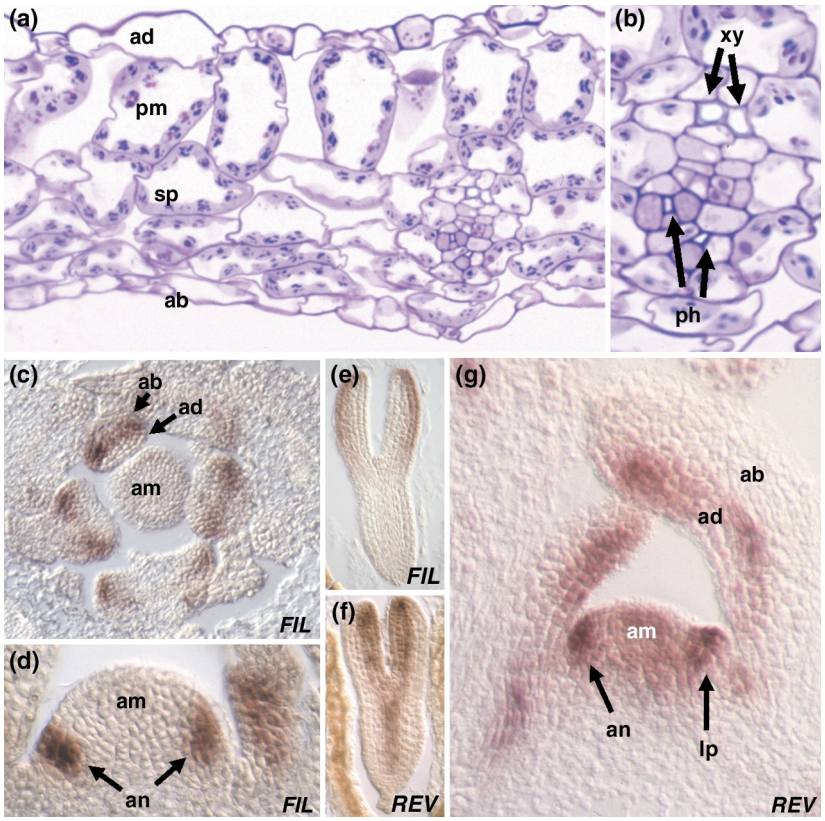

Fig. 2. Positional relationship between lateral-organ primordia and the apical meristem in seed plants. (a,b) Lateral organs, such as this *Arabidopsis* leaf, are often curved over the apical meristem early in their development. As the leaf expands, however, the adaxial surface becomes the top of the fully differentiated leaf and the abaxial surface the bottom. Polarity is evident in the distribution of cell types within the epidermis and also the mesophyll, where the palisade mesophyll (pm) and xylem (xy) are located adaxially and the spongy mesophyll (sp) and phloem (ph) abaxially. (c) Because lateral organs develop from the flanks of apical meristems (am), there is a fundamental positional relationship between lateral-organ primordia and the meristems from which they are derived. The adaxial (ad) side of the primordium is directly adjacent to the cells of the meristem, whereas the abaxial (ab) region of a primordium is at a distance from the meristem. Experiments in which an incipient leaf primordium or anlage (an) was separated by an incision from the shoot apical meristem suggested that the apical meristem might be the source of a signal required for proper adaxial/abaxial development of the leaf [2,3]. The leaf abaxial domain is marked by *FIL* (c), which is initially expressed throughout anlagen (d) but as primordia emerge, becomes restricted to the abaxial domains of developing leaves [18,19]. (g) By contrast, *REV* is expressed in the central zone of the apical meristem (am), throughout leaf anlagen (an), and becomes restricted to the adaxial regions of developing leaves. Once leaf primordia (lp) have become distinct from the apical meristem, the domains of *FIL* and *REV* expression are complementary. This is most easily seen in the embryos (e,f) where *FIL* expression is restricted to the abaxial regions of the cotyledons, whereas *REV* expression is localized to the adaxial regions of the cotyledons, the apical meristem and the developing vasculature.

symmetrical organs (Fig. 3b), as did isolated primordia in the incision experiments [2,3].

The interpretation that *phan* mutant leaves are abaxialized suggests that *Phan* normally promotes adaxial identity. *Phan* encodes a transcription factor of the MYB family, whose mRNA is expressed throughout lateral-organ anlagen and primordia implying post-transcriptional control in its promotion of adaxial cell fates [7]. The activity of *Phan* is at least partially duplicated by that of *Handlebars* (*Hb*), and the double mutant shows abaxialized, radialized cotyledons and leaves [8]. Strikingly, *phan hb* seedlings lack a functional apical meristem, suggesting a role for these genes in meristem formation or maintenance [8]. In the case of *Phan*, this activity is non-cell autonomous, indicating that there is two-way communication between the lateral organs and the meristem.

Signal perception

In contrast to *phan* mutations of *Antirrhinum*, semidominant *phabulosa* (e.g. *phb-1d*) and *phavoluta* (e.g. *phv-1d*) mutations of *Arabidopsis* act in a dose-dependent manner to cause an adaxialization of lateral organs (Fig. 3c) [9,10]. Adaxialized lateral organs of *phb1-d* homozygotes are filamentous and radially symmetrical [9], supporting the view that a juxtaposition of adaxial and abaxial domains is required for lamina outgrowth. *PHB* and *PHV* encode members of the plant-specific homeodomain/leucine zipper (HD-ZIP) family of proteins (Table 1) [10]. Strikingly, in class III HD-ZIP proteins, such as PHB and PHV, and the closely related protein encoded by the *REVOLUTA* (*REV*) gene, a domain of about 200 amino acids carboxyl to the HD-ZIP domain has a similar sequence to that of mammalian sterol/lipid-binding proteins [10–12]. The semidominant *phb* and *phv* lesions lie within the putative sterol/lipid-binding domain, and can be considered gain-of-function mutations [10]. This interpretation is supported by the observation that ectopic expression of wild-type PHB is insufficient to alter cell fate, which is consistent with limitation of the protein's activity by availability of a ligand [10]. This led McConnell *et al.* [10] to propose that the semidominant *phb* mutations in the putative sterol/lipid-binding domain render the protein ligand independent and constitutively active.

PHB is expressed throughout lateral-organ anlagen, but its expression becomes restricted to adaxial regions as the lateral-organ primordia become morphologically apparent [10]. McConnell *et al.* [10] raise the possibility that *PHB* encodes a receptor for, or its activity is dependent upon, the proposed signal that emanates from the apical meristem and establishes adaxial fates in lateral-organ primordia. In this scenario, PHB is 'activated' when it binds a ligand whose concentration is determined by proximity to the meristem. The detection of *PHB* mRNA in a gradient in lateral-organ primordia, with the highest concentration in the most adaxial regions, suggests that its action might be dose dependent. In addition, *PHB* activity might be maintained by a positive feedback loop, as *PHB* mRNA is expressed in much higher concentration in *phb-1d* mutants than in wild type [9]. Such a feedback loop, if accompanied by capacity for positive regulation of ligand synthesis or stability, or both, would enable maintenance of a new reference point for adaxial position. The reference point could function as the developing leaf becomes independent of its original source of positional information [10]. This arrangement would allow the autonomous development of primordia isolated from the apical meristem after becoming morphologically distinct from it [2,3]. *PHB* is also expressed at high concentrations in the central domain of the apical meristem, and in groups of cells that lie between the central domain and developing

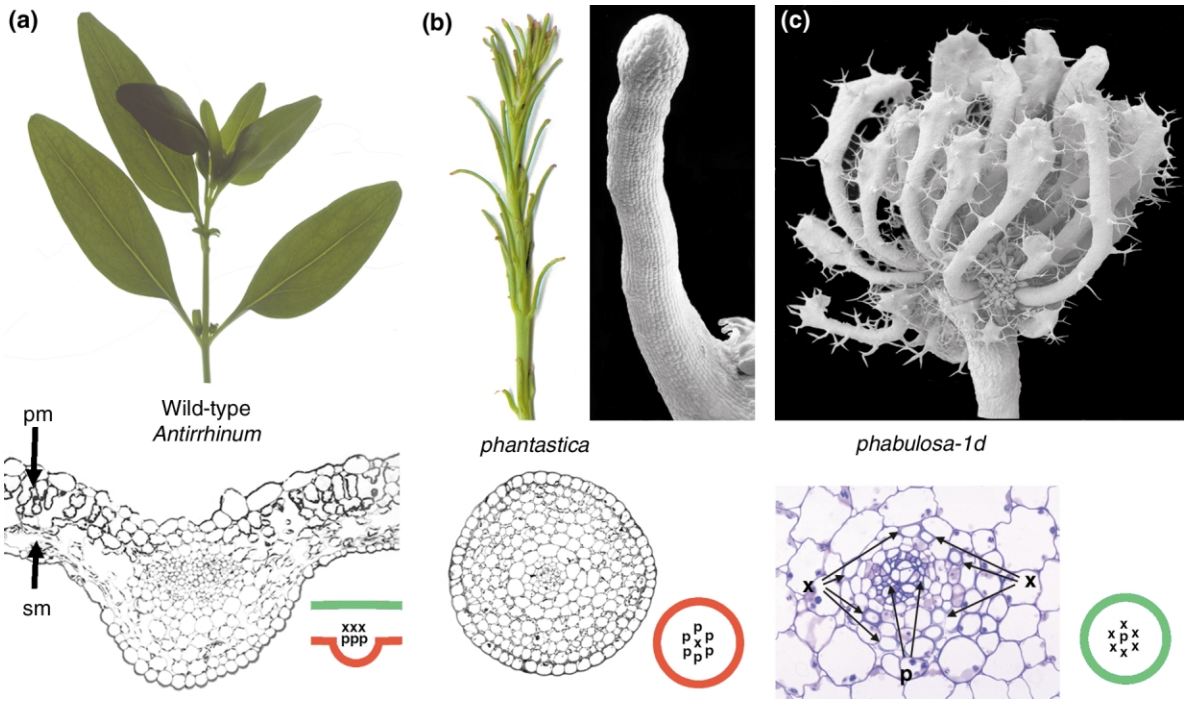

Fig. 3. Specification of adaxial identity in the leaves of seed plants. In *Antirrhinum* leaves (a), as in *Arabidopsis* leaves, polarity is manifested by development of an adaxial palisade mesophyll and an abaxial spongy mesophyll. In many genera, including *Arabidopsis* and *Antirrhinum*, leaf vasculature also has a characteristic polarity, with xylem (x) towards the adaxial side and phloem (p) towards the abaxial side (green, adaxial; red, abaxial). Leaves of loss-of-function *Antirrhinum phan* mutants can be said to be abaxialized on the basis of their epidermal cellular morphology (b) [6]. In addition, radial *phan* leaves have a central xylem array surrounded by phloem elements (b). The phenotype of gain-of-function *Arabidopsis phb-1d* mutants is the converse of that of *phan* mutants [9]. The radial leaves of *phb-1d* homozygotes can be described as adaxialized on the basis of epidermal cell features such as colour and an abundance of trichomes (c). Strikingly, the arrangement of vasculature in some leaves of *phb-1d* mutants is the opposite of that in *phan*: there is a central phloem element surrounded by strands of xylem. Lateral organs in both mutants lack blade outgrowth (b,c), supporting the idea that lamina formation depends on a juxtaposition of adaxial and abaxial fates [6]. Both genes promote adaxial fates, but only *PHB* expression is localized to the adaxial regions of developing lateral organs [7,10]. pm, palisade mesophyll; sm, spongy mesophyll. (a) and (b) courtesy of Andrew Hudson.

lateral-organ primordia [10]. It is tempting to speculate that these expression patterns reflect communication pathways between cells of the apical meristem and the lateral organs, and mark the functional concentration of the putative ligand.

A striking feature of *phb-1d* mutants is the development of axillary meristems, which normally are found only adaxially in the leaf axil, around the entire circumference at the base of the adaxialized leaves. The presence of these meristems suggests that their formation is associated with adaxial cell fate [9]. Conversely, the absence of axillary meristems in loss-of-function *rev* mutants [13] could be interpreted as a partial loss of adaxial identity. As *REV* encodes a closely related HD-ZIP family member [14] and is expressed adaxially in lateral organs [15], it is likely that *REV* acts with *PHB* and *PHV* to promote adaxial identity in lateral organs. This is consistent with the presence of a link between the development of axillary meristems and the differentiation of adaxial leaf tissues [16,17].

The view from below: establishment of abaxial identity

The meristem-based signalling model predicts concentration-dependent suppression of abaxial promoting factors by interpreters of the signal. In the absence of the signal, for example when the leaf anlage is separated from the meristem, leaves develop with uniform abaxial characteristics, implying that abaxial-promoting factors remain active throughout leaf development. Members of the YABBY and KANADI gene families are candidate abaxial promoting factors because they have been implicated in the promotion of abaxial cell fates, as observed in loss-of function or gain-of-function mutant alleles, or both (Fig. 4) [18–23].

A common theme among the YABBYs and KANADIs, as with PHB and its closely related genes, is one of redundancy (Table 1). The *Arabidopsis* YABBY gene family comprises six members that probably encode transcriptional regulators [18–20,23,24]. Each member of the family is expressed in a polar manner in one or more above-ground lateral organs, and in every asymmetrical above-ground lateral organ at least one family member is expressed. Three members (*FILAMENTOUS FLOWER* [*FIL*], *YABBY2* [*YAB2*], *YAB3*) are expressed in a polar manner in all lateral organs produced by the apical and flower meristems [18,19]. By contrast, *CRABS CLAW* (*CRC*) [20,24,25] and *INNER NO OUTER* (*INO*) [23] are more specialized, because their expression is restricted to the carpels and nectaries or outer integuments, respectively. The KANADI genes, members of the GARP gene family, encode transcriptional regulators. Although the GARP gene family has many members, only three closely related

Table 1. Phenotypes of *Arabidopsis* gene mutations

Arabidopsis gene	Family of protein	Expression in lateral organs	Loss-of-function phenotype[a]	Gain-of-function phenotype	Refs
PHABULOSA	Class III HD-ZIP	Adaxial	Unknown	Adaxialized	[9,10]
PHAVOLUTA	Class III HD-ZIP	Adaxial	Unknown	Adaxialized	[10]
REVOLUTA	Class III HD-ZIP	Adaxial	Abaxialized?	Unknown	[13–15,21]
FILAMENTOUS FLOWER	YABBY	Abaxial	Adaxialized?	Abaxialized	[18,19]
YABBY2	YABBY	Abaxial	Unknown	Unknown	[18]
YABBY3	YABBY	Abaxial	Adaxialized?	Abaxialized	[18]
CRABS CLAW	YABBY	Abaxial	Adaxialized	Abaxialized	[20,24,25]
INNER NO OUTER	YABBY	Abaxial	Adaxialized	Unknown	[23]
KANADI1	GARP	Abaxial	Adaxialized	Abaxialized	[20–22]
KANADI2	GARP	Unknown	Adaxialized	Abaxialized	[21]
KANADI3	GARP	Unknown	Unknown	Abaxialized	[21]
ARGONAUTE	eIF2C?	Uniform	Abaxialized?	Adaxialized?	[16,32]
PINHEAD	eIF2C?	Adaxial	Abaxialized?	Unknown	[16,33,34]
PETAL LOSS	Unknown	Unknown	Petal orientation reversed	Unknown	[37]
ASYMMETRIC LEAVES1	MYB	Uniform	Not clear	Unknown	[42–45]

[a]Loss-of-function phenotype as deduced from either a single mutant, or a multiple mutant combination.

members (*KANADI1* [*KAN1*], *KAN2* and *KAN3*) have been implicated in promoting abaxial cell fates [20–22]. Both *KAN1* and *KAN2* promote abaxial fates in all lateral organs produced by apical and flower meristems and in the ovule outer integument [21].

Loss-of-function alleles *kanadi1* (*kan1*) were originally identified in a screen for genetic enhancers of *crabs claw* (*crc*) mutations [20]. *CRC* is the only member of the YABBY gene family that is expressed in the medial regions of the carpels [24], yet *crc* single mutants show no conspicuous loss of polarity in the carpels [25]. The striking mirror-image duplication of adaxial tissues in abaxial positions of *crc kanadi* double mutants indicates that *KANADI* and *CRC* promote abaxial identity in a non-biochemically redundant manner [20]. Whereas *kanadi1* loss-of-function mutants do not show a striking phenotype [20–22], all lateral organs are adaxialized when the activity of *KANADI2* is also compromised [21]. All *kan1 kan2* floral organs are severely adaxialized, but leaves of the double mutant exhibit a novel phenotype with ectopic, blade-like outgrowths developing from their abaxial surfaces. Gain-of-function alleles of *KAN1*, *KAN2* and *KAN3* result in radialized organs with abaxial tissues differentiating in adaxial positions [21,22]. In accord with a proposed role in promoting abaxial identity, *KAN1* is expressed abaxially in all lateral organs [22].

As with the KANADIs, ectopic expression of each of the YABBY gene family members tested promotes the differentiation of abaxial tissues in adaxial positions [18–20]. Transcripts of at least some of the YABBY gene family members, such as *FIL*, are detectable throughout lateral-organ anlagen, and then become restricted to the abaxial domains of primordia as they emerge from the flanks of the apical meristem (Fig. 2) [18,19]. YABBY genes such as *FIL* and *CRC* are activated normally in *kan1 kan2* lateral-organ anlagen, but as the primordia emerge YABBY gene expression is not properly localized to the abaxial domains of the developing organs [21]. So, KANADI activity is not necessary for the initial activation of YABBY gene expression, but is required for its proper abaxial localization. Furthermore, *YAB3* activity is sufficient for differentiation of abaxial cell fates in a *kan1 kan2* background, suggesting that YABBY function does not require KANADI activity [21], and that YABBY function might, at least in part, be downstream of KANADI function.

Genetic redundancy has made phenotypes of loss-of-function alleles of YABBY gene family members less informative [18,26–28]. However, loss of polar expression of YABBY genes in *fil yab3* double mutants results in a loss of polar differentiation of cell types in lateral organs [18]. The radial nature of these organs is again consistent with the hypothesis that juxtaposition of abaxial and adaxial cell fates is required for lamina outgrowth [6]. Both loss- and gain-of-function alleles of YABBY and KANADI gene family members implicate these genes as promoters of abaxial cell fates. However, the dramatically different appearance of *kan1 kan2* plants when compared with *fil yab3* plants suggests that YABBYs and KANADIs act on both distinct and common targets.

Top and bottom meet

A molecular explanation for the framework of interactions between lateral organs and the meristems from which they develop, established nearly 50 years ago [2,3], is beginning to emerge (Fig. 5). Both adaxial and abaxial promoting factors are initially expressed throughout lateral-organ anlagen [10,18,19,22]. Subsequently, their expression patterns become restricted to their respective complementary domains, as lateral-organ primordia emerge from the flanks of the apical meristem [10,18,19,22], consistent with the temporal acquisition of polarity as determined by surgical experiments [2,3]. One attractive hypothesis is that once PHB is 'activated' by signals derived from the apical meristem, KANADI and YABBY gene expression is repressed in the cells within the anlagen that are closest to the meristem. These cells, which are destined to give rise to the adaxial portion of the lateral organ, would have a higher level of 'activated' PHB/PHV because of their proximity to the source of the signal. *FIL* expression is nearly undetectable in *phb-1d* homozygotes [18], supporting the idea that an 'activated' version of PHB [10] is sufficient to repress *FIL* expression. Hence, the 'default' state of abaxial identity would be a consequence of KANADI/YABBY activation throughout lateral-organ anlagen without subsequent downregulation by factors promoting adaxial identity (e.g. 'activated' PHB/PHV). Likewise, constitutive ectopic KANADI activity is sufficient to promote abaxial identity, suggesting it might be able to regulate PHB/PHV negatively. Once established, signalling between the two domains, the nature of which is unknown, leads to lamina outgrowth via localized cell divisions.

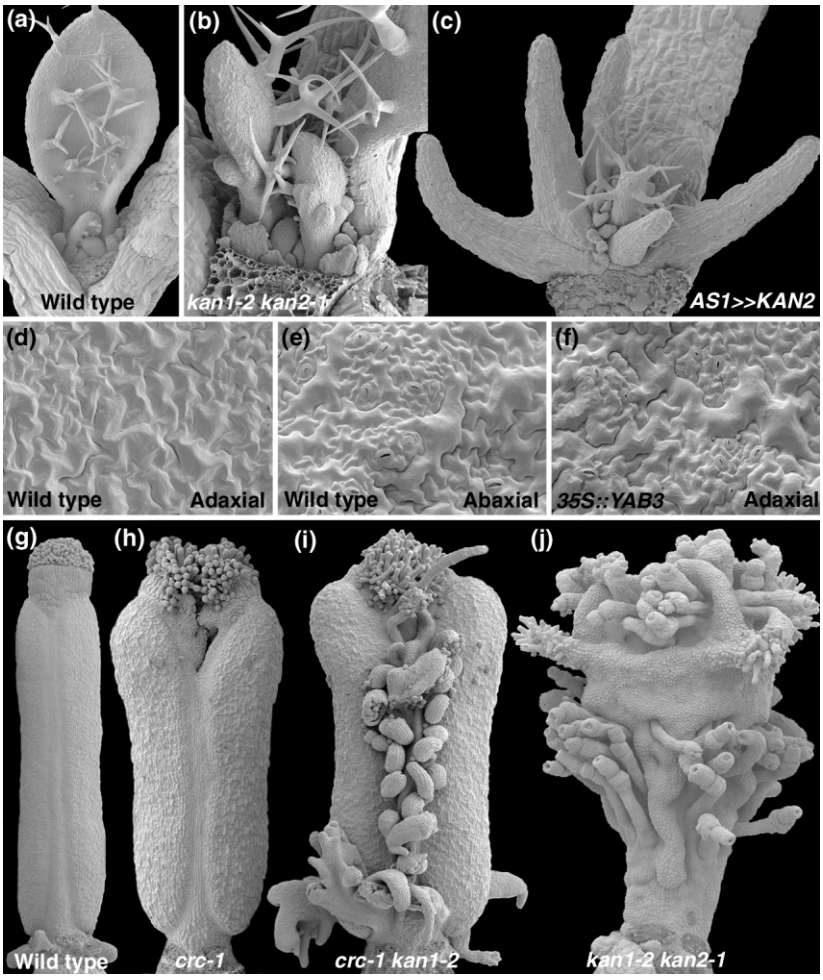

Fig. 4. Specification of abaxial identity: loss- and gain-of-function phenotypes of the YABBY and KANADI genes in *Arabidopsis*. Wild-type leaves (a) exhibit lamina expansion along the left–right axis. On the first-formed leaves of *Arabidopsis*, trichomes are confined to the adaxial surface (a). On the later-formed leaves (e.g. after the fifth leaf) the density of trichomes on the abaxial surface gradually increases. When the activities of *KAN1* and *KAN2* are missing (b), leaves are only partially adaxialized: first-formed leaves fail to develop trichomes on the abaxial surfaces, and lamina expansion is limited [21]. An additional feature of *kan1 kan2* leaves is formation on their abaxial surfaces only of ectopic outgrowths, which might result from ectopic adaxial–abaxial boundaries [21]. Conversely, ectopic expression of *KAN1* or *KAN2* throughout leaf primordia, as occurs in *AS1>>KAN2* plants (c), causes the leaves to be abaxialized: the leaves are radial and those first formed fail to develop trichomes [21,22]. Later-formed leaves do differentiate trichomes, indicating that heteroblastic leaf morphology changes are maintained in these plants. Ectopic expression of members of the YABBY gene family in the adaxial regions of lateral organs is sufficient for adaxial epidermal cells to differentiate with abaxial fates [18–20]. The epidermal cells of adaxial (d) and abaxial (e) surfaces of cotyledons display distinct morphologies. Constitutive expression of *YAB3*, as in *35S::YAB3* plants, is sufficient to cause the adaxial epidermis of cotyledons to assume an abaxial fate (f). Carpels of *crc* (the founder YABBY gene) single mutants (h) do not display obvious polarity defects [25], but carpels of *crc kanadi1* double mutants (i) have adaxial (inside) tissues duplicated in abaxial (outside) positions [20] – compare with wild type (g). In *crc kanadi1* carpels, adaxial tissues such as septum tissue and placentae-bearing ovules occur in both adaxial and abaxial positions. The carpels of *kan1 kan2* double mutants (j) consist entirely of adaxial tissues, such as septum and placentae-bearing ovules [21].

The formation of leaf lamina in angiosperms is similar in many respects to the development of laminar structures in metazoans. During *Drosophila* wing development, the juxtaposition of dorsal and ventral cells results in the secretion of factors at the boundary that act from a distance to specify cell fates in the dorsal and ventral compartments [29–31]. The extent to which the boundaries between abaxial and adaxial domains act as organizing centres in the lateral organs of plants remains to be determined.

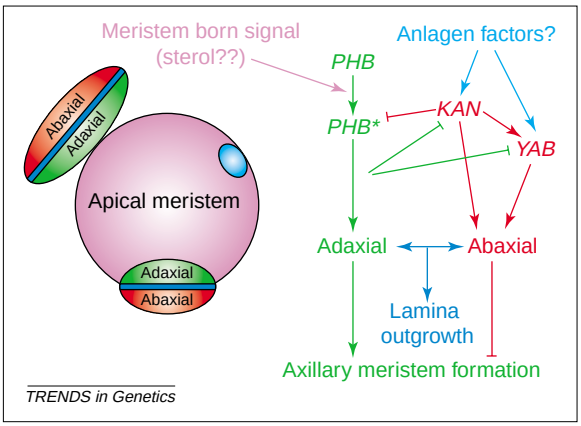

Fig. 5. Signalling in the establishment of cell fates in lateral organs of seed plants. The emerging picture from classical and molecular genetic analyses is that as incipient lateral-organ primordia develop from the flanks of the shoot apical meristem, factors both intrinsic and extrinsic to the organ primordia (anlagen and leaf primordium) contribute to the specification of cells as adaxial (green) or abaxial (red). The apical meristem (purple) itself probably provides a signal(s) that promotes adaxial cell fate [2,3], the perception of which might be mediated through PHB (activated PHB protein is shown as PHB*) [10]. Abaxial cell fate might be a 'default' in the absence of signal, for example when the lateral-organ primordia are separated from the apical meristem [2,3]. This 'default' state could be the result of the failure to repress genes that promote abaxial identity (e.g. YABBY and KANADI genes) and are initially activated throughout the anlagen (light blue) [18,19,22]. On the basis of epistasis experiments, it is thought that KANADI activity might mediate between PHB and YABBY activities. However, the precise relationships between these pathways are unclear. *Phan* is required for adaxial identity in *Antirrhinum* [6], but its role in other species is less clear [42–45]. Subsequent interactions between the juxtaposed adaxial and abaxial domains, mediated by relative levels of KANADI and YABBY activities, are required for lamina outgrowth (dark blue) [6]. Several key questions remain. If PHB is activated by a ligand, how is this activation restricted in space and time, and what is the ultimate source and biochemical nature of the ligand? For example, *PHB*, *FIL* and *KAN1* are all expressed in the leaf anlagen, but their expression becomes confined to mutually exclusive domains as the primordia form. So, if the ligand source is the meristem, why doesn't activated *PHB* downregulate *FIL* and *KAN1* in the anlagen? It remains to be determined whether the KANADI or YABBY genes are direct targets for suppression by 'activated' PHB/PHV, and whether *PHB* and *PHV* too are direct targets of the abaxial promoting factors. The antagonistic regulation could be indirect, with, for example, KANADI activity regulating the stability of the putative PHB ligand rather than PHB itself. In any event, as YABBY gene function might be downstream of KANADI activity, KANADI expression is perhaps more likely to reflect a direct response to levels of 'activated' PHB. Finally, the communication pathways between the abaxial and adaxial domains required for lamina expansion remain enigmatic. In *Nicotiana* leaves, the non-cell autonomous action of the *LAM1* gene product in lamina development implies that *LAM1* has a role in this process [46].

Other players

Several additional genes have been implicated in the establishment or maintenance of polarity in lateral organs of angiosperms. For example, *argonaute1* (*ago1*) and *pinhead* (*pnh*) mutants of *Arabidopsis* produce lateral organs that could be interpreted as partially abaxialized [16,32–34]. As *AGO1* and *PNH* encode similar proteins and are expressed in partially overlapping patterns [16,32,35], it is likely that they act redundantly. Although *AGO1* and *PNH* display sequence similarity to translation initiation factors and *AGO1* has been implicated in gene silencing [35,36], their *in vivo* biochemical function is unclear. One attractive speculation is that they might

Fig. 6. Establishment of zygomorphy in *Antirrhinum* flowers. The dorsal side of the flower is that closest to the plant apex (the top of the flower) and the ventral side is that furthest away from the apex (the bottom of the flower). The relative position of the apical meristem is indicated by the white dot. Wild-type *Antirrhinum* flowers have two asymmetrical dorsal petals (d), two asymmetrical lateral petals (l) and one bilaterally symmetrical ventral petal (v). In *cyc* mutants the dorsal and lateral petals become partially or completely ventralized, but remain asymmetrical, whereas in *dich* mutants the dorsal petals are no longer asymmetrical. All petals are ventralized and bilaterally symmetrical in *cyc dich* double mutants. In addition, petal number is increased from five to six in the double mutant. Photographs courtesy of Enrico Coen.

regulate the stability or translatability of mRNAs of other genes that play a part in establishing lateral-organ polarity.

PETAL LOSS (*PTL*) has a role in establishing the proper orientation of lateral organs [37]. A fraction of the petals in *ptl* mutants develop in a reverse orientation, 180° from normal, with adaxial cell types now occurring abaxially, and vice versa [37]. Mutations in *PISTILLATA* or *APETALA3* enhance this phenotype such that nearly all of the second-whorl organs arise in a reverse orientation in *ptl pi* and *ptl ap3* double mutants [37]. The defect in *ptl* mutants appears to be upstream of the establishment of polar YABBY gene expression, because *FIL* expression correlates with abaxial cell types [18]. Griffith *et al*. [37] argue that *PTL* might have a role in the perception of, or response to, signals emanating from other regions of the floral meristem that are required to establish proper orientation of petal primordia. One feature that *ptl* mutants have in common with other mutants in which polarity of lateral organs is disrupted (e.g. *phb-1d*, *pnh*) [9,33] is the formation of tubular or trumpet-shaped organs. The formation of these organs probably results from alterations in the positioning of the adaxial–abaxial boundaries, as evidenced by YABBY gene expression [18]. Such positional variation in boundary establishment could account for the development of peltate leaves in many angiosperm species.

Establishment of dorsal–ventral and left–right axes in *Antirrhinum* flowers

ZYGOMORPHIC flowers can exhibit asymmetries in the arrangement of lateral organs around the floral axis and within individual organs. For example, petal and stamen morphology is asymmetrical along the dorsal–ventral axis of the *Antirrhinum* flower: the two dorsal (upper) petals, the two lateral petals and the ventral (lower) petal all have different morphologies (see Fig. 6 for positional definitions) [38]. Furthermore, asymmetry exists within individual organs along the left–right axis in the dorsal and lateral petals.

Mutations in either *cycloidea* (*cyc*) or *dichotoma* (*dich*) result in semipeloric flowers, whereas *cyc dich* double mutants have completely peloric (radially symmetrical) flowers in which the lobes of all petals resemble those of the lower petals of wild type (Fig. 6). These observations suggest that the genes act to promote dorsal identity [38,39]. Although *cyc* and *dich* flowers are both semipeloric, their phenotypes differ in detail. In *cyc* flowers, the dorsal petals have a combination of dorsal and lateral characteristics and the lateral petals are ventralized [38], whereas in *dich* flowers the dorsal petals are symmetrical along their left–right axes. In accordance with the observation that the genes' action is partially redundant, *cyc* and *dich* encode closely related members of the TCP family of transcription factors [38,39]. Consistent with *cyc* and *dich* establishing dorsal fates throughout the flower, both genes are expressed in the dorsal regions of flower meristems [38,39]. As the floral-organ primordia emerge, the expression of *cyc* is restricted to the adaxial regions of the two upper petals and stamen, whereas *dich* expression is even more restricted, being confined to the adaxial region of the dorsal portions of the upper petal lobes. The differences between *cyc* and *dich* expression during this later phase are correlated with the proposed functions of the genes; that is, *cyc* confers dorsal identity to the upper petals and influences the morphology of the dorsal regions of the lateral petal lobes [38], whereas *dich* promotes asymmetrical growth and differentiation within the upper petals [39]. Although the early expression phases of *cyc* and *dich* confer dorsal identity on regions of the entire flower and are functionally redundant, the mutant phenotypes and the late expression patterns indicate that the two genes also have unique functions at a later stage in development [39]. Thus, *cyc* and *dich* promote asymmetry within meristems and within lateral organs. One of the roles that *cyc* and *dich* share is to repress a gene, *divaricata* (*div*), that promotes ventral petal identity, and thus in *cyc dich* double mutants all petals acquire ventral identity [40]. In the absence of both dorsalizing and ventralizing factors, such as in *cyc dich div* triple mutants, all petals have lateral identity, suggesting this could be a 'default' state [40].

Luo *et al*. [39] point out that the two phases of expression of the two closely related genes indicate that lateral-organ asymmetry can arise through a series of steps, progressively increasing throughout development; in this case, they suggest, the developmental progression might reflect a sequence of evolutionary events. An example of how such genes might play a part in the origin and loss of zygomorphy over evolutionary time is the loss of *cyc* function by epigenetic means in a peloric form of *Linaria vulgaris*, first described by Linnaeus more than 250 years ago [41].

Epilogue

The enormous variation in morphology among the lateral organs of angiosperms results largely from

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differential growth along three axes: adaxial–abaxial, proximal–distal and left–right. Is the development of these axes linked in any way? Whenever the establishment of the adaxial–abaxial axis is disrupted, the proximal–distal axis of the lateral organs is also altered. For example, lateral organs in *phan*, *phb*, *kan1 kan2* and *fil yab3* plants are significantly shorter than those of wild type [6,8,17,20], suggesting that the proper establishment of the adaxial–abaxial axis and that of the proximal–distal axis might be interdependent. In addition, there are indications that some genetic machinery is shared in the establishment of these axes; for example, *dich* affects the adaxial–abaxial axis of the flower and the left–right axis of floral organs, and *hb* affects the adaxial–abaxial axis of leaves and the left–right axis of floral organs [8,39]. The conspicuous polarity of lateral organs contrasts sharply with the radial symmetry of the evolutionarily more-ancient stems; it is tempting to speculate that the evolution of adaxial–abaxial polarity in lateral organs has allowed the generation of the diverse laminar structures seen in vascular plants. It might be that taxon-specific differences in the complex interactions between and within various members of the three plant-specific gene families (class III HD-ZIPs, YABBYs and KANADIs) that establish adaxial–abaxial polarity account for a substantial part of the morphological variation in lateral organs of seed plants.

Finally, it is worth noting that mutations in genes that control floral meristem asymmetry or direct establishment of adaxial–abaxial polarity often result in changes not only in organ symmetry, but also in organ initiation. This implies that these genes, or the generation of asymmetry itself, might have a role influencing the activity of the meristem from which the lateral organs derive [6,8,38,39].

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