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Molecular cartography of leaf development — role of transcription factors

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Organ elaboration in plants occurs almost exclusively by an increase in cell number and size. Leaves, the planar lateral appendages of plants, are no exception. Forward and reverse genetic approaches have identified several genes whose role in leaf morphogenesis has been inferred from their primary effect on cell number and size, thereby distinguishing them as either promoters or inhibitors of cell proliferation and expansion. While such classification is useful in studying size control, a similar link between genes and shape generation is poorly understood. Computational modelling can provide a conceptual framework to re-evaluate the known genetic information and assign specific morphogenetic roles to the transcription factor-encoding genes. Here we discuss recent advances in our understanding of the roles of transcription factors in the planar growth of leaf lamina in two orthogonal dimensions.

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Introduction

Leaves evolved from ancestral branching systems multiple times among the vascular plant lineages [1]. Angiosperm leaves share a common phylogenetic origin, which is reflected in the conserved sequence of developmental events leading up to the initiation of a rod-shaped bulge called primordium on the flanks of the shoot apical meristem (SAM), having distinct upper and lower sides [2]. Yet, angiosperm leaves are distinguished by their tremendous architectural diversity in the final size, shape, and complexity [2,3]. This is attributed to the variation in the post-initiation growth patterns along the 'base-to-tip' and the 'middle-to-margin' axes [2,4]. The simple leaves of the winter annual Arabidopsis, the lobed leaves of lettuce, the compound leaves of tomato and even the intricately-designed insect-trapping leaves of the bladderwort arise by the modification of the growth parameters, such as growth duration and growth direction, along these two perpendicular axes.

Growth in a leaf primordium occurs by an increase in cell number and cell size, which is regulated in space and time primarily through the activities of growth-promoting and growth-repressing transcription factors, sometimes themselves expressed in gradients, as a result of their transcriptional regulation and/or post-transcriptional control by the upstream regulatory microRNAs. Transcriptional output is often a modulation of cellular properties and response to hormones, which act as intercellular messengers. These molecular players lay down the blueprint for growth patterns in space and time. The challenge is to decipher the connection between proximal effects of gene activities and its ultimate manifestation on organ growth. The current review highlights the attempts made over the past decade in joining the dots by making use of the insights gleaned from computational models.

How do leaves grow?

A leaf is characterized by a flat, bifacial lamina with a stalk-like base. Laminar growth proceeds by division and expansion of cells thought to arise from a short-lived meristematic activity localized at the base of the primordium [5]. Initially, active proliferation occurs throughout the primordium but is quickly limited to the base by the sudden appearance of an arrested zone at the distal end [6–9]. Cells distal to the proliferation-differentiation boundary, also called arrest front, contribute to growth by undergoing expansion and maturation, whereas cells proximal to the arrest front continue to increase in number till the complete cessation of proliferating activity. Thereafter, growth is propelled by post-mitotic cell expansion alone till the mature size is achieved. This linear gradient of cell division and expansion along the proximo-distal axis, though common to most monocot and dicot model species is, however, not universal and several other types of growth gradient exist, at least among the eudicot species (Box 1, Figure 3) [10,11••]. Nevertheless, whereas the link between the base-to-tip growth gradient and final leaf size has been well-studied [12,13], how the

Box 1 Divergent growth polarity patterns and their evolution

Leaves of all model plants studied so far display a common 'basipetal' pattern of progression of the cell-proliferation arrest from the distal tip towards the proximal base [6,7,15,76]. Even though such a growth pattern was initially assumed to be universal in plants, a broader study using a large number of dicot species revealed three other types of proximo-distal gradient: firstly, *acropetal* pattern where the arrest front progresses in the base-to-tip direction, secondly, *bi-directional* pattern where two simultaneous arrest fronts progress from base-to-tip and tip-to-base and thirdly, *diffuse* growth where there is no progression of the growth arrest and cells throughout the lamina proliferate and differentiate simultaneously (Figure 3) [11••]. These divergent patterns are also closely associated with the expression of growth promoting transcription factors (such as the GRFs) and growth repressing genes (such as miR396) (Figure 3) [11••]. A phylogenetic analysis showed that some of these growth patterns evolved independently in several plant lineages [32•]. It has been speculated that the direction of the growth pattern is under the control of a conserved gene regulatory module, which was co-opted to novel expression domains during evolution to create diverse growth gradients, either by mutation in the proximal-regulatory genes (e.g. *CIN-TCP*s that regulate miR396 and hence *GRF* expression) or in the regulatory sequences of the individual genes (e.g. *GRF*s) [11••].

patterns of cell division and expansion generate species-specific shapes is still an enigma.

Classical studies tracking laminar growth by observing the movement of artificial landmarks during development or by clonal analysis in fig, tobacco and cotton leaves, had already established that the growth of a primordium does not occur by uniform enlargement of the primordial bulge [14–18]. Rather, different regions of the primordium grow at different rates and orientations that can be quantified using computational tools [19,20•]. Growth orientations diverge at the leaf base and converge towards the tip [21••]. Not only do the growth rates progressively decrease from base to tip as reflected in the patterns of cell division and cell expansion, they differ along the medio-lateral axis as well, with more growth in the lateral than in the medial domain [21••]. Recently, tracking lamina growth in fluorescently-labelled Arabidopsis leaf cells has revealed that the growth pattern is established early on during the primordium development [21••].

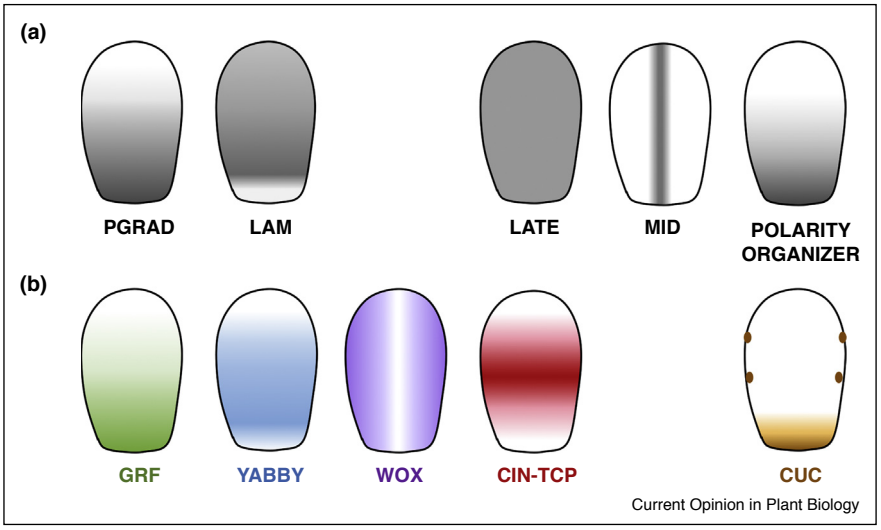
Although such studies provide a roadmap for describing growth patterns, the major challenge is to understand how they are specified at the genetic level [4,22]. Gene expression patterns in space and time, when combined with mutant phenotypes, inform us how a gene specifies growth rate and/or orientation locally. However, cells in a tissue are mechanically constrained by being connected to their neighbours and a specified growth pattern of cells in a region may conflict with that in the neighbouring regions leading to buckling of the tissue out of plane generating curvature or bending [4]. Thus, a 'resultant' growth pattern is an emergent property of any anisotropically-growing organ and may not be intuited from studying gene mutant phenotypes and/or expression domains in isolation [4].

Modelling leaf growth predicts a molecular toolkit for shape specification

Time-lapse growth analyses in combination with computational modelling can reveal coherent links between gene activity and the resultant growth patterns [8,21••,23,24••,25•]. While elegant reports described modelling growth in the petals or leaf margin [24••,25•], Kuchen et al built a model to simulate the observed leaf shape and growth patterns in Arabidopsis by incorporating two key systems — a network of factors specifying the *rate* of growth and a factor for determining the *orientation* of growth along the two orthogonal laminar axes [21••] (Figures 1a and 2 a). Two growth-promoting factors, PGRAD that is expressed in a decreasing gradient from base to tip, and LAM that is expressed uniformly, promote growth along the proximo-distal and the medio-lateral axes, respectively. In addition, two growth-inhibitory factors were introduced into the model to account for the cessation of leaf growth — a uniformly-distributed late-acting factor LATE and a midline-restricted factor MID that repress growth along the proximo-distal and the medio-lateral axes, respectively. Finally, a tissue polarity organizer, expressed in a decreasing base-to-tip gradient, was included that determined the direction of growth (Figure 1a). The regulation of growth *rate* was thus uncoupled from that of growth *orientation*. Further, the distribution of these factors changed in space and time with growth, allowing a feedback from tissue deformation to specified growth pattern. This 'deforming growth-orientation organizer' model could accurately reproduce shape changes and growth patterns observed experimentally in a wild-type Arabidopsis leaf. Varying the parameters of the model generated a variety of shapes that are observed among the simple-leaved species with smooth margins, indicating that the specification of growth patterns through interactions of growth-modulating and polarity-determining factors along the orthogonal axes underlies diverse simple leaf forms. An independent study incorporated the directional growth of veins as the major determinant of the orientation of specified growth and the resulting model accounted for the generation of diversity in complex leaf shapes, including serrations, lobes and leaflets [26•].

The value of computational modelling lies in its testable predictions about gene action in morphogenesis, leading to new insights. For example, modelling the development of a petal primordium that shows divergent growth pattern at its tip relative to its base, in contrast to the leaf, led to the prediction of the existence of a distally-expressed polarity organizer in addition to a proximally-expressed organizer [24••]. Based on its expression domain and mutant phenotype which matched the requirement of the model, the

Figure 1



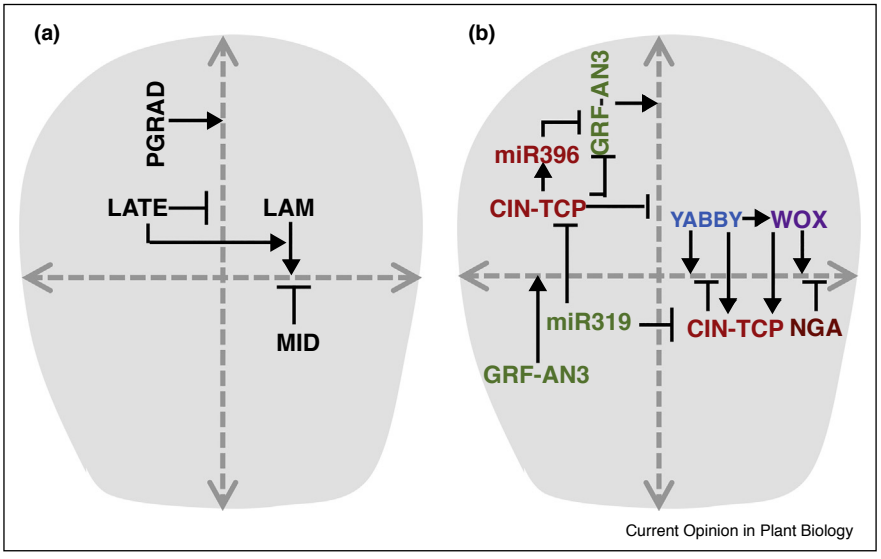
Spatial expression domains of the growth-regulating factors along the longitudinal axis of the early leaf primordia represented as rod-shaped structures. Both the hypothetical factors of the growing polarized tissue framework model in Kuchen *et al.* (**a**) and the proposed counterparts discussed in the text **(b)** are shown. The expression domains depicted in **(b)** are approximated from published studies on GRF, [30]; YABBY, [45••]; WOX, [63•]; CIN-TCP, [7]; CUC, [25•]. The primordium depicted in **(b)** is assumed to be at an early growth stage (up to ~2.0 mm in length), reflecting the dynamic expression of the growth-regulatory genes described here.

JAGGED gene encoding a Zn-finger transcription factor was identified and validated as the distal polarity organizer in addition to its previously-known function in petal cell division [24••]. Likewise, the model proposed by Kuchen et al. suggests candidate transcription factor-encoding genes that could function as the 'leaf architects', based on their known expression patterns and developmental roles [21••] (Figures 1b and 2 b). Following is a discussion on such factors and their modes of action.

Growth-promoting factors along the proximo-distal axis

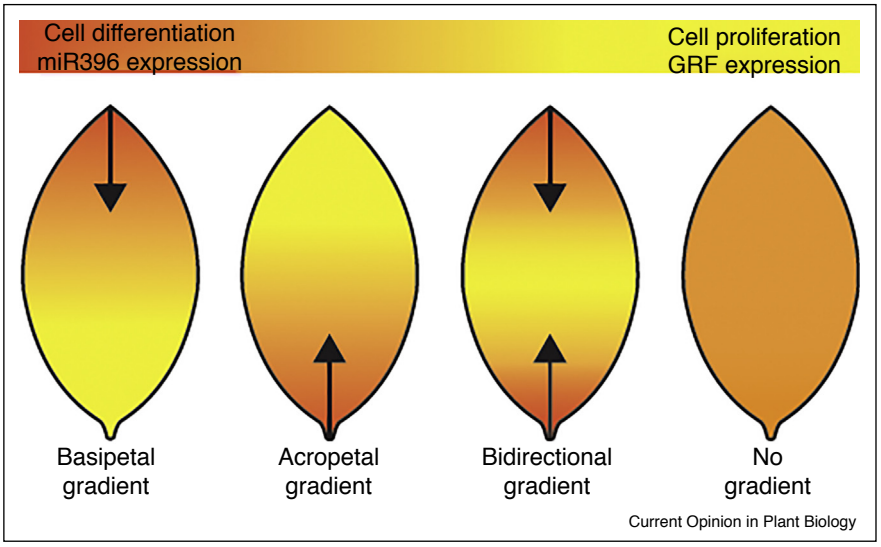
GROWTH-REGULATING FACTORS (GRFs) — These are a conserved group of plant-specific transcription factors that

Figure 2



Growth-regulatory network consisting of positive and negative regulatory factors in the early leaf primordium as hypothesized by Kuchen *et al.* **(a)** and as discussed in the text (**b**). The proximo-distal and the medio-lateral axes are represented by the orthogonally intersecting broken grey lines, along which growth is regulated by transcription factors as indicated.

Figure 3



Multiple growth polarities in eudicot leaves. The young leaf primordia show cell proliferation throughout the organ at inception. A pattern of cell differentiation is later superposed on this proliferating sheet of cells. The direction of the progression of the arrest front (black arrow) determines leaf growth polarity. The arrest front progresses from tip-to-base (basipetal gradient), base-to-tip (acropetal gradient) or from both ends to the middle (bidirectional gradient). In some cases, cells throughout the leaf exit proliferation simultaneously, thereby showing no growth polarity. The differentiating regions of the leaves show a strong correlation with the expression of miR396 while the proliferating region shows a gradient of GRF expression.

promote lateral organ growth [27]. Arabidopsis *grf* mutants have smaller leaves with reduced size and cell number, pointing towards a role in enhancing cell proliferation, although some family members also enhance cell size [28,29]. GRF expression is strongly associated with the base-anchored proliferation zone in the lamina [30] and is down-regulated in differentiated cells at the tip due, at least in part, to the activation of miR396 that targets seven of the nine GRFs in Arabidopsis [30,31]. The expression gradient of the miR396-GRF module along the proximo-distal axis is conserved in other species with basipetal growth pattern, suggesting that this module is a primary factor in promoting growth in this axis, similar to the PGRAD factor in the Kuchen *et al.* model [21••] (Figures 1b and 2 b). However, *grf* mutants have narrower leaves, indicating that growth along the medio-lateral axis (perpendicular to midvein in the Kuchen *et al.* model) is also affected, leading to a change in shape. Remarkably a recent study has identified a correlation between the polarity of miR396-GRF expression gradient and that of the leaf growth gradient (Box 1) (Figure 3) [11••,32•].

How GRFs stimulate proliferation directly is at present unclear. They seem to enhance the duration of cell proliferation and the number of cells undergoing proliferation [33•]. GRFs are proposed to regulate their target genes through a conserved interaction with GRF-INTERACTING FACTORs (GIFs) (Figure 2b). Loss of *GIF*s also results in narrower leaves with reduced cell number [34–36]. They function as transcriptional co-activators that lack a DNA-binding domain [34,35], though GIF1/ANGUSTIFOLIA3 (AN3) interacts and co-regulates its target genes with several components of the SWI/SNF chromatin remodelling complex containing BRAHMA or SPLAYED ATPases [37•]. Furthermore, *AN3* transcript is specifically enriched in the mesophyll cells and its protein product moves to the epidermis, coordinating cell proliferation across the clonally distinct cell types [38•]. Transcriptome profiling of the *35S:AN3-GR* transgenic line revealed up and down-regulation of transcripts that are inversely regulated during the transition from proliferation-driven to expansion-driven growth phases, supporting a role for AN3-GRF in balancing proliferation *versus* differentiation in developing leaves [12,37•]. In addition, *AN3* promotes its own transcription and that of *GRF3/5/6*, thereby providing a molecular basis for synergistic effect of simultaneous overexpression of *AN3* and *GRF* in leaf development [29,37•]. Interestingly, in maize, ZmAN3 is associated preferentially with ZmGRF1 in proximal division zone and with ZmGRF10 in the distal expansion zone; this preference reflects the mRNA and protein abundance of the respective ZmGRF partners [39]. Because ZmGRF1 stimulates and ZmGRF10 limits cell proliferation, it is hypothesized that the competition for ZmAN3 binding by different GRFs along the proximo-distal axis determines the position of the transition zone between that of division and expansion in growing leaves [39,40].

GRFs may promote laminar outgrowth by other indirect mechanisms. A study suggests that GRFs repress class I *KNOX* genes in both monocots and dicots [41]. Overexpression of GRF5 in Arabidopsis and GRF2 in *Brassica napus* leads to an increased chloroplast division and chlorophyll accumulation with strong induction of the *PORA* gene encoding an enzyme in the tetrapyrrole pathway for chlorophyll biosynthesis [33•,42]. Enhanced chlorophyll content is linked to the promotion of cell-cycle progression as the intermediates of the chlorophyll biosynthesis activate cyclin-dependent kinases through chloroplast-to-nucleus signalling, suggesting that GRFs link chloroplast division with cell division [33•,43]. Further, AtGRF7 represses several stress-response genes under normal conditions which would otherwise be detrimental to growth [44].

Growth-promoting factors along the medio-lateral axis

The YABBY and WUSCHEL RELATED HOMEOBOX (WOX) transcription factors — These proteins promote lamina outgrowth downstream to the adaxial-abaxial polarity establishment [45••,46]. *YABBY*s encode small proteins with zinc-finger and helix-loop-helix domains, conserved among all seed plants [47]. Arabidopsis plants mutated for four abaxially-expressed, vegetative *YABBY* genes form leaves with severe to moderate loss of lamina and marginal tissues with polarity defects [45••]. A subgroup of *WOX* genes encoding transcriptional repressors, comprising of the *PRESSED FLOWERS* (*PRS*) and the *MAEWEST/WOX1* subclades, regulate laminar expansion specifically along the medio-lateral axis [46,48,49••]. These factors are expressed in the so-called middle domain between the adaxial and the abaxial domains [49••,50•]. The *prs wox1* double mutant in Arabidopsis produces narrow leaves with perturbed polarity but unaltered length [49••]; similar phenotype is associated with the loss of *WOX1* homologues in other dicots and that of *PRS* homologues in maize [46,48,51].

Given their lamina-wide expression pattern and role as 'lamina identifiers', the *YABBY* and the *WOX* genes qualify as the LAM factor that determines growth along the medio-lateral axis in the Kuchen et al model (Figure 1). *YABBY* activity may be upstream to that of *WOX*, as the *YABBY* member *FILAMENTOUS FLOWER* (*FIL*) was shown to up-regulate *WOX1* expression [49••]. Vegetative *YABBY*s regulate a broad lamina-specific genetic program involving the repression of SAM identity and maintenance genes (*WUSCHEL* and *KNOX1*), promoting expression of the polarity and lamina maturation markers [45••,52]. The YABBY factors may act in concert with *AINTEGUMENTA* (encoding an AP2/ERF family transcription factor), as the *fil ant* and the *yab3 ant* double mutants have reduced lamina growth compared to the single mutants [53]. Whereas, the *WOX* genes mainly control cell proliferation along the medio-lateral axis, possibly by recruiting transcriptional repressors like TOPLESS to its targets, resulting in the indirect activation of growth-promoting transcription factors (SCARECROW-like), enzymes (KLU/CYP78A5), cell-cycle factors (D-type cyclins) and metabolic pathways leading to auxin biosynthesis and cytokinin signalling [46,49••,54]. Both YABBY and WOX factors regulate lamina outgrowth non-cell autonomously, affecting differentiation of tissues lacking their expression [45••,49••,52,55,56]. It is possible that they generate a mobile signal, such as the phytohormones. YABBYs have been postulated to control auxin response and flux along the leaf margin; the quadruple *yabby* mutant in Arabidopsis shows perturbed venation and lack of marginal structures [45••]. The *WOX1* homologue in *Nicotiana sylvestris*, *LAM1*, promotes auxin biosynthesis and co-application of auxin and cytokinin can rescue lamina growth defect in the *lam1* mutant [46].

Growth-repressing factors — common to proximo-distal and medio-lateral axes?

CINCINNATA-LIKE TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTORS (CIN-TCP) transcription factors — These belong to the plant-specific, non-canonical, bHLH domain-containing TCP family that redundantly repress growth [57]. Five Arabidopsis CIN-TCPs are post-transcriptionally co-regulated by microRNA319 (miR319) [58]. Perturbation of the conserved miR319-TCP module either by mutation of *CIN-TCP*s or by ectopic expression of miR319 results in larger leaves with altered shape and loss of flatness due to prolonged cell proliferation phase more towards the margin [7,58,59]. On the other hand, premature or increased activation of these factors leads to precocious cellular, organ and organism maturation, suggesting that they are heterochronic regulators of morphogenesis [60–62].

CIN-TCP expression occurs in a dynamic spatio-temporal gradient during the primordium development, as an output of their transcriptional and the miR319-mediated post-transcriptional control [63•] (Figure 1b). Initially, the *CIN* expression in snapdragon and *TCP4* expression in Arabidopsis starts at the tip and later gets restricted to the leaf base, overlapping with the base-anchored proliferation zone, before disappearing with the cessation of mitotic activity [7,63•]. Given the relatively late onset of CIN-TCP growth-repressor activity, compared to the growth-promoters like YABBYs, CIN-TCPs could serve as the late-acting growth-inhibitor LATE (Figure 2b). However, LATE is proposed to be uniformly distributed and retards growth along proximo-distal axis while promoting growth along medio-lateral axis. This is inconsistent with the expression pattern and activity of CIN-TCPs which restrict growth along both the axes, as evident from phenotypes of their gain-of-function and loss-of-function mutants [61].

CIN-TCPs are postulated to mainly promote the onset of cell maturation program, thereby indirectly inhibiting cell proliferation [61]; although some studies suggest a direct link with cell-cycle suppression [64,65]. CIN-TCPs also activate miR396 expression leading to temporal and spatial decline in its cognate *GRF* target levels [30,65] (see Box 1). They also down-regulate GRF5/6 and AN3 expression independent of miR396 [30], thus efficiently repressing the overall growth-promoting activity of the GRF-AN3 complex along the proximo-distal axis, similar to the antagonistic activities proposed for the PGRAD and LATE factors (Figure 2). In addition, CIN-TCPs regulate the level and/or response to several growth-regulating phytohormones such as auxin, cytokinin, gibberellic acid and jasmonic acid among others [66,67].

In snapdragon, *CIN* expression shows a strong medio-lateral gradient; more expression at the margin than at the centre of lamina. This is consistent with more de-repression of growth and mitotic marker expression at the margin than at the centre in the *cin* mutant, suggesting that CIN-TCPs also function as a component of the proposed MID that inhibits growth along the medio-lateral axis, though the observed CIN-TCP expression pattern is in contrast to that hypothesized for MID [7,21••]. Two recent studies have shed light on the relevance of CIN-TCP activity in the medio-lateral axis to the regulation of leaf morphogenesis [68••,69••]. Transcriptome profiling of young wild-type snapdragon and *cin* mutant leaves allowed the identification of *AmHISTIDINE KINASE4* (encoding a homolog of Arabidopsis cytokinin receptor) and *AmIAA3/SHY2* (encoding a homolog of the Arabidopsis AUX/IAA repressors of auxin signalling), as the direct downstream targets of CIN [68••]. Interestingly, these two genes are expressed more strongly at the margins than at the medial region, similar to *CIN*, raising the possibility that CIN regulates growth suppression at the margins by modulating the balance between auxin and cytokinin signals [70]. On the other hand, direct identification of the margin and centre-enriched genes in Arabidopsis enabled an analysis of their differential regulation in the *tcp2/3/4/10* quadruple mutant [69••]. The margin-enriched genes (genes expressed more in the margins than in the centre) were more down-regulated in the *cin-tcp* mutant than the centre-enriched genes and included several transcription factors known to control margin development, such as the *NGATHA*, *STYLISH* and even the miR319-resistant *CIN-TCP* family members. Other differentiation markers such as photosynthesis-related genes, shown to be expressed during the proliferation-to-expansion transition [9], were more down-regulated at the margins than at the centre of *cin-tcp* mutant; in contrast, the mitosis markers and the growth-promoting transcription factors such as *ANT* and *WOX1* were up-regulated [69••]. These studies have established CIN-TCPs as the major growth repressors not only along the proximo-distal axis but also along the medio-lateral axis; thus CIN-TCPs could serve as the LATE and MID factors that inhibit growth (Figure 2b).

Another study has revealed a novel role of *CIN-TCP*s and *NGA* genes in regulating determinate leaf growth [63•]. Simultaneous down-regulation of miR319-targeted *CIN-TCP*s and four *NGA* genes, either throughout the lamina or only at the margin, results in a dramatic indeterminate growth with sustained *de novo* organogenesis at the margin, whose molecular signature resembles that of undifferentiated initiating leaf primordia. Strikingly, there was no ectopic expression of SAM-specific genes at the margin; the authors proposed that the phenotype rather results from the de-repression of a short-lived *bonafide* 'leaf meristem', which is normally active only transiently at the leaf base and is kept suppressed in the distal margins by the coordinated CIN-TCP and NGA activity [63•]. CIN-TCPs have been shown to directly activate *NGA* genes; however, the phenotype of their combined down-regulation, absent from individual *cin-tcp* and *nga* mutants, suggests a synergistic relationship between these two family members [63•,71]. Possibly, CIN-TCPs interact with NGA to co-regulate margin-enriched genes for determinate growth [69••] (Figure 2b).

Growth orientation-determining factors

CUP-SHAPED COTYLEDON transcription factors — These are the NAC (NAM, ATAF1/2, CUPULIFORMIS) domain-containing transcription factors, characterized by their roles in organ boundary formation and serration development [72,73]. *CUC2* and *CUC3* are expressed at the leaf base and margin, demarcating the boundaries of incipient serrations [73]. Anisotropic growth requires a mechanism for the cells to determine the orientation of growth in response to growth regulatory factors. In the Kuchen *et al.* model, this information is derived from a polarity organizer expressed at the leaf base and growth occurs along the axes parallel and perpendicular to its proximo-distal gradient (Figure 1a). The *CUC* genes could serve as the candidate organizer based on their expression pattern and function [25•] (Figure 1b).

Cellular anisotropy may result from unequal or polarized distribution of molecules within the cytoplasm (e.g. microtubule cytoskeleton) or at the plasma membrane (e.g. receptors) [4]. CUC activity is associated with the generation of membrane anisotropy. *CUC2* promotes re-orientation of the plasma-membrane-localized auxin efflux carrier protein PINFORMED1 (PIN1) in the epidermis to form PIN1 convergence points that result in auxin maxima formation at the margins and the subsequent serration outgrowth [25•]. Whether a similar CUC activity regulates growth polarity during laminar outgrowth requires further experimental validation.

Interestingly, CUC activity is repressed by miR319-regulated CIN-TCPs. CIN-TCPs activate the transcription

of miR164 that targets *CUC2* transcript for degradation [74]. In addition, TCP4 interacts with and inhibits CUC2-CUC3 dimerization and dampens their transactivation potential. This effect is ameliorated by the sequestration of TCP4 by the miR156-targeted SPL transcription factors in the adult vegetative-phase leaves, causing age-dependent changes in the leaf margin shape [75•]. Thus, the polarity organizer activity may be subject to regulation by growth-regulatory factors.

Concluding remarks

Computational models provide a useful prism through which a complex biological phenomenon can be viewed in order to reveal its underlying component network(s) of interacting molecules. On the other hand, mutant analyses provide valuable mechanistic details that may not be predicted by modelling alone. For example, the *yabby* and the *wox1* mutants (in Arabidopsis and *Medicago truncatula*, respectively) show down-regulation of *CIN-TCP* expression, suggesting that the induction of growth-repressors by the growth-promoters is required for balanced determinate growth [45••,46]; although Kuchen et al. hypothesized that the LATE factor enhances the extent to which LAM promotes medio-lateral growth, there is no evidence that CIN-TCPs directly regulate *YABBY* and *WOX* genes. Likewise, inhibition of CUCs by CIN-TCPs indicates that the determination of growth orientation may not be independent of the growth regulatory network. It would be interesting to model these interactions and observe the impact on the simulated leaf shape.

Leaf form is a complex trait that requires several genetic regulators [77], of which only a few have been discussed in this review. Transcription factors such as ANT, STRUWWELPETER (a component of RNA Polymerase II-associated Mediator complex), AUXIN RESPONSE FACTOR2 and SPATULA control the duration of proliferation or the number of cells undergoing proliferation; most of these mutants show alteration in size but not shape, suggesting that they are general regulators of growth [78–81]. The PEAPOD transcription factors, on the other hand, control the onset of a secondary proliferation arrest of the dispersed meristematic cells, more at the centre than the margins [82]. Thus, they can form a component of the putative LATE or MID factors. Another candidate polarity organizer is JAGGED, which serves a similar function in petal growth [24••]. Detailed spatio-temporal analysis of these genes coupled with time-lapse growth analysis of their mutants should clarify their morphogenetic role.

Though modelling and experimental approaches together explain how diverse and complex leaf shapes can be generated, many questions still remain. Firstly, what is the evolutionary significance of this diversity. Indeed, the existence of any strong selection pressure on leaf shape in a specific environment is still debated [2]. Secondly, what is the advantage of evolving diverse growth gradients, as many species grow leaves without any gradient [11••]. It has been suggested that specific growth patterns confer adaptive advantage depending on the ecological niche of the plant species [32•]. A detailed 'eco-evo-devo' approach will be required to gain deeper insights.

Conflict of interest statement

Nothing declared.

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- • of special interest
- •• of outstanding interest

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