

Formation of primordia and phyllotaxy

Andrew J Fleming

Leaves are made in an iterative pattern by the shoot apical meristem. The mechanism of this pattern formation has fascinated biologists, mathematicians and poets for centuries. Over the past year, fundamental insights into the molecular basis of this process have been gained. Patterns of auxin polar transport dictate when and where new leaf primordia are formed on the surface of the apical meristem. Subsequent events are still obscure but appear to involve both alteration of cell wall characteristics (to facilitate a new vector of growth) and a cascade of spatially co-ordinated transcription factor activity (to determine the fate of cells that are incorporated into new lateral organs). The co-ordinated signalling events involved in these processes are beginning to be elucidated.

Addresses

Department of Animal and Plant Sciences, University of Sheffield,
Western Bank, Sheffield S10 2TN, UK

Corresponding author: Fleming, Andrew J (a.fleming@sheffield.ac.uk)

Current Opinion in Plant Biology 2005, 8:53–58

This review comes from a themed issue on
Growth and development
Edited by Liam Dolan and Michael Freeling

Available online 25th November 2004

1369-5266/\$ – see front matter
© 2005 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.pbi.2004.11.013

Abbreviations

clv3	<i>clavata3</i>
CUC	<i>CUP-SHAPED COTYLEDON</i>
CUP	<i>CUPULIFORMIS</i>
KNOX	KNOTTED-like homeobox
NS	<i>NARROWSHEATH</i>
PHB	<i>PHABULOSA</i>
PHV	<i>PHAVULOSA</i>
PIN	<i>PINFORMED</i>
PRS	<i>PRESSED FLOWER</i>
wus	<i>wuschel</i>

“Wusst’ ich genau, wie dieses Blatt aus seinem Zweig herauskam, schwieg ich auf ewige Zeit still, denn ich wüsste genug.”

“If I knew how a leaf from a branch grew precisely, forever silent would I remain, for my knowledge would suffice”

Hugo von Hoffmannsthal

Introduction

Immediately after germination, plants have an apparently simple structure. During the subsequent lifetime of the plant, this structure can become exquisitely ornate. This is as a result of the ability of the plant to continually generate organs from a specialised structure, the apical meristem. The most common organ formed is the leaf. These organs are not generated in a random fashion, rather in a consistent pattern over space and time, producing the regular architecture of the plant. The meristem is, thus, a pattern-generating machine. Recent research, described in this review, has provided key insights into the operation of this machine.

Meristems provide a field of cells from which leaves can be made

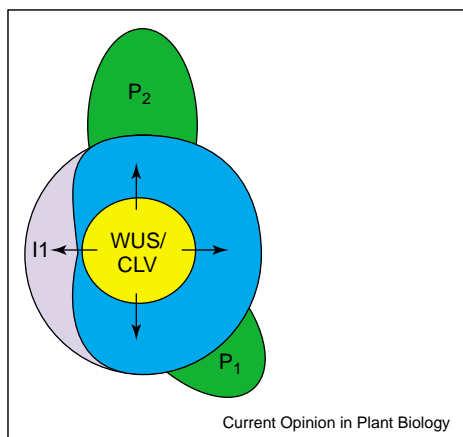
The shoot apical meristem consists of a population of cells that functions to maintain itself and, at periodic intervals, to generate a new organ (a leaf primordium) from its lateral boundary (Figure 1). This process of organ formation involves loss of cells from the meristem; yet, under normal circumstances, meristem size is maintained, with a tendency towards meristem enlargement as the plant ages. This indicates that a mechanism exists whereby meristem size is sensed and whereby alteration from a set norm leads to automatic re-adjustment. Investigations of the *wuschel* (*wus*) and *clavata3* (*clv3*) mutants of *Arabidopsis* have provided key insights into the molecular processes that underpin this mechanism, with the following paradigm emerging [1,2]. Cells towards the centre of the meristem express a homeodomain transcription factor termed WUS. This protein acts at a distance to promote the expression of a gene that encodes a small secreted peptide, CLV3, in the cells situated above the WUS-expressing domain. CLV3 acts to promote cell proliferation and growth and also feeds back to suppress WUS gene expression, thus suppressing growth. The interaction of WUS and CLV3 acts to maintain meristem size. How this size is initially set and the nature of the signalling process between these two proteins is still open to discussion.

The WUS–CLV paradigm provides a mechanism by which a meristem of particular size is maintained, but does not provide information on how particular regions of the meristem are selected for leaf formation. Recent data have indicated that the plant growth regulator auxin plays a central role in this process.

Auxin and patterning in the apical meristem

Auxin is a small molecule that has long been implicated in the control of plant growth. In the past few years, tremendous advances have been made in our understanding

Figure 1



The apical meristem is a pattern-generating machine. The WUS/CLV engine (yellow) maintains a supply (arrows) of cellular material to the rest of the meristem (blue) to maintain its size and shape. At periodic intervals (termed plastochron), meristem tissue becomes determined to form a leaf primordium at a site termed incipient primordium1 (I1; mauve). The position of I1 is determined by the positions of the previously formed leaf primordia, in this example P1 and P2 (green).

of the molecular action of this growth factor [3]. One key facet of auxin biology is that movement of the growth regulator occurs in a vectorial fashion. The pioneering studies of Rubery and Sheldrake [4] led to a model in which polar auxin transport could be mediated via asymmetric localisation of an auxin-efflux carrier. This model was the focus of experimental investigation for almost 30 years, with various physiological and biochemical data to support it. In the past few years, results from molecular genetic analyses have identified key components of the system. Most notably, research has led to the characterisation of a series of PINFORMED (PIN) proteins whose expression is intimately related to polar auxin transport [5]. Although these proteins show many of the characteristics expected of auxin-efflux carriers, there is still some discussion as to whether they represent the actual carrier or whether they are auxin-efflux regulators [6•]. Irrespective of this important point, disruption of *PIN* genes (either singularly or in multiple knockout mutants) disrupts polar auxin transport ([5,6•,7]; see review by Weijers and Jürgens, this issue). Recently, the PIN1 efflux carrier was shown to be expressed in a specific pattern in the shoot apical meristem [8•]. The protein is restricted to cells of the outer cell layer (L1 or outer tunica). The cells in this layer are characterised by a precise and consistent pattern of anticlinal cell division, leading to a layer of cells on the outside of the meristem dome. PIN protein is localised within these tunica cells in an asymmetric pattern that is thought to indicate the direction of flux of auxin through these cells. Remarkably, the polarity of PIN protein localisation within this cell layer is such that the predicted flux of auxin congregates

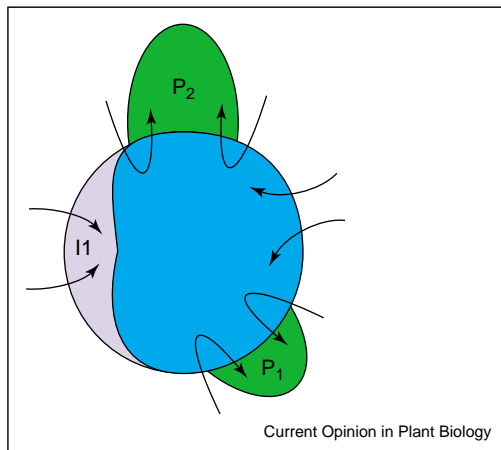
on one region of the meristem, the region that is destined to alter its vector of growth to generate a leaf primordium [8•].

The observed pattern of PIN protein distribution in the apex fits with a model in which auxin flux towards the meristem from adjacent tissue is restricted to the outer cell layer (the epidermis, L1-derived cells; Figure 2). If a young primordium acts as a sink for auxin, then auxin will be diverted from the neighbouring epidermis, creating a local minimum concentration of auxin within the apical meristem adjacent to the primordium. Depending on the pattern of previously formed primordia around the meristem and their relative strength as sinks for auxin (which might itself be dependent on factors such as distance from the meristem, primordium size and so on), a pattern of auxin minima will be generated around the meristem leading, *de facto*, to an area of auxin maximum in a region most distant from the strongest auxin sinks. If a local elevated level of auxin leads to organ initiation, leaf formation will occur at a site most distant from the most recently formed primordia. Formation of a primordium leads automatically to a new auxin sink and, thus, to the establishment of a new pattern of auxin minima within the meristem.

Several data support this model. Localised application of auxin is sufficient to trigger primordium formation [9] and the pattern of auxin efflux carriers in newly formed primordia is consistent with their acting as auxin sinks [10•]. Ablation of the L1 layer blocks primordium formation [11] and genetic ablation of *PIN* genes leads to the formation of inflorescence meristems that do not form lateral organs [7]. Local application of auxin to these naked stems is sufficient to restore lateral organ formation [9]. Finally, in addition to the efflux-associated PIN proteins, an auxin influx-associated protein (AUX1) shows a pattern of expression in meristem L1 cells that is consistent with a flux of auxin being directed towards the site of incipient leaf formation [8•].

On the basis of the auxin-patterning model, several mechanisms can be envisaged by which the pattern of organ formation could be altered. For example, increasing the field of cells from which primordium formation can occur (i.e. increasing the meristem size) might alter the position and number of sites of auxin minima. Indeed, altered meristem size is frequently associated with altered phyllotaxy [12,13•]. The finding that the *ABPHYL1* gene (mutation of which leads to increased meristem size and altered phyllotaxy) encodes a protein that has the potential to impinge on the perception of cytokinin indicates a role for this growth regulator in setting the field size within which the auxin-patterning system can function [13•]. The *ABPHYL1* expression pattern suggests that the protein might function to set field-size at a relatively early stage of embryo development when the gene is expressed

Figure 2



Channelled flux of auxin determines the incipient primordium (I1) position. Auxin moves towards the meristem from more proximal tissue predominantly in the outer cell layers (arrows). Newly formed primordia (P₁, P₂) act as sinks and draw auxin towards them. By default, the region of the meristem furthest away from newly formed primordia becomes a locus of auxin maximum. The localised accumulation of auxin within the meristem determines the site of incipient leaf formation.

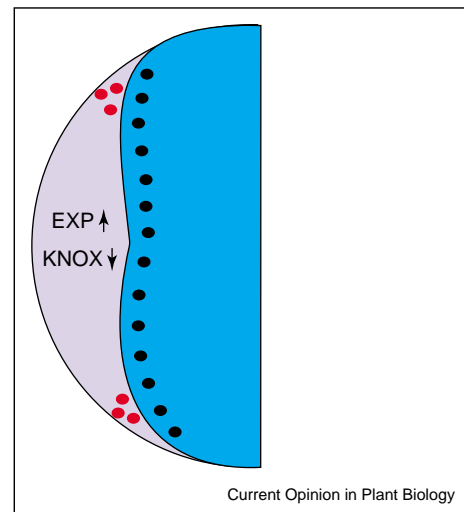
throughout the meristem. At later developmental stages, *ABPHYL1* expression becomes restricted to the region of the incipient primordium, implying that any influence on meristem size must be indirect.

Parameters that alter either the transport dynamics of the pattern-generating machine or the sensitivity of the field of cells to the organogenic signal might lead to altered phyllotaxy without overt change in meristem size. The *BELLRINGER* (*BLL*) protein might belong to this category. *BLL* encodes a TALE homeodomain protein that can interact with members of the *KNOTTED*-like homeobox (*KNOX*) family of homeodomain transcription factors [14]. As *KNOX* proteins have been implicated in defining the competence of meristem cells to form leaves [15], the observed influence of altered *BLL* expression on phyllotaxis might occur via a *KNOX*-mediated influence on competence for organogenesis.

The initial events of leaf morphogenesis and differentiation

A long-held view was that altered pattern of cell division is a key effector in leaf initiation. Indeed, it is intuitive that increased cell proliferation is a driving force for growth. Local promotion of cell proliferation within the meristem alone does not, however, lead to leaf initiation [16], neither does disruption of cell-division pattern disrupt meristem function [17]. By contrast, local alteration of cell wall extensibility by altered expression of the cell wall protein expansin is sufficient to induce leaf initiation [18], and expansin genes are specifically expressed at the

Figure 3



Early events in primordium formation. Selection of leaf initiation site is accompanied by changes in gene expression that allow outward growth of the tissue (e.g. upregulation of expansin gene expression [EXP]) and a cascade of transcription factors that lead to acquisition of appropriate tissue identity (e.g. downregulation of *KNOX* genes). The extent of meristem tissue incorporated into the new primordium is delineated by factors including *CUC/CUP* proteins (black circles) and *PRG/NS* gene products (red circles).

site of incipient leaf formation [19,20]. These data fit with the view that, providing sufficient turgor pressure and metabolic activity are present, it is the architecture of the cell wall that restrains plant growth ([21]; Figure 3). Whether expansin is an endogenous target of auxin during leaf formation is unknown. In the context of cell wall architecture, it has been suggested that biophysical patterns function not only at the local level in the execution of the organogenic program, but also that they could form the basis of pattern formation itself within the entire meristem [22]. Certainly, theoretical considerations indicate that the rapid establishment of energetically stable physical configurations could function as a robust means of dictating patterns of organogenesis [23]. However, experimental data to support these theories remain limited.

The formation of an organ implies that something must control the amount of meristem tissue that is incorporated into the new leaf primordium. Appropriate expression of the *CUP-SHAPED COTYLEDON* (*CUC*)/*CUPULIFORMIS* (*CUP*) family of transcription factors is necessary in the presumptive boundary tissue to prevent the formation of fused organs [24,25]. The mechanism by which these boundaries are set (i.e. the relationship with the predicted auxin maxima that define sites of leaf initiation) is unknown. The observation that the *CUP* protein can interact with a TCP-domain protein [25] provides an initial lead to a possible mechanism by which boundary-defining gene products might exert their influence, as

TCP-domain proteins have been implicated in various aspects of the control of growth [26].

Investigation of leaf lateral boundary formation has revealed a potentially significant difference between monocot and dicot species. It appears that, in monocots, the extent of the lateral leaf margin is set very early within the meristem during the process of leaf inception, whereas in dicots, leaf lateral margin growth is defined essentially post-primordium inception. Thus, the *NARROWSHEATH* mutations in maize (*NS1* and *NS2*) lead to narrower leaves, whereas mutation of the orthologous gene in *Arabidopsis* (*PRESSED FLOWER* [*PRS*]) leads in leaf tissue only to loss of stipules (i.e. small lateral appendages at the base of the leaf) [27]. These data are consistent with the view that the majority of the dicot leaf structure is derived from the distal region of the incipient primordium, whereas the monocot leaf is derived mainly from the proximal part of the incipient leaf [28].

Although the lateral boundaries of a leaf can be set very early in leaf formation, the final size of a leaf appears to be dependent on the action of genes expressed over a longer period of developmental time. The recent characterisation of the product of the novel *ARGOS* gene promises to shed light on this fundamental question of size control [29]. It is noticeable that the expression of the *ARGOS* gene is auxin sensitive, again implying a role for this ubiquitous growth regulator in leaf patterning.

Leaf morphogenesis is normally concomitant with differentiation. Even before overt formation of a primordium, changes in gene expression occur at the site of incipient leaf formation. Thus, decreased expression of *KNOX* genes presages the site of primordium formation [15]. How or whether this pattern of gene expression is linked to the flux of auxin that is predicted to dictate the site of leaf initiation is unclear. What is clear, however, is that an altered pattern of *KNOX* gene expression is able to set in train a cascade of transcription factor switching, whose appropriate patterning is required for growth and differentiation of the leaf (described in the review by Byrne, this issue). The process of differentiation and morphogenesis in the incipient leaf can be separated. Thus, altered cell division pattern in the meristem leads to altered *KNOX* gene expression in the absence of morphogenesis [17].

Once a cell has left the domain of the apical meristem, drastic and rapid processes of differentiation occur. The most obvious involve a rapid increase in cell size, as a consequence of vacuolation, and the synthesis of the photosynthetic machinery that allows the cell to become autotrophic. Our understanding of this switch from heterotrophic to autotrophic metabolism, which appears to be a key element in the transition from meristem to non-

meristem state (as distinct from a meristematic to non-meristematic state), remains negligible.

Signalling from meristem to leaf and back again

Spatially restricted expression of the related PHABULOSA (PHB)/PHAVULOSA (PHV) homeodomain leucine zipper proteins along the plant radial axis is required for the acquisition of adaxial/abaxial identity in the leaf [30]. Because the encoded proteins contain a potential site for sterol or lipid binding, it has been proposed that sterol-like compounds might act as a signal emanating from the meristem to the leaf [30]. In this model, binding of a sterol would lead to protein activation and to the acquisition of adaxial identity in a gradient following that of the sterol activator. More recently, microRNAs (miRNAs) have been identified that can bind to the mRNAs that encode PHB/PHV [31]. Binding of the miRNAs can target these transcripts for degradation [32]. If miRNAs were present in a gradient across the leaf, this would provide a post-transcriptional mechanism for generating a gradient of PHB/PHV protein. Intriguingly, the binding site of miRNA covers the region encoding the sterol-binding site alluded to above. Data have been reported which indicate that miRNAs are present in a gradient between the meristem and the leaf, and that misexpression of these miRNAs leads to altered morphogenesis [33,34]. Coupled with data showing miRNA-regulated expression of transcription factor genes that influence other aspects of leaf growth [35], a novel level of regulation of leaf formation and growth is being revealed.

Inappropriate patterns of early leaf differentiation can lead to cessation of meristem growth and primordium formation [36]. Because the events of differentiation occur in the leaf but the observed altered growth response occurs in the meristem, signalling from leaf to meristem must occur. The nature of this signal is unknown. It is interesting to note that the *PLASTOCHRON1* gene of rice is expressed in young primordia but leads to altered meristem size and rate of primordium initiation [37]. This gene encodes a cytochrome P450 enzyme whose endogenous substrate is unknown. Nevertheless, it provides further evidence of events in newly formed leaves signalling back to the organogenic centre of the meristem.

In addition to the movement of small molecules, many macromolecules can move within the shoot apex. Recent data suggest that diffusion via plasmodesmata might be the default process for many proteins [38], with selected proteins showing more restricted or directional movement [39]. As several of these proteins clearly have the potential to be important information carriers, it seems that signalling within the apex might be very complex. In addition, signal movement to the apex from the rest of the plant might be gated, with plasmodesmata acting as gatekeepers [40]. How such supracellular regulation of

macromolecular signals is integrated with the other signalling processes that are involved in meristem function and leaf formation remains unclear.

Conclusions

Research from the past year has provided major insights into the patterning mechanism involved in primordium formation at the shoot apex. At the same time, intriguing hints have been discovered that point to novel and complex signalling mechanisms both within the meristem and between the meristem and newly formed primordia. Deciphering these clues will lead to fundamental advances in our understanding of the intricate yet robust system that underlies leaf formation and the promise that, in the foreseeable future, our knowledge will 'suffice'.

Acknowledgements

Thanks go to Cris Kuhlemeier (University of Bern) for critical reading of the manuscript and David Jackson (Cold Spring Harbor) for providing data before publication.

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

1. Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T: **The stem cell population of *Arabidopsis* shoot meristems is maintained by a regulatory loop between the *CLAVATA* and *WUSCHEL* genes.** *Cell* 2000, **100**:635-644.
 2. Brand U, Fletcher JC, Hobe M, Meyerowitz EM, Simon R: **Dependence of stem cell fate in *Arabidopsis* on a feedback loop regulated by *CLV3* activity.** *Science* 2000, **289**:617-619.
 3. Leyser O: **Auxin signalling: the beginning, the middle and the end.** *Curr Opin Plant Biol* 2001, **4**:382-386.
 4. Rubery PH, Sheldrake AR: **Carrier-mediated auxin transport.** *Planta* 1974, **118**:101-121.
 5. Galweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K: **Regulation of polar auxin transport by *AtPIN1* in *Arabidopsis* vascular tissue.** *Science* 1998, **282**:2226-2230.
 6. Friml J, Vieten A, Sauer D, Schwarz H, Hamann T, Offringa R, •• Offringa R, Jürgens G: **Efflux dependent auxin gradients establish the apical-basal axis of *Arabidopsis*.** *Nature* 2003, **426**:147-153.
- An elegant series of experiments in which the pattern of PIN protein expression in several multiple knockout mutants indicates an essential role for auxin flux during the earliest stages of plant embryogenesis.
7. Okada K, Ueda J, Komaki M, Bell C, Shimura Y: **Requirement of the auxin polar transport system in early stages of *Arabidopsis* flowering.** *Plant Cell* 1991, **3**:677-684.
 8. Reinhardt D, Pesce E-R, Stieger P, Mandel T, Baltensberger K, Bennett M, Traas J, Friml J, Kuhlemeier C: **Regulation of phyllotaxis by polar auxin transport.** *Nature* 2003, **426**:255-260.
- Using a combination of methods to visualise patterns of gene expression and molecular genetic tools, pharmacological agents and micro-techniques to manipulate auxin gradients, the authors provide convincing evidence that auxin flux within the meristem plays a determining role in the selection of the site of incipient leaf formation. The results are incorporated into a model in which newly formed primordia act as sinks for auxin and, thus, create patterns of growth regulator concentration within the meristem.
9. Reinhardt D, Mandel T, Kuhlemeier C: **Auxin regulates the initiation and radial position of plant lateral organs.** *Plant Cell* 2000, **12**:507-518.
 10. Benkova E, Michniewicz M, Sauer M, Teichmann T, Seifertova D, •• Jürgens G, Friml J: **Local, efflux-dependent auxin gradients as a common module for plant organ formation.** *Cell* 2003, **115**:591-602.
- In conjunction with the data described in [8**], this paper proposes that PIN-mediated control of auxin flux is a unifying mechanism in determining the site of organogenesis in plants.
11. Reinhardt D, Frenz M, Mandel T, Kuhlemeier C: **Microsurgical and laser ablation analysis of interactions between the zones and layers of the tomato shoot apical meristem.** *Development* 2003, **130**:4073-4083.
 12. Running MP, Lavy M, Sternberg H, Galichet A, Gruissem W, Hake S, Ori N, Yalovsky S: **Enlarged meristems and delayed growth in *plp* mutants result from lack of CaaX prenyltransferases.** *Proc Natl Acad Sci USA* 2004, **101**:7815-7820.
 13. Giulini A, Wang J, Jackson D: **Control of phyllotaxy by the • cytokinin-inducible response regulator homologue *ABPHYL1*.** *Nature* 2004, **430**:1031-1034.
- Cloning of the *ABPHYL1* gene (mutation of which leads to decussate as opposed to the normal alternate phyllotactic pattern in maize) indicates that it encodes a response-regulator type protein that is implicated in the perception of cytokinin. The mutant *abphyl1* phenotype is associated with an increased meristem size, suggesting that cytokinins may play a role in regulating the field of cells from which primordia can form and, thus, phyllotaxis.
14. Byrne ME, Groover AT, Fontana JR, Martienssen RA: **Phyllotactic pattern and stem cell fate are determined by the *Arabidopsis* homeobox gene *BELLRINGER*.** *Development* 2003, **130**:3941-3950.
 15. Jackson D, Veit B, Hake S: **Expression of maize *KNOTTED1* related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot.** *Development* 1994, **120**:405-413.
 16. Wyrzykowska J, Pien S, Shen WH, Fleming AJ: **Manipulation of leaf shape by modulation of cell division.** *Development* 2002, **129**:957-964.
 17. Wyrzykowska J, Fleming AJ: **Cell division pattern influences gene expression in the shoot apical meristem.** *Proc Natl Acad Sci USA* 2003, **100**:5561-5566.
 18. Pien S, Wyrzykowska J, McQueen-Mason S, Smart C, Fleming AJ: **Local induction of expansin is sufficient to induce the entire process of leaf development and to modify leaf shape.** *Proc Natl Acad Sci USA* 2001, **98**:11812-11817.
 19. Reinhardt D, Wittwer F, Mandel T, Kuhlemeier C: **Localised upregulation of a new expansin gene predicts the site of leaf formation in the tomato meristem.** *Plant Cell* 1998, **10**:1427-1437.
 20. Cho H-T, Kende H: **Tissue localization of expansins in deepwater rice.** *Plant J* 1998, **15**:805-812.
 21. Cosgrove DJ: **Loosening of plant cell walls by expansins.** *Nature* 2000, **407**:321-326.
 22. Green PB: **Expression of pattern in plants: combining molecular and calculus-based biophysical paradigms.** *Am J Bot* 1999, **86**:1059-1064.
 23. Shipman PD, Newell AC: **Phyllotactic patterns on plants.** *Phys Rev Lett* 2004, **92**:168102.
 24. Vroemen CW, Mordhorst AP, Albrecht C, Kwaaitaal MACJ, de Vries SC: **The *CUP-SHAPED COTYLEDON3* gene is required for boundary and shoot meristem formation in *Arabidopsis*.** *Plant Cell* 2003, **15**:1563-1577.
 25. Weir I, Lu J, Cook H, Causier B, Schwarz-Sommer Z, Davies B: ***CUPULIFORMIS* establishes lateral organ boundaries in *Antirrhinum*.** *Development* 2004, **131**:915-922.
 26. Nath U, Crawford CW, Carpenter R, Coen E: **Genetic control of surface curvature.** *Science* 2003, **299**:1404-1407.
 27. Nardmann J, Jiabing J, Werr W, Scanlon MJ: **The maize duplicate genes *narrow sheath1* and *narrow sheath2* encode a conserved homeobox gene function in a lateral domain of shoot apical meristems.** *Development* 2004, **131**:2827-2839.

28. Kaplan DR: **The monocotyledons: their evolution and comparative biology. VII. The problem of leaf morphology and evolution in the monocotyledons.** *Q Rev Biol* 1973, **48**:437-457.
29. Hu Y, Xie Q, Chua N-H: **The *Arabidopsis* auxin-inducible gene • ARGOS controls lateral organ size.** *Plant Cell* 2003, **15**:1951-1961.
The authors identify a novel gene product whose manipulation allows directed control of organ size.
30. McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK: **Role of PHABULOSA and PHAVOLUTA in determining radial patterning in shoots.** *Nature* 2001, **411**:709-713.
31. Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP: **Prediction of plant microRNA targets.** *Cell* 2002, **110**:513-520.
32. Tang G, Reinhart BJ, Bartel DP, Zamore PD: **A biochemical •• framework for RNA silencing in plants.** *Genes Dev* 2003, **17**:49-63.
Plants contain several miRNAs that have the potential to bind to endogenous mRNA sequences. Strikingly, several of these mRNAs encode proteins that have developmental significance [31]. This paper demonstrates that binding of miRNAs to target sequences can direct the breakdown of these sequences (i.e. can provide post-transcriptional regulation of gene expression via miRNAs).
33. Kidner CA, Martienssen RA: **Spatially restricted microRNA • directs leaf polarity through ARGONAUTE1.** *Nature* 2004, **428**:81-84.
The authors demonstrate that specific miRNAs accumulate in specific patterns during leaf formation and lead to the spatially regulated differential expression of target genes that are involved in the acquisition of leaf polarity.
34. Juarez MT, Kui JS, Thomas J, Heller BA, Timmermans MCP: **• MicroRNA-mediated repression of rolled leaf1 specifies maize leaf polarity.** *Nature* 2004, **428**:84-88.
The authors propose that miRNAs act as a short-range signal to regulate the expression of genes that are involved in determining leaf polarity.
35. Palatnik JF, Allen E, Wu X, Schommer C, Schwab R, Carrington JC, •• Weigel D: **Control of leaf morphogenesis by microRNAs.** *Nature* 2003, **425**:257-263.
Abnormal leaf morphogenesis resulting from altered expression of TCP-domain transcription factors is shown to be regulated via miRNAs that target the breakdown of specific TCP-encoding transcripts.
36. Waites R, Hudson A: **Phantastica: a gene required for dorsiventrality of leaves in *Antirrhinum majus*.** *Development* 1995, **121**:2143-2154.
37. Miyoshi K, Ahn B-O, Kawakatsu T, Ito Y, Itoh J-I, Nagato Y, Kurata N: **Plastochron1, a timekeeper of leaf initiation in rice, encodes cytochrome P450.** *Proc Natl Acad Sci USA* 2004, **101**:875-880.
38. Wu X, Dinneny JR, Crawford KM, Rhee Y, Citovsky V, Zambryski PC, Weigel D: **Modes of intercellular transcription factor movement in the *Arabidopsis* apex.** *Development* 2003, **130**:3735-3745.
39. Kim J-Y, Yuan Z, Jackson D: **Developmental regulation and significance of KNOX protein trafficking in *Arabidopsis*.** *Development* 2003, **130**:4351-4462.
40. Foster TM, Lough TJ, Emerson SJ, Lee RH, Bowman JL, Forster RLS, Lucas WJ: **A surveillance system regulates selective entry of RNA into the shoot apex.** *Plant Cell* 2002, **14**:1497-1508.