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Phyllotaxis

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Phyllotaxis, the regular arrangement of leaves or flowers around a plant stem, is an example of developmental pattern formation and organogenesis. Phyllotaxis is characterized by the divergence angles between the organs, the most common angle being 137.5°, the golden angle. The quantitative aspects of phyllotaxis have stimulated research at the interface between molecular biology, physics and mathematics. This review documents the rich history of different approaches and conflicting hypotheses, and then focuses on recent molecular work that establishes a novel patterning mechanism based on active transport of the plant hormone auxin. Finally, it shows how computer simulations can help to formulate quantitative models that in turn can be tested by experiment. The accumulation of ever increasing amounts of experimental data makes quantitative modeling of interest for many developmental systems.

A numbers problem in development

Following the stem of a tomato plant from the root junction upward, one encounters a succession of leaves in a developmental and temporal gradient. The bottom leaves are the oldest and may already be senescing. Moving up the plant, the leaves become smaller and younger until the close packing of young leaves obstructs further access to the tip. Located at the tip is the shoot apical meristem, a fragile, dome-shaped tissue of 80–150 μm in diameter, where the lateral organs – leaves at first, flowers after the onset of reproductive development – are continuously initiated. Organ initiation in plants is an iterative process and the meristem can remain active over the life span of the plant.

The lateral organs are positioned in distinct patterns, and this arrangement around the stem is called phyllotaxis. The most prevalent patterns are distichous, spiral, decussate and whorled patterns (Figure 1). Phyllotactic patterns can be disrupted by experimental interference, but, within limits, they will quickly recover and reestablish the original arrangement [1]. On the one hand, apparently, aberrant positioning can be corrected. On the other hand, transitions between patterns, for instance from decussate to spiral, occur frequently during the life of a single plant (Figure 2a,b), indicating that developmental switches can override the self-correction mechanism.

Models of phyllotaxis must explain its *de novo* establishment in the radially symmetric embryo, the stable maintenance of the different arrangements and the observed transitions between phyllotactic patterns. Most importantly, they must explain the specific divergence angles of 180°, 90° and 137.5°, and, in rarer cases, other angles as well. This quantitative aspect makes phyllotaxis an unusual developmental problem.

The geometry of phyllotaxis

The types of phyllotactic arrangements (Figure 1) can be fully described by the number of organs that is simultaneously initiated (jugacy), the angular divergences between primordia, and their spacing along the apical–basal axis [2]. Most common in nature are spiral patterns with divergence angles of ~137.5°. This is the golden angle, which is obtained when a circle is sectioned according to the golden ratio of 1.618 [3,4].

Higher order patterns arise when the size of the primordia is small relative to the circumference of the apex. Figure 2c shows such a system with divergence angles of ~137.5° and with the leaf primordia arranged in eight right- and 13 left-winding spirals. The numbers of these so-called visible contact parastichies are not arbitrary, but are given by the Fibonacci series (1, 1, 2, 3, 5, 8, 13, 21...), in which each term is the sum of the two previous ones. The ratio of two consecutive Fibonacci numbers tends to the golden ratio [3]. In simple spiral patterns, such as seen in an *Arabidopsis* seedling, the average divergence angle between successive leaves or flower primordia can vary by several degrees from the theoretical angle, and an angle between any two consecutive leaves can vary by even more [5,6]. This variation in individual angles is a logical consequence of the cellular make-up of the meristem. Assuming an average of 25 cells in the circumference of the *Arabidopsis* meristem, a lateral shift of primordium initiation by a single cell would cause a change in angle by 360°/25 = 14.4°. By contrast, in higher order phyllotactic patterns (Figure 2c), a deviation of <1° would disturb the pattern [3]. Another curious aspect of higher order patterns is that the geometry of the apical surface and the size and shape of the organs affect the subjective perception of the pattern [7,8].

Why are Fibonacci spirals so common in nature? Why is, for instance, a hexagonal arrangement or a divergence angle of 30° rare? Mathematical modeling suggests that spiral leaf arrangements are superior for light capture, at least under model conditions [9,10]. However, the establishment of phyllotaxis is largely insensitive to environmental conditions, and adaptations to the light environment occur primarily at the post-meristematic level through adjustment of divergence angles, leaf shape, leaf inclination and petiole length. Thus, it seems unlikely that phyllotactic patterning is of major adaptive significance with respect to light capture.

Another view emphasizes the role of phyllotaxis as a solution to a packing problem. In 1873, Hubert Airy [11]

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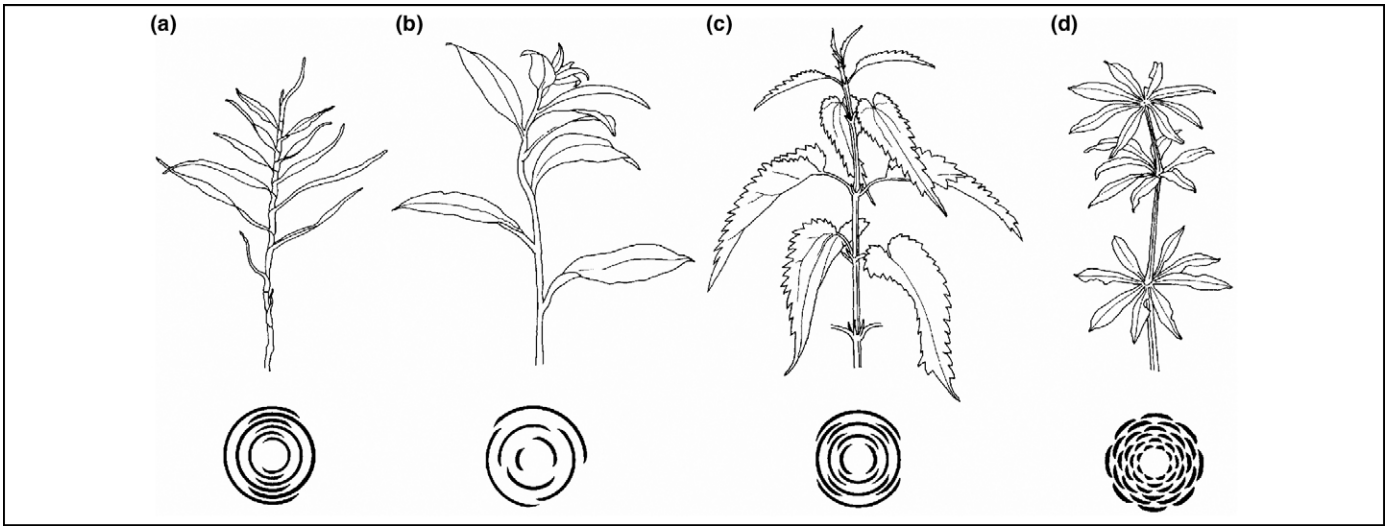

Figure 1. Phyllotactic patterns. **(a)** Distichous or alternate: one leaf per node with 180° divergence angles (*Trisetum distichophyllum*). **(b)** Spiral: angle is ~137.5° (*Lysimachia dethroides*). **(c)** Decussate: two opposite leaves per node at 90° angles between the pairs (*Urtica dioica*). **(d)** Whorled: multiple leaves per node (*Galium odoratum*). Drawings by Peter Leuthold.

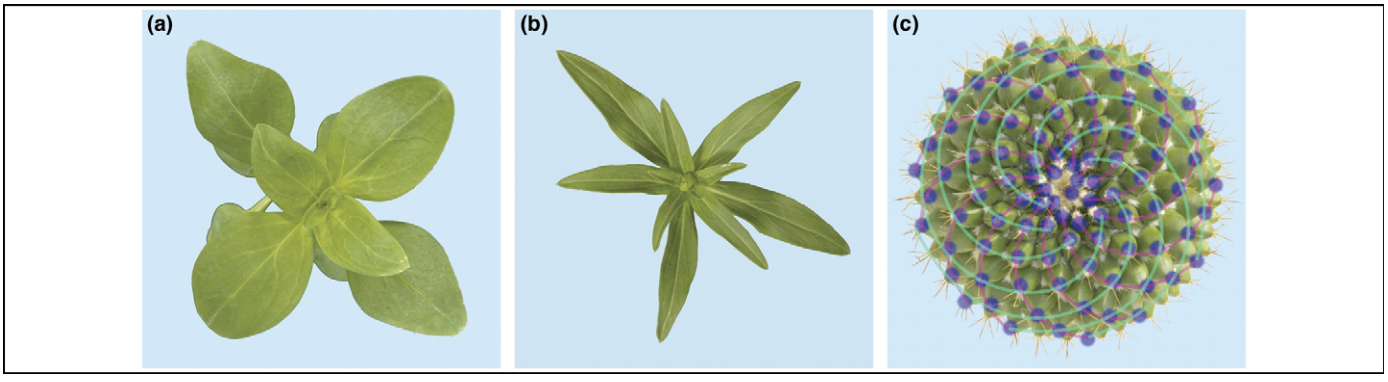

Figure 2. Phyllotactic transitions and higher order spirals. **(a)** Young vegetative *Antirrhinum majus* with decussate phyllotaxis. **(b)** Top part of older plant with spiral phyllotaxis. **(c)** *Mammillaria marksiana* with eight right-winding and 13 left-winding visible contact parastichies. Photographs by Peter von Ballmoos.

argued that the adaptive significance must lie in the bud itself, where the patterns are most regular: 'It is *for economy of space*, whereby the bud is enabled to retire into itself and present the least surface to outward danger and vicissitudes of temperature'. This hypothesis is interesting but also hard to prove experimentally.

A third idea is that the prevalence of Fibonacci spirals is a consequence of the molecular mechanism underlying phyllotaxis. The molecular mechanism might well constrain the number of geometric options that is biologically feasible. This might ultimately explain why and under which conditions spiral patterns are preferred. In the next sections the experimental data and their use as a basis for quantitative models will be reviewed.

Plant stem cells

The shoot apical meristem, first observed by Caspar Wolff in 1759 [12] (Figure 3), is a fragile, dome-shaped tissue of 80–150 μm in diameter. It remains active after the end of embryogenesis, and all mature aerial tissues originate from the meristem, be it an annual plant or a 1000-year old tree.

The meristem consists of a few hundred small, rapidly dividing cells (Figure 3). The cells in the central zone at the tip of the dome do not differentiate, but their daughters in the peripheral zone have the option to either retain their identity or differentiate into the cell types of the central axis and lateral organs. The analogy with animal stem cells is now obvious [13]. The shoot apical meristem is an attractive system for studying fundamental questions of stem cell research because it is a spatially well-defined structure and neither cell migration nor cell death complicate matters. Cells divide symmetrically and, with the exception of the cells on the surface, the orientation of cell division appears random.

The stem cells are defined at the molecular level by the expression of CLV3, a small peptide that signals to the underlying WUS expressing cells. The WUS center promotes stem cell fate and can therefore be compared to a stem cell niche [13,14]. Negative feedback between the homeobox protein WUS and the CLV ligand-receptor system is thought to regulate the size of the stem cell population. WUS and CLV3 are expressed in distinct subdomains of the meristem. Genetic approaches have been essential for understanding the interactions between WUS and CLV, but they are not entirely conclusive in explaining how the expression domains can be so sharply delimited in space. Interestingly, mathematical models can explain the positioning of the WUS domain and the phenotype of mutants and surgical alterations with simple and realistic assumptions [15].

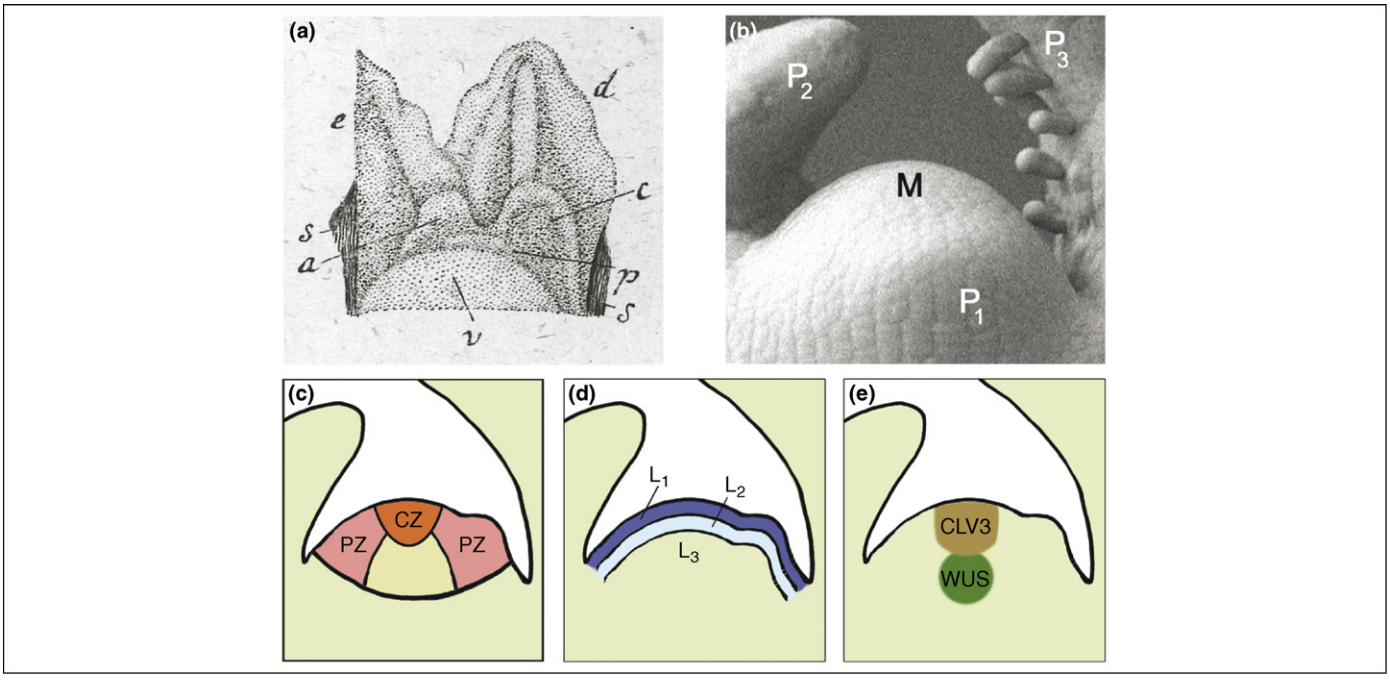

Figure 3. The shoot apical meristem. **(a)** Shoot apical meristem (*v*) of *Brassica capitata* (cabbage) as first seen by Caspar Wolff in 1759 [12]. Leaf primordia are labelled *p, a, c, d, e.* **(b)** Meristem of tomato visualized by low vacuum scanning electron microscopy. Abbreviations: M, Meristem; P_1, P_2, P_3 primordia. Photograph by Didier Reinhardt. **(c)** Subdivision of the meristem into zones. The central zone (CZ) contains the stem cells; organ initiation takes place in the peripheral zone (PZ). **(d)** Subdivision of the meristem into concentric layers, L_1, L_2 and L_3. **(e)** The WUSCHEL–CLAVATA feedback loop. The CLV3 expression domain defines the stem cells.

The analogy with animal stem cells is justified and conceptually useful, yet should not obscure the unique properties of plant stem cells and plant development. Complete ablation of animal stem cells has serious consequences. Not so in plants. Cell ablation experiments illustrate this point. It has long been known that major injury to the central zone has only mild effects, and growth and leaf development continue essentially as normal and without delay [16,17]. After laser ablation of the central zone including all WUS (and presumably CLV3) expressing cells, cells in the peripheral zone re-express WUS and presumably adopt a stem cell fate [18]. Similarly, in *clavata* mutants, the central zone expands through respecification of peripheral zone cells [19]. These experiments show that cells can switch fate and regain stem cell character. This is a manifestation of a general feature of plant cells that is carried to the extreme in plant cell tissue culture, where single differentiated cells can be reprogrammed with high efficiency [20]. Might it be possible that efficient developmental reprogramming contributes to stem cell homeostasis in the undisturbed shoot apical meristem as well?

The orientation of cell divisions in the shoot meristem appears to be mostly random. The one exception allows for a functional subdivision of the meristem into layers (Figure 3). In dicot plants, there are usually three concentric tissues: L_1, L_2 and L_3. The outer L_1 layer will differentiate into the epidermis, and the L_2 and L_3 layers each make variable contributions to the inner tissues [21]. In particular, the cells of the L1 layer divide almost exclusively perpendicular to the surface. These oriented cell divisions of the L_1 cause a million-fold increase in leaf surface area, whereas the epidermis remains a single cell layer in depth.

As we have seen above, cells of the peripheral zone can quickly and efficiently reprogram and acquire stem cell fate. Switches between the L_1 and L_2 layers occur in only one direction. In the rare cases that an L_1 cell divides parallel to the surface, the displaced cell loses L_1 identity and takes on L_2 identity according to its position [21]. By contrast, L_2 cells seem to be unable to acquire L_1 fate. When L_1 cells are destroyed by laser ablation, the underlying L_2 cells do not differentiate into L_1 cells, instead they enlarge and terminally differentiate [18]. Similarly, expression of a cellular toxin in the L_1 layer leads to morphological defects in the epidermis but not to respecification of underlying L_2 cells [22]. Such experiments suggest that L_1 fate cannot be acquired by reprogramming of L_2 cells. An interesting possibility is that the L_1 produces a mobile signal that prevents differentiation in the underlying layers. If this is correct, the L_1 layer can be considered a stem cell niche.

Experiments on phyllotaxis

In his 1868 textbook on general plant morphology, Wilhelm Hofmeister [23] describes his detailed observations on organ initiation in large numbers of plant meristems. He concludes that each leaf arises in the largest gap between the edges of the previous leaf primordia. Variations of this simple concept remain the basis of all theories of phyllotaxis and have inspired subsequent experiments. In mechanistic terms, it suggests that existing primordia are the source of a signal that prevents establishment of primordium identity in the vicinity of the source.

If older primordia provide positional information, removing them should change the position of new primordia. Surgical experiments performed on *Lupinus albus* meristems in the 1930s suggest that when the inhibiting

influence of an older primordium is removed, the new leaf initial (I_1) closest to the gap can move towards the gap. Although this experiment has been a cornerstone of phyllotaxis research, it needs to be interpreted with care. Similar experiments in tomato recapitulate the original experiments, but atypical stem elongation growth is also evident [24]. More precise removals of I_1 by infrared laser ablation show that in most cases a new primordium forms in the vicinity of the ablated position and then is the starting point for a normal spiral (Figure 6c).

A biophysical patterning mechanism is unlikely

So what is the molecular nature of the inhibiting influence exerted by pre-existing primordia? The first concept is that the control mechanism is not based on a signaling molecule at all. Spiral patterns exist outside biology [11,24,25], and the regularity of phyllotaxis might simply be a consequence of physical forces operating on a growing system. Although genetic analysis over the past half century has amply demonstrated the preeminence of specialized regulatory mechanisms, the physical constraints on developmental programs should not be neglected.

In its most extreme form, the biophysical theory posits that differences in tension between the L_1 surface layer and the inner tissues lead to tissue buckling [26]. The position of the bulges is determined by the mechanical properties of the meristem, and once the bulges are formed, they will grow and acquire new developmental identity. This concept is fascinating. Differential tissue tension provides an elegant mechanism for *de novo* pattern formation based on simple engineering principles, with phyllotactic patterning an emergent property of the system. In biological terms, it puts the emphasis at the control of growth, that is, at the cell wall. Local application or gene induction of expansin, a protein that regulates wall extensibility *in vitro*, induces primordia at ectopic positions [27,28]. This suggests that wall properties can indeed influence organ positioning, but does not prove that expansin controls positioning. If it were the primary signal, one would expect expansin to be differentially expressed between the L_1 and the underlying layers. Instead, the expansin mRNA is preferentially expressed in incipient primordia, consistent with expansin expression as the readout of a primary signal [29].

If global tissue tension drives organogenesis, local manipulation of that tension should affect neighboring tissue and affect organogenesis. This is not the case. Removal of the L_1 layer from an incipient primordium by infrared laser ablation completely abolishes its outgrowth. However, the observed effects are strictly local. Even the removal of large stretches of L_1 tissue has no global effects and leaves continue to form at positions with intact L_1 [30]. Therefore, there is no convincing evidence that biophysical mechanisms constitute the principal regulatory mechanism. However, they might well have a more limited role in morphogenesis [31].

The patterning mechanism is based on active transport of the plant hormone auxin

The obvious alternative to a biophysical regulatory mechanism is chemical signaling. All evidence now points towards the plant hormone auxin, or indole-3-acetic acid (IAA). This simple amino acid derivative is structurally related to the neurotransmitter serotonin and, similar to serotonin, it is actively transported. In *Arabidopsis*, a family of at least six transporters, the PIN proteins, catalyzes auxin export from cells [32,33]. Each of the PINs has a distinct expression pattern and a characteristic asymmetric subcellular localization. PIN polarization can proceed by different mechanisms [34,35], but the mechanism most relevant for phyllotaxis is PIN polarization by auxin itself. Auxin promotes retention of PIN in the plasma membrane [36] and, hence, an auxin concentration gradient across the cell could promote asymmetrical localization of the transporter.

Inhibition of active auxin transport, either through chemical inhibition or by mutations in the PIN1 transporter, specifically inhibits organogenesis. This leads to the formation of a naked meristem that grows normally but is entirely devoid of lateral organs. The defect can be rescued by the application of a microdroplet of auxin to the peripheral zone of such a pin-shaped meristem [37]. Auxin accumulates at sites of incipient organ formation [6,38] and localized application of auxin can induce ectopic organs [37]. Hence, auxin is required for the induction of lateral organs.

As well as being required for lateral organ induction, it appears that auxin also provides positional information. In the *pin1* mutant, all transcripts including the *Pin1* mRNA itself are uniformly present throughout the peripheral zone [39,40], indicating the absence of a prepattern. Furthermore, the position and concentration of the auxin applied to a *pin1* meristem determine the position and size of the induced primordium. Synthetic auxins with different transport properties induce organogenesis but the organs are not correctly positioned [41]. Proper positioning requires IAA. In summary, all auxins can induce organogenesis, but correct positioning requires the endogenous hormone.

PIN1 is predominantly expressed in the L_1 surface layer of the shoot meristem and in the incipient and young primordia. PIN1 is induced by auxin and the expression maxima coincide with maxima of auxin concentration [6,38,42]. Modeling of auxin transport combined with a comprehensive analysis of PIN1 expression indicates that the observed subcellular polarization of PIN1 will cause auxin to accumulate at incipient primordia [43].

The combined experimental results suggest that auxin or, more precisely, the naturally occurring auxin IAA, instructs the meristem as to organ patterning [18,37]. The results also suggest a simple model in which an autoregulatory loop between auxin, PIN1 expression and polar localization of PIN1 creates auxin maxima by transport against concentration gradients (Figure 4a,b). The auxin maxima can activate downstream processes through specific receptors and the combinatorial action of members of two large families of transcription factors (ARF and IAA/AUX) [61,62].

Interestingly, and rather puzzling, during phyllotactic patterning in the L_1, PIN1 polarizes towards auxin concentration maxima, whereas during vein formation it seems to orient away from them [6,38,44]. How this

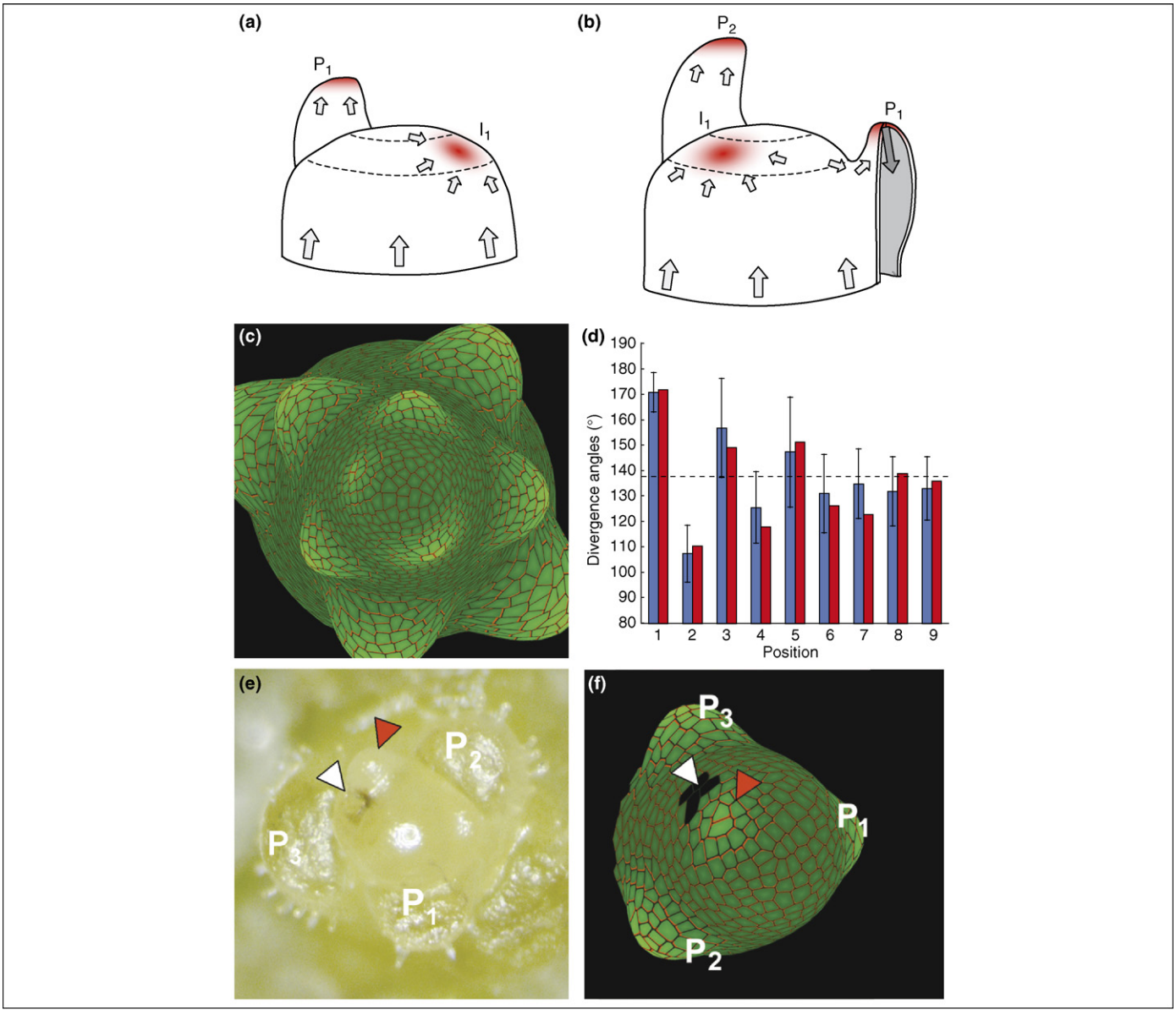

Figure 4. Models of phyllotaxis. **(a,b)** Experimental model of phyllotactic patterning. **(c,d)** Computer simulation of spiral phyllotaxis and model validation. **(a)** PIN1 orientation directs auxin fluxes (arrows) in the L_1 layer, leading to accumulation of auxin (red color) at the initiation site (I_1) in the peripheral zone. This accumulation eventually results in organ induction. **(b)** Later, basipetal PIN1 polarization inside the bulging primordium (P_1) drains auxin into inner layers, depleting the neighboring L_1 cells. As a consequence, another auxin maximum is created in the peripheral zone at position I_1 removed from primordia P_1 and P_2. **(c)** The simulation by Smith *et al.* [6] can initiate spiral phyllotactic patterning starting *de novo* from the radially symmetric embryo. Lighter green signifies higher auxin concentration. PIN1 is depicted in red. For the animation, see supporting movie 4 [6] at http://www.pnas.org/content/vol0/issue2006/images/data/0510457103/DC1/10457Movie4.mpg. An animation based on the model by Jönsson *et al.* [53] can be found as supporting movie 1 at: http://www.pnas.org/content/vol0/issue2006/images/data/0509839103/DC1/Movie1.mov. (To view the movie, please paste the URL into your web browser rather than clicking the URL.) **(d)** Comparison of the divergence angles: angles measured in *Arabidopsis* with standard error bars (blue), and angles generated by the spiral phyllotaxis model (red). Reproduced, with permission, from Ref. [6]. **(e)** Ablation of incipient primordium I_1 (white arrowhead) leads to the initiation of a new primordium in close vicinity (red arrowhead). Reproduced, with permission, from Ref. [18]. **(f)** The simulation model reproduces the effects of the ablation shown in (e). Reproduced, with permission, from Smith *et al.* [6].

happens is unclear. The molecular mechanism might involve a protein kinase such as PINOID, which can cause reversal of PIN1 orientation [45].

Phyllotactic transitions

Phyllotactic patterns often change during development. In *Antirrhinum*, for instance, the early vegetative plant is decussate, but later on the pattern changes to spiral (Figure 2). There are numerous mutants that affect phyllotaxis but most of them result in irregular leaf arrangements. Such defects are often caused by perturbations of the meristem, for instance in the *clavata* mutants where the enlarged meristem has a tendency to fasciate [46]. 'Homeotic' mutations, which change one regular pattern into a different one, are scarce. This suggests that regular phyllotactic transitions are under polygenic control.

The only well-studied homeotic mutant is *abphyl1* in maize, which changes the distichous leaf arrangement of the wild type (one leaf per node, 180° angles) into decussate (90° angles between opposite leaf pairs), although not completely – reversals to distichous are frequently observed [47,48]. ABPHYL1 encodes an ARR protein, a negative regulator of cytokinin action. In *Arabidopsis*, a septuple *arr* mutant shows slight irregularities in phyllotaxis [49].

Several ARR proteins are direct downstream targets of WUS, which is expressed in a radially symmetric domain below the central zone and is probably not directly involved in leaf positioning. If cytokinin simply increases the size of the meristem, this changes the ratio between meristem size and primordium size. This provides a model for phyllotactic transitions that is in line with classical theories [7]. Additional regulators are likely to be involved in maize and *Arabidopsis*.

Virtually nothing is known about the molecular mechanisms that underlie the transitions between different spiral systems (e.g. 5/8 to 8/13 parastichies), except that larger meristems seem to have higher Fibonacci numbers. A major question is how higher order patterns such as those seen in sunflower heads are generated at all. It seems more likely that the position of organs is determined by their nearest neighbors in space than in developmental time.

Quantitative approaches to phyllotaxis

The model presented in Figure 4a,b is qualitative in nature and reflects the lack of quantitative experimental data. This is not a specific shortcoming of this particular model but pertains to most developmental models. However, in phyllotaxis, qualitative models are particularly deficient because phyllotaxis is in essence a quantitative phenomenon. A good model should: (i) generate a wide variety of phyllotactic patterns; (ii) generate robust patterns that are insensitive to noise and can recover from perturbations; but (iii) at the same time generate phyllotactic transitions, such as the frequently observed change from decussate to spiral (Figure 2); (iv) generate patterns *de novo* from a radially symmetric embryo; and (v) be based on plausible assumptions about the molecular mechanisms involved [6].

Most models [24,50–52] are in some way or another guided by Hofmeister's rule, that older primordia inhibit the emergence of new initials in their vicinity [23]. In a recent example, the inhibition exercised by each primordium is inversely proportional to its distance from a given position, and decreases exponentially with the age of that primordium [2]. At positions where the sum of the inhibitory effects exerted by the primordia falls below a predefined threshold, a new primordium will arise. The model recreates distichous and spiral patterns in a robust manner. The introduction of a second inhibitory function that is similar to the first, except that it decays more rapidly over time, makes it possible to simulate decussate and whorled systems. Variation in the short-range-inhibition parameters also allow for phyllotactic transitions. Thus, with only minimal assumptions about the molecular mechanisms involved, it is possible to recreate all the important aspects of phyllotactic patterning.

The construction of mechanistic quantitative models that incorporate the accumulating molecular data is now feasible. Two recent computer simulation models are based on data on auxin and auxin transport [6,53]. Both assume that patterning occurs in the L_1 surface layer, that auxin is uniformly available in the meristem, that polarization of PIN proteins is based on auxin concentrations in the neighboring cells and that the rate of auxin transport depends on diffusion and active transport. They differ in several details (Box 1), most importantly the algorithms used to model cell division and the equations describing the dependencies of PIN polarization and auxin transport on cellular auxin concentration. Linear dependencies on auxin concentration produced phyllotactic patterns of limited stability [6,53]. Stability improved when strongly nonlinear equations were used to capture PIN polarization and active transport. Moreover, in the model by Richard Smith *et al.* [2] an additional equation was introduced to increase PIN polarization exclusively within the primordia. With these modifications, the model can start from a radially symmetric embryo, produce opposite cotyledons, and then settle into a Fibonacci spiral [6]. The patterns are stable and reproduce *in vivo* measured angles within one standard deviation. The model also faithfully recapitulates the phenotype of the *pin1* mutation and the effects of selected experimental manipulations (Figure 4).

Questions raised by the mechanistic models

Mathematics, physics and biology have all made substantial contributions to phyllotaxis research but, until recently, they have had surprisingly little interaction. Mathematical models were based on simplified representations of anatomical structures and on molecular mechanisms with little experimental basis. Even though technology is rapidly advancing, the lack of experimental data remains a bottleneck. Many of the molecular components are probably not yet known, for example, ABC transporters, import carriers and protein kinases such as PINOID and transcription factors. In fact, we cannot even measure auxin concentrations with cellular resolution. Knowing more about the nuts and bolts of the system should enable the development of more refined models.

Even when experimental data are available, they tend to be qualitative. The assumptions made about PIN polarization and auxin transport are plausible, but the exact forms of the equations used for quantitatively describing PIN polarization and auxin transport are the outcome of the modeling efforts. For instance, the models predict a nonlinear dependence of PIN polarization on auxin concentration, and a molecular mechanism for measuring auxin in neighboring cells. Similarly, the inclusion of a 'factor X' that modifies PIN polarization specifically within incipient primordia is based on the enhanced stability it provides to the model. Such predictions serve to guide experimentation. They illustrate the usefulness of quantitative modeling when only limited and qualitative data are available.

Finally, the published simulation models make the assumption that all relevant patterning events take place in the outer cell layer of the meristem and that the inner tissues can be neglected. This is a useful but potentially unjustified simplification. For instance, most of the PIN1 molecules are not located at the meristem surface but in internal cells [40]. When auxin exits the L_1 layer, it induces vein formation, but by a mechanism that has been proposed to depend on auxin flux rather than on concentration [54,55]. This vein will connect to the primary vasculature of the stem. Can we exclude the old idea [56] that the stem vasculature dictates, or at least influences organ position? All recent experiments and simulations are consistent with

Box 1. Quantitative models of phyllotaxis

The models by Henrik Jönsson *et al.* [53] and Richard Smith *et al.* [6] are similar in many aspects. In both models, organ patterning is the result of dynamic interactions between existing and incipient primordia in a growing apex, mediated by actively transported auxin. Some of the key elements are:

(1) The dependence of PIN1 polarization on auxin concentration in neighboring cells

In both models, the distribution of the PIN1 in cell *i* towards the membranes facing each of the four surrounding cells j_1–j_4 depends on the relative auxin concentrations in these neighboring cells (Figure I). According to Smith *et al.* [6] the relationship is strongly non-linear: the amount of PIN1 in cell *i* that is localized in the membrane towards cell j_n is proportional to: $b^{[IAA]_j}$, where b is a constant and the exponent $[IAA]_j$ is the IAA concentration in the neighboring cell j_n. In the simplified example below, the auxin concentrations are set at 1, 2, 2, and 3. If b = 3, the partitioning of PIN1 over the four sides of cell *i* will be: 3^1: 3^2: 3^2: 3^3, or a threefold difference in auxin concentration translates into a ninefold difference in PIN1 polarization. In the simulations presented by Jönsson *et al.* [53], the relationship is linear with a saturation term. In both models, an unknown short-range signal must communicate the auxin concentration from the neighboring cells j_n to cell *i*. Cell walls are depicted in green and PIN1 is depicted in red.

(2) The dependence of active auxin transport on auxin concentration

In both models, the rate of auxin transport depends on auxin concentration. In the Jönsson model, the relationship is linear (with a saturation term), whereas according to Smith *et al.* the transport rate is proportional to the square of the cellular auxin concentration.

(3) Auxin transport within primordia

The Smith model makes the *ad hoc* assumption that within incipient primordia, PIN1 polarization is slightly different in the sense that PIN1 orients more strongly towards the center of the primordium. In biological terms, this is equivalent to introducing a regulatory factor X.

(4) Cellular template

In both models, cellular growth is modeled, either by representing the cells as circles with mechanical interactions [53], or with a cell division algorithm [6] that mimics the division pattern seen in live apices [19]. Full descriptions of these growth and division models are presented in the supplementary materials of Refs [6,53].

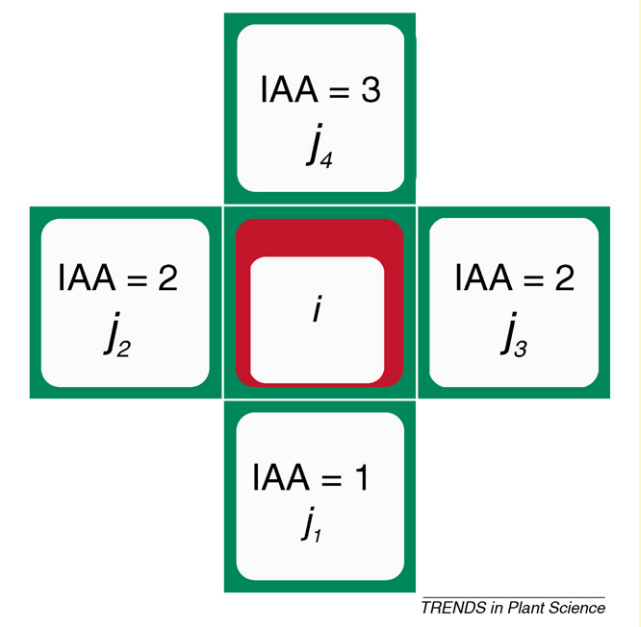

Figure I.

a phyllotactic patterning mechanism in which the meristem is autonomous and does not receive information from the mature tissues below. However, to definitively answer such questions, a model is needed that integrates the events at the surface with those in the internal layers.

Conclusions

The cells of the shoot apical meristem express many molecular components that interact to initiate lateral axes of growth. How should one order these components and build meaningful mechanistic models? In developmental biology, the approach has traditionally been intuitive and largely qualitative. This is in contrast to many other branches of experimental biology, such as physiology and population genetics that have a long tradition of quantitative approaches. A good example is metabolic control analysis that over the past decades has provided us with a new understanding of the regulation of biochemical pathways [57,58]. The fascination of phyllotaxis is that it is a numbers problem that has always been the subject of mathematical treatment. It is now becoming possible to create quantitative models with realistic assumptions based on molecular data. This is necessary if we want to order the mass of accumulating molecular data and, at the same time, understand the geometrical and physical constraints on development [59].

Quantitative modeling might also help us to identify the molecular targets for artificial and natural selection. Most of our developmental mutants would not survive outside the growth room because most changes in complex regulatory networks are likely to incur severe fitness costs. Selection for developmental novelty without loss of fitness seems to favor selected molecular components and often involves subtle quantitative differences [60]. Quantitative developmental control analysis might identify such targets and should help in understanding the evolution of development.

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