A mathematical basis for plant patterning derived from physico-chemical phenomena

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The position of leaves and flowers along the stem axis generates a specific pattern, known as phyllotaxis. A growing body of evidence emerging from recent computational modeling and experimental studies suggests that regulators controlling phyllotaxis are chemical, e.g. the plant growth hormone auxin and its dynamic accumulation pattern by polar auxin transport, and physical, e.g. mechanical properties of the cell. Here we present comprehensive views on how chemical and physical properties of cells regulate the pattern of leaf initiation. We further compare different computational modeling studies to understand their scope in reproducing the observed patterns. Despite a plethora of experimental studies on phyllotaxis, understanding of molecular mechanisms of pattern initiation in plants remains fragmentary. Live imaging of growth dynamics and physicochemical properties at the shoot apex of mutants displaying stable changes from one pattern to another should provide mechanistic insights into organ initiation patterns.

Keywords:
auxin; computational modeling; mechanical properties; pattern; phyllotaxis; plant; shoot meristem

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Introduction

When we walk through a garden, colorful flowers and leaves certainly catch our attention but one just cannot stop admiring the meticulous arrangement of leaves and flowers along the central stem axis of plants. This precise pattern of organ arrangement is known as phyllotaxis. In plants, at the shoot apex, the central zone of the meristem contains a slow-growing stem cell niche that produces cells for the peripheral zone. Lateral organs such as leaves and flowers are produced at the periphery of the shoot apex where cells actively divide, and are precisely positioned [1–5]. For example, the arrangement of leaves can be in a spiral fashion (Fig. 1A) where, in many species, successive leaves are separated by the golden angle (divergence angle of 137.5°). Most plants, including *Arabidopsis* – a model dicot species, display some degree of variation from the golden angle [6]. Divergence angles other than 137.5° are observed in some plants that display a spiral pattern; an example is *Costus* where the divergence angle between successive leaves is ~40°–50° [7, 8]. The divergence angle between successive leaves in another spiral pattern is ~99.5° (Lucas angle) [9]. The lateral organ primordia are arranged into a lattice, in which two sets of spirals called parastichies arise, one running in the clockwise and the other in the counter-clockwise direction [10]. The number of parastichies in each direction are often consecutive terms in the Fibonacci series, which starts with the numbers 1 and 2, and consists of terms that represent the sum of the previous two numbers (1, 2, 3, 5, 8, 13, etc.) [10, 11]. The number of parastichies depends on the angle, and the displacement of successive primordia from the center of the apex of tip. In a sunflower capitulum, where the florets are small relative to the large flower disc, the numbers can be as high as 34 or 55 [12]. The leaves could also be arranged in a paired fashion, arising simultaneously in opposite directions with the next pair of opposite leaves positioned at 90° (decussate; Fig. 1B) or at ~68° (bijugate phyllotaxis) relative to the first pair. In other arrangements, the leaves can arise in opposite directions but in alternate fashion (distichous; Fig. 1C) or multiple leaves can arise simultaneously from the same node (whorled or multijugate systems; Fig. 1D) [13].

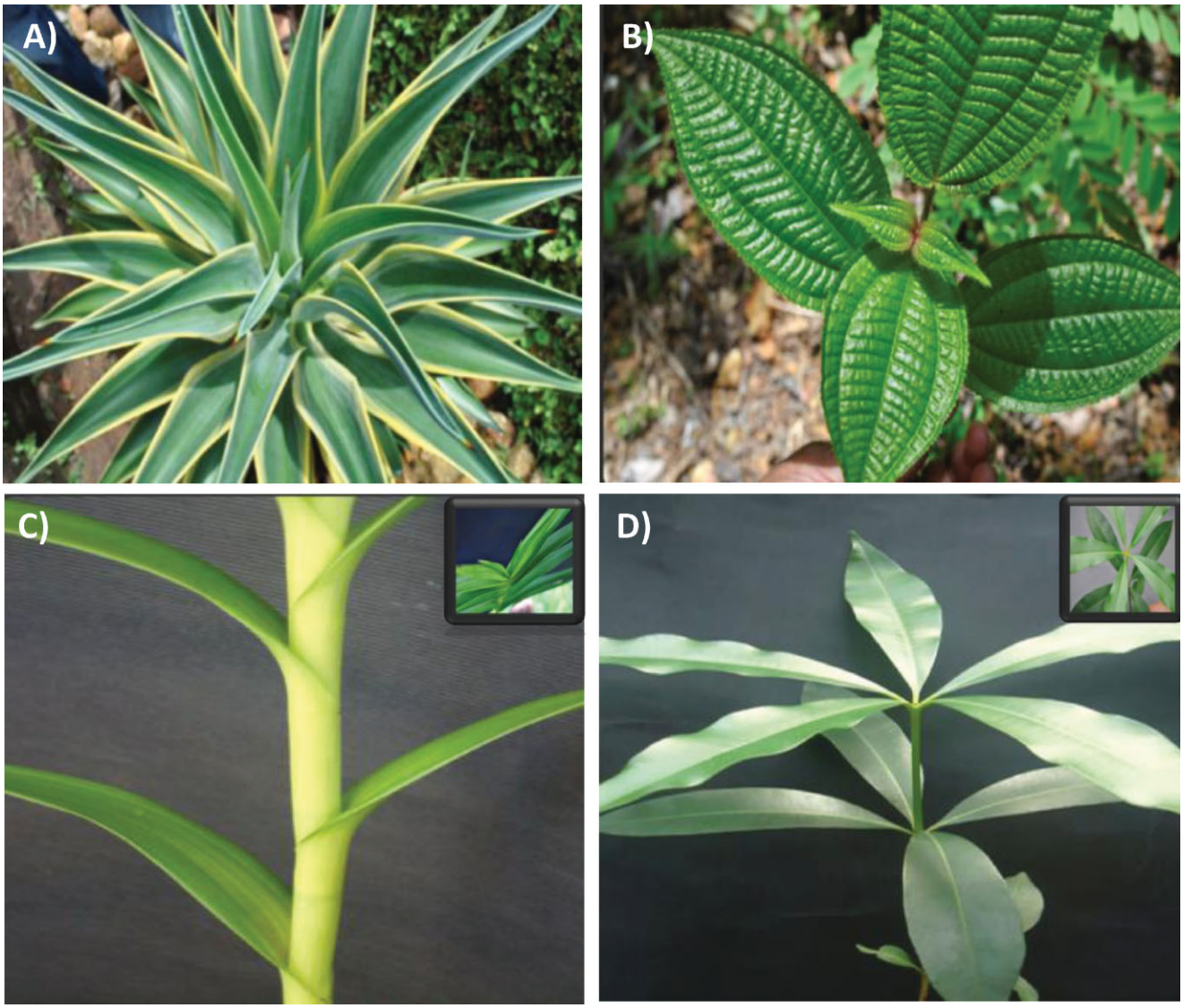

Figure 1. Predominant phyllotactic patterns observed in nature. **A:** An *Agave* species represents the spiral pattern where successive leaves are positioned at the 137.5° golden angle. **B:** *Clidemia hirta* shows a decussate pattern in which a pair of opposite leaves arises at a node and the successive pair arises perpendicular to previous one. **C:** *Arundinagramini folia* is an example of the alternate (distichous) pattern in which one leaf arises per node with a divergence angle of 180° between successive leaves. Inset shows top view of the pattern. **D:** *Alstonia scholaris* shows whorl pattern in which several leaves arise concurrently from a single node. Inset shows top view of the whorl pattern.

A central question that arises is how do the various patterns arise in nature? Modeling studies shed light on this. Previous mechanistic models [14, 15] focused on a particular parameter and its maximization/minimization in a field located on the meristem. Hofmeister [15] was one of the first to propose this kind of constraint stating that new leaves form in regions of the meristem with the most space [11]. Similarly, Douady and Couder [14, 16, 17] showed by experimental analogy and modeling that spiral phyllotaxis could appear by a form of ''energy minimization'', where primordia ''repel'' each other and space themselves regularly. Prior to this work, Schoute [18] had put forward the concept of a growth inhibitor released by primordia. These earlier models were of the ''reaction-diffusion'' type pioneered, among others by Alan Turing, the father of modern computers [19, 20], where the causes and inhibitors of organ formation interact with each other while being transported spatially by diffusion. Mechanisms unique to living organisms like active transport were typically not considered in such models.

The idea of an inhibitory field is a potent conceptual tool in constructing mathematical models for predicting organ positioning. The presence of an inhibitory field around a newly formed organ prevents the formation of another organ close to it. The extent of the inhibitory field, relative to the size and geometry of the growing tip of the plant (meristem) therefore determines where the next organ can form. A primordium with a large inhibitory field will prevent the formation of other primordia, while one with a weak field will

allow multiple primordia to appear simultaneously. The interplay between the range of the inhibitory field, the rate of growth of the meristem, and the size of the meristem, etc., have been explored numerically by several authors so as to reproduce the observed phyllotactic patterns [10, 13, 21]. To fully address the question of pattern formation one has to go a step further and understand how the inhibitory field is actually realized in a plant. Both chemical and physical effects have been proposed as possible mechanisms that give rise to the inhibitory field. Here we focus mainly on how the plant growth hormone auxin, its concentration, distribution, and transport can present a picture of the origin of a plausible inhibitory field. All current models posit a threshold value of the plant growth hormone auxin in a cell for primordium initiation. When low-auxin regions are formed by the action of various forces (directional transport, heterogeneous auxin-response within meristem, etc., Table 1), these become regions where primordia are least likely to develop, or are maximally inhibited.

Local accumulation of plant growth hormone auxin regulates the organ positioning

The growth hormone auxin is crucial for organogenesis in plants [29–33]. The most common, naturally occurring auxin is indole-3-acetic acid (IAA). It is a simple molecule, related to tryptophan and generates the majority of the auxin effects in plants. A growing body of evidence emerging from recent computational modeling and experimental studies in *Arabidopsis thaliana* suggests that polar auxin transport and dynamic accumulation of auxin in the cells can regulate organ positioning [26, 27, 30, 34]. The role of auxin in triggering initiation of a lateral primordium was established through a remarkable experiment by Kuhlemeier's group in which the local application of auxin at the tip of a naked shoot meristem of *Arabidopsis pin1* mutant defective in polar auxin transport [29, 35–37] sufficed to initiate the flower primordium [24]. The dynamic accumulation of auxin in the meristem is generated by polar auxin transport, which in turn is facilitated by the membrane protein PIN1 [31, 33, 38]. The control of organ initiation by polar transport of auxin is at the root of elegant hypotheses that have been developed in silico via computational modeling [22, 26–28]. These modeling studies are based on the central theme that the local auxin accumulation at the site of primordium emergence is required for its initiation. One of the most attractive hypotheses explaining the dynamic nature of the auxin peak at the periphery of the shoot apex is that PIN1 gets polarized toward cells with high auxin concentration, thereby amplifying the existing auxin content there, and eventually generating auxin maxima at the organ initiation sites [26, 34]. For example, in the work of Smith et al. [26], PIN1 localizes towards cells with high auxin concentrations (Fig. 2). In this model, primordia prevent other initiums from developing by producing the most auxin locally, thus causing all the surrounding PIN1 proteins to orient themselves to the current primordia. Modeling studies by Smith et al. [26] produced spiral, decussate, distichous and whorled patterns. Like Smith et al. [26], Jönsson et al. [27], using the same PIN1 localization rule, were able to recreate spiral-like patterns and decussate phyllotaxy. These two models, among other cell-level simulations [22, 24, 25] that use different PIN1 localization rules, indicate the importance of directional transport in creating auxin peaks and troughs in the meristem. Another majorly used PIN localization rule is that PIN1 localizes onto cell membranes with the most ''flux'', also known as the ''canalization'' mechanism, proposed by Sachs in 1969 [39]. Flux is the rate of auxin flow between two adjacent cells along their common membranes. The fluxes themselves are set up through initial passive transport, variations in cellular auxin synthesis rates, and different cellular geometries. An initial auxin flow between cells is amplified by a positive feedback between auxin flux and the accumulation of auxin transporters (PIN) and their polarization in the direction of the flux (Fig. 2). This has been modeled by Stoma et al. [25], who were able to propagate a spiral phyllotaxis from confocal sections of labeled PIN. The preferential localization of PIN1 proteins leading to directional auxin transport provides a micro-level mechanism for the behavior of the meristem-level inhibitory field. It has been proposed that auxin-accumulating lateral organ primordia act as auxin sinks, which leads to depletion of auxin from the surrounding cells. This contributes to the inhibitory field, defining the spacing between successive leaves or flowers [30].

In the spiral pattern, auxin foci are generated at regular intervals at a divergence angle of 137.5° and in opposite or whorl pattern, auxin maxima originate simultaneously. Disrupting polar auxin transport, either pharmacologically using 1-*N*-naphthylphthalamic acid (NPA), which inhibits auxin transport, or genetically using a *pin1* mutant, abolishes the dynamic appearance of auxin peaks at the meristem periphery [26, 29, 31]. This results in a naked shoot stem with no lateral organ initiation. PIN1 has been implicated in the control of phyllotaxis [30]. Interestingly, lowering the dose of PIN1 transcripts in a hypomorphic *pin1* mutant delays the transition from the first pair of opposite leaves to a spiral pattern in *Arabidopsis* [40]. Furthermore, inhibition of polar auxin transport by NPA application changes the phyllotactic pattern [40–43]. These studies demonstrate the key role of polar auxin transport in controlling the phyllotactic pattern. Here, compromising polar auxin transport is likely to have reduced the inhibitory field, allowing the emergence of two primordia simultaneously. Furthermore, a combination of AP2-containing plant specific transcription factors PLETHORA (PLT) control the lateral organ positioning by regulating the local auxin biosynthesis in the central domain of shoot apex and act synergistically with polar auxin transport in this process [40, 44]. The efficient polar distribution of PIN1 required to position the auxin maxima in the meristem appears to be under the control of auxin influx carriers AUX1 and its paralogs, as evident from largely uniform PIN1 distribution or loss of auxin peaks in the *aux1*,*lax1*,*lax2* triple mutant inflorescence apex [45]. These experimental studies [40, 45] fit with chemical-based models, which have identified polar auxin transport as an important parameter in generating the patterns [24–27]. How do influx carriers control the PIN1 distribution? A plausible explanation is that influx carriers maintain the required level of auxin in the cell and the auxin feedback on PIN1 to regulate its localization. Irrespective of the mechanisms by which influx carriers contribute to positioning the lateral

Table 1. An overview of simulation studies of phyllotaxis[a]

Reference	Mechanism being modeled	De novo evolution of model?[b]	Auxin synthesis	Auxin degradation/ removal	Cellular PIN synthesis/cycling to membrane	Cellular PIN degradation/ cycling to endosome	PIN localization mode	All cells in meristem subject to same rules/all cells in meristem uniform?	Kinds of patterns generated
[22]	Auxin concentration and auxin transport by PIN proteins	No de novo evolution. Initiate model with confocal data and let auxin transport occur until equilibrium	Constant, in all cells and from external source	Constant in all cells/flow directed downwards	None mentioned	None mentioned	Static	Yes	Spiral (auxin maxima at initium sites)
[23]	Auxin concentration and auxin transport by PIN proteins	Yes	From external source	Increased in primordium	Fixed cellular concentration, cycling between endosome and membrane	Fixed cellular concentration, cycling to endosome	Dynamic, polarized toward the cell with highest auxin concentration	Yes	Whorl pattern with similar numbers and positions of reproductive organs as in *A. thaliana*
[24]	Mechanical strain	Yes	Constant	Constant rate	Fixed cellular concentration, distribution only on cell walls	None explicitly stated, PIN assumed to be in ''quasi-equilibrium''	Dynamic, toward the side with most relaxed cell wall	Yes	Spatially uniform patterns
[25]	Flux based	No de novo evolution. Use of data from confocal images to initiate simulations	Constant, in all cells	Constant and CZ degrades at higher rate	Rate of insertion into membrane positively dependent on flux through it	Constant rate of removal from membrane	Dynamic, in the direction of highest flux	Cells grouped into CZ and PZ with different rules	Spiral, ~137°
[26]	Auxin concentration and auxin transport by PIN proteins	Yes	Zone-based rates, 0 in CZ and higher in ''proximal'' and peripheral zones, and in primordia	Constant rate	Positive regulation controlled by auxin concentration	Constant	Dynamic and polarized to the side with the highest auxin concentration	Cells grouped into CZ and PZ with different rules	Spiral, distichous, decussate, tricussate
[27]	Auxin concentration and auxin transport by PIN proteins	No de novo evolution. Use of data from confocal images to initiate simulations	Constant, additional production dependent on concentration of ''inducer''	Constant rate	Fixed cellular concentration, cycling between endosome and membrane	Fixed cellular concentration, cycling to the endosome	Dynamic, and polarized to the side with highest auxin concentration	Cells grouped into CZ and PZ with different rules where an auxin inducer acts only outside the CZ	Decussate, spiral-like – not always stable
[28]	Mechanical strain as well as auxin concentration	Yes	Fluctuations in the auxin concentration about a mean level are modeled	Constant rate but with modulations due to mechanical stress	Continuum model considered with no division into cells. Continuum limit of a model with fixed concentration	Continuum model	Continuum model	Auxin and mechanical stress fields coupled together. Auxin dynamics at each point depends on the mechanical stress at that point	Spiral pattern in certain types of cactus

[a] CZ, central zone; PZ, peripheral zone.

[b] Yes indicates that the structure, shape, and size of the cells, the initial orientation and concentrations of the PIN proteins, and the initial auxin concentration, etc. were generated as part of the simulation. No de novo evolution indicates that the initial data was chosen based on observations and confocal images, and there are typically restrictions on the variety of initial conditions that can be computationally evolved to see the patterns that arise.

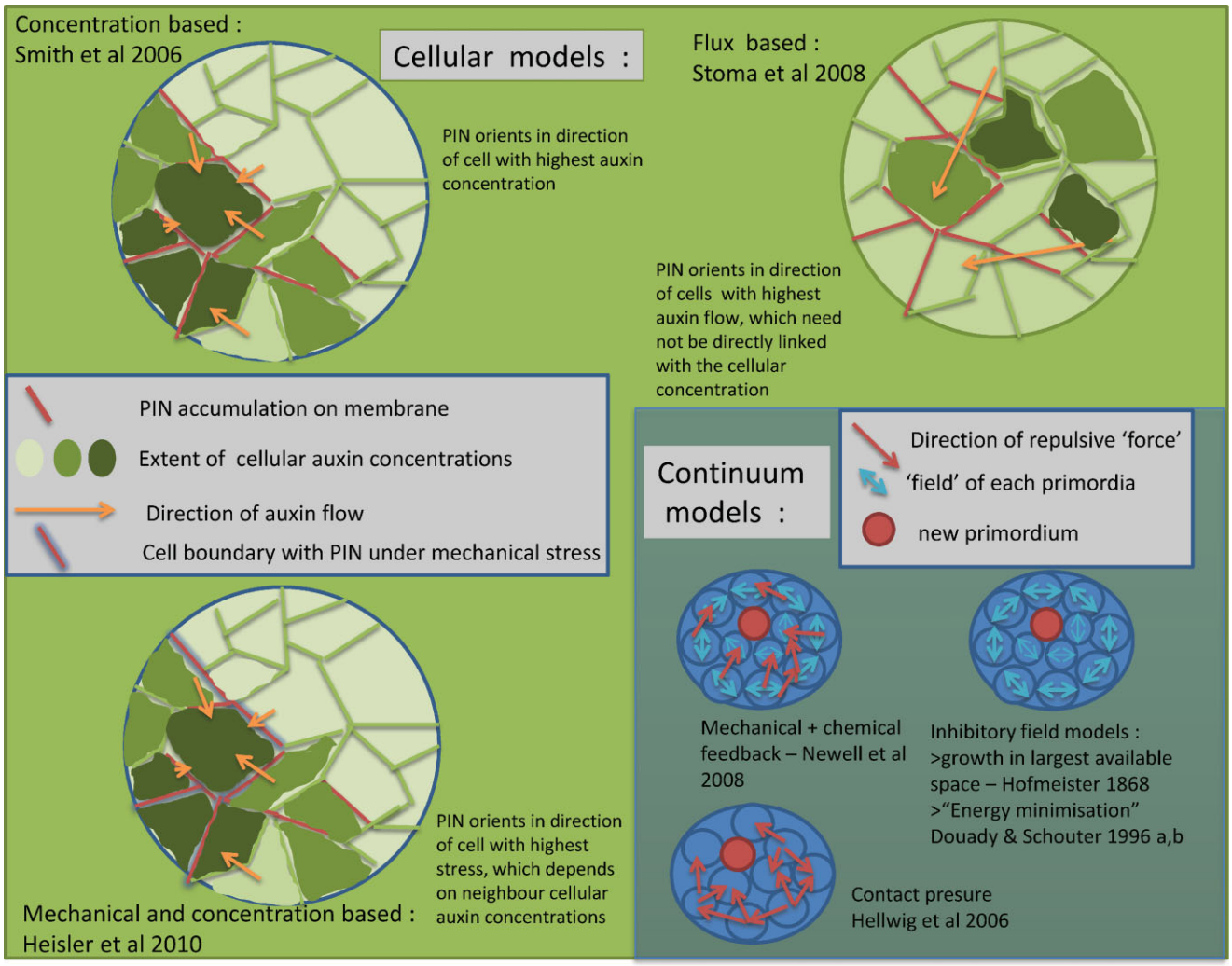

Figure 2. Schematic representation of modeling efforts and their assumptions to simulate pattern formation. For simplicity PIN has been shown only in those cells where it gets oriented in response to auxin concentration, auxin flux or mechanical strain (please see the main text for details).

organs, the role of PIN1 polarity in regulating phyllotaxis demands further interrogation. It will be informative to examine the effects of changes in PIN1 polarity on phyllotaxis by localized delivery of the non-polar PIN1, i.e. PIN1 mutated at residues phosphorylated by the PIN polarity regulator PINOID, in an inducible *pin1*-knockdown background.

Both experimental and theoretical studies have captured the role of polar auxin transport in controlling *Arabidopsis* phyllotaxis. Strikingly, functional polar auxin transport has not yet been detected in the gametophytic shoots of several mosses (non-vascular plants), which exhibit phyllotaxis [46]. However, a hypothetical auxin transport mechanism in moss by carrier-dependent auxin storage in the endoplasmic reticulum has been proposed [47]. Further, an auxin signaling pathway is conserved in *Physcomitrella patens* (moss, a non-flowering plant) [48]. It is plausible that distinct polar auxin transport and/or local auxin synthesis and its accumulation contribute to gametophytic shoot phyllotaxis in *P. patens*.

Meristem geometry influences the pattern formation

How do the chemical properties of cells at the meristem surface influence morphogenetic fields to control phyllotaxis? Surgical experiments together with laser ablation at the site of incipient primordia suggest that morphogenetic fields in the shoot meristem dictate positioning of the primordia [49, 50]. Inhibitory signals can arise either from the center of the meristem or from the primordia to generate a morphogenetic field. Early theoretical work identified the relation between the size of the central zone of the meristem and the size of primordia as an important parameter for pattern formation [14]. Variation in the size of the central zone and the capability of changing the distance between auxin peaks by fine-tuning the relative strength of active and passive auxin transport has been observed in several models [21, 24, 26, 27]. Signals arising from pre-existing primordia direct the next primordium to initiate farthest from existing primordia. The inhibitory field generated in the meristem can be reduced either by reducing the ramp up of PIN1 in the incipient primordia, thus changing the auxin flux, or by increasing the meristem size. Large meristem surface may allow two or more primordia to arise

simultaneously (in opposite or whorl pattern) by making more space available and thus reducing the inhibition between primordia.

Several *Arabidopsis* mutants with enlarged meristems, such as *clv3* or *fas*, display irregular positioning of organ primordia instead of a defined opposite or whorl pattern [51–53]. Besides other factors, the growth hormone cytokinin can regulate the meristem size in plants. Cytokinin oxidase/dehydrogenase enzymes control meristem size; loss of *ckx3*,*ckx5* alone or together with the negative regulator of cytokinin signaling *ahp6* enlarges the shoot meristem [54] and displays random initiation of flowers. There are several examples in grass species, both in maize and rice, where a random organ initiation pattern accompanies the change in meristem geometry. For example, abnormal leaf initiation pattern is associated with the abnormal meristem geometry of maize *terminalear1* mutant [55]. Although the change in the meristem size can have profound effects on organ initiation pattern, it may not suffice to regulate the phyllotactic pattern. For example, rice plastochron1 (*pla1*) and *pla2* mutants with large meristem do not display any change in phyllotactic pattern [56, 57]. It is important to note that, although the size of the meristem of the *pla* mutant is altered, the shape remains invariant, suggesting the meristem size alone may not be sufficient for changes in the pattern, but alteration in shape and size together influence the pattern initiation. Further, the shoot apex of the shoot organization (*sho*) mutant, which displays random phyllotaxy, is not only wide but also has variable shape [58].

Unlike these mutants that do not display a switch from one specific pattern to another, the maize *abphyl1* (*abph1*) mutant shows a clear change in the pattern, with the leaves arising in a decussate fashion instead of the distichous pattern of leaf arrangement typical of grasses [59]. *ABPH1* encodes for a cytokinin response regulator. Interestingly, *PIN1* expression is reduced in the *abphyl1* mutant, favoring a decrease in inhibitory field [60, 61]. These results also suggest that cytokinin signaling can regulate the inhibitory field. In addition, the rice *decussate* (*dec*) mutant shows an increase in the shoot meristem size. A *dec* mutant displaying a change from a distichous to a decussate pattern of leaves further supports the notion that change in the meristem geometry can impact the phyllotactic pattern [62]. Interestingly, OsRR5, the closest homolog of maize *ABPH1*, is down-regulated in *dec* mutant, further supporting a role of cytokinin signaling in regulating phyllotactic pattern in grasses [62]. Unlike the clear switch in maize *abphy11* (*abph1*), a shuttle effect on phyllotactic pattern was seen when several *Arabidopsis* cytokinin A-type response regulators were mutated, demonstrating that their redundant activities do not suffice to shift a spiral pattern to another one. Although the ''on'' and ''off'' ectopic meristem activity in the *wuschel* mutant makes it difficult to assess its effect on lateral organ positioning, the direct control of cytokinin response regulators by WUSCHEL provides, at least in part, a plausible link between stem cell activity and patterning [63]. It is interesting to note that ''meristem size regulation'' by cytokinin signaling in both monocots and dicots is reflected in the control of organ positioning, suggesting a conserved regulatory action of cytokinin signaling in both species. To further understand how different phyllotactic patterns arise in nature, analysis of dynamic changes in expression pattern of meristem growth regulators that specifically affect either stem cells or peripheral zone of the meristem and changes in auxin accumulation pattern should also be carried out in mutants of different model plant species, such as rice and maize, which switch their distichous pattern to decussate.

Changes in physical properties of cells can impact the organ initiation

Besides the altered chemical properties of cells such as auxin accumulation, physical properties such as cell mechanics influence organ initiation by changing the growth of the meristem. The view that biophysical forces can contribute to organ initiation [64] is well supported by a study in which the application of the cell wall expansion-promoter, expansin, resulted in the localized outgrowth of organ primordia and also disrupted the normal phyllotactic pattern to some extent in tomato apices [65, 66].

Recent studies further add to the role of mechanical properties of a cell in controlling organ initiation and growth [67–69]. Interestingly, chemical modification of the cell wall component pectin alters the number of initiating primordia as well as their pattern. Employing atomic force microscopy it has been shown that presuming certain criteria, such chemical modification alters the mechanical properties of cell wall and thus the stiffness at the meristem. The peripheral zone of the meristem, where organs are formed, is less stiff than the central zone [68, 70]. Ectopic expression of pectin methyl esterase, which causes demethylesterification of pectin, results in ectopic initiation of primordia at aberrant positions. Conversely, reduced demethylesterification (stiffer meristem) decreases primordium formation and, in severe cases, completely abolishes the outgrowth [67]. These studies suggest that changes in the chemistry of cell wall components at the primordia inception site are crucial for outgrowth. An interesting question is whether the mechanical changes and the prerequisite of dynamic auxin accumulation are linked, or whether they work independently to trigger primordia initiation. Emerging evidence suggests that mechanical properties of cells can influence PIN localization. Interestingly, mechanical interference with cell wall affects the polar distribution of PIN proteins [71]. Further, mechanical stress induced by the chemical changes in the cell wall upon isoxaben treatment influences PIN1 localization within the cell. Additionally, upon laser ablation in the shoot meristem, PIN gets repositioned away from stressed cells surrounding the ablated region of the shoot meristem [24]. Both experiments and simulations indicate that mechanical signals might be regulating PIN1 polarization and thus auxin transport (Fig. 2) [24]. Remarkably, recent findings demonstrate that growth-induced mechanical strain up-regulates PIN1 localization to the plasma membrane and auxin accumulation, which can in turn promote further growth [72]. PIN1 reorients similarly to microtubules in response to laser ablation. It has been suggested that their localizations are regulated by a common upstream factor such as mechanical signals [24, 73]. Supporting this notion, changes in mechanical properties of the cell wall by local up-regulation of a pectin methyl esterase is sufficient to induce ectopic flowers [24, 67].

Presuming that the mechanical properties of the cell wall influence polar auxin transport in triggering organ initiation, one would expect ectopic demethylesterification at the meristem periphery to initiate a whorl pattern. However, this was not observed on ectopically expressing *pectin methyl esterase* in the meristem. Instead, a more random initiation of ectopic primordia was observed together with occasional simultaneously arising pairs of primordia. This suggests that altering the mechanical properties cannot force all of the polar auxin transport machinery to reorient toward the sites with altered cell wall mechanics, and some site preference of temporal auxin accumulation still remains. Although biomechanical regulation has been proposed to act upstream to polar auxin transport, the pathway need not be linear. Auxin increases expansin activity in the cell wall [74]. It is possible that changes in auxin concentration can feed back to cell mechanics. Newell et al. [28] modeled the feedback interaction between the mechanical and biochemical pathways that determine organ positioning (Fig. 2). Auxin concentrations are directly linked with meristem expansion and vice versa. Merging the individual cells into a continuum made it possible to model the mechanical stresses in a straightforward manner, while the dynamics of the auxin concentration field was treated by taking the continuum limit of the discrete difference equation used in the cell-based model proposed by Jönsson et al. [27]. A coupling between the two was introduced, leading to a closed set of differential equations for the model. They were able to observe complex phyllotactic patterns like those observed in cacti and other atypical phyllotaxis with ridged leaves. From experimental and modeling studies we surmise that the mechanical properties of cells can impact the pattern formation. Despite the emerging evidence of mechanical strain affecting auxin accumulation, it remains largely unknown whether the mechanical properties of cells control the phyllotaxis by regulating the auxin-accumulation pattern.

How is the floral organ positioning controlled? Lessons learnt from old mutants

Flower meristem identity genes control the floral organ positioning

Remarkably, various plant species display a stable change in organ positioning pattern during their life cycle. Such a switch of pattern is conspicuous upon transition from the vegetative to the reproductive phase. For example, plants with spiral or opposite leaves exhibit the whorl pattern of floral organs. In *Antirrhinum*, leaves are produced in a decussate pattern during the vegetative phase and upon transition to the reproductive phase, the leaf-like organs (bracts) and flowers are produced in spiral pattern. Interestingly, floral organs are produced in a whorled phyllotaxy [7]. Similarly, in *Arabidopsis*, leaves are produced in spiral fashion and floral organs (sepals, petals, stamens, and carpels) all arise in whorl pattern (Fig. 3A–C). An obvious question that arises is how do the plants that produce lateral organs in a specific pattern on the shoot meristem switch to an entirely different, yet stable,

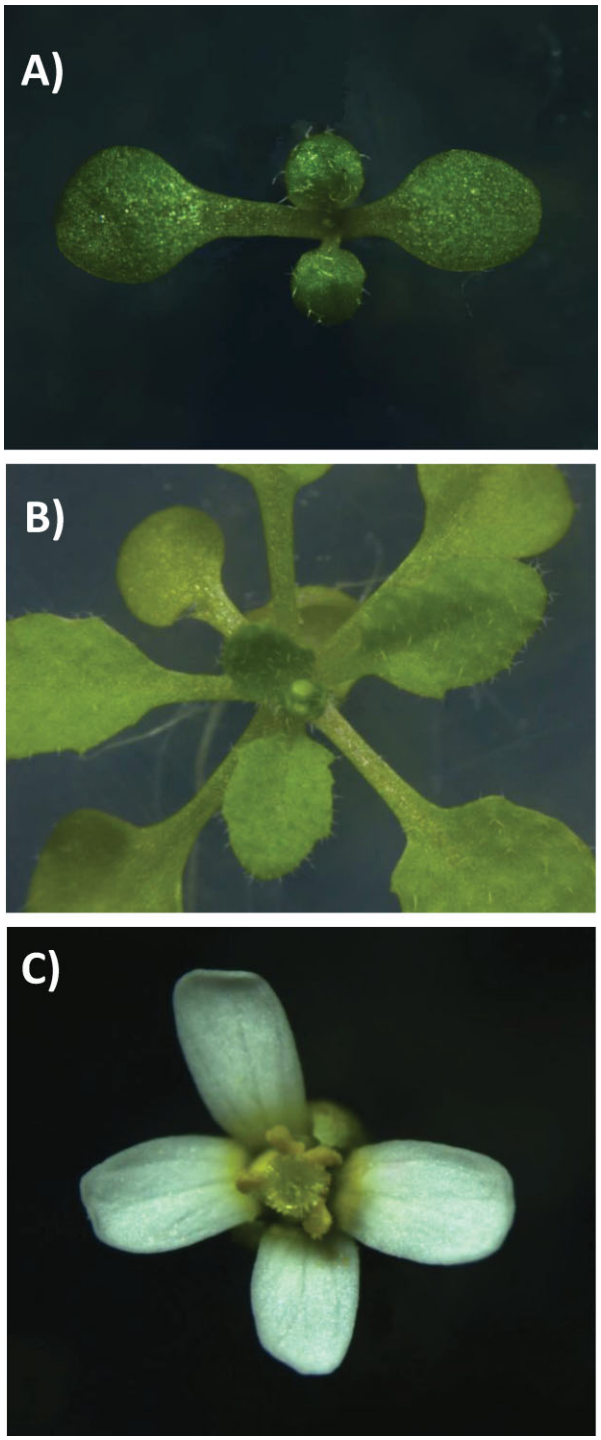

Figure 3. Transition of phyllotactic patterns in *Arabidopsis thaliana*. **A:** Cotyledon and first pair of *Arabidopsis* leaves show a decussate pattern. **B:** Transition from the decussate to a spiral pattern can be seen when the rosette leaves emerge at the shoot apex. **C:** Upon transition from the vegetative to the reproductive phase, flowers are produced and the floral organs are positioned in a whorl. Sepals, petals, stamens, and carpels in the flower are arranged in a whorl pattern.

pattern on the floral meristem? Interestingly, the nature of these two meristems is different; while the shoot meristem is indeterminate, the flower meristem is determinate. Mutation in floral meristem identity genes such as Arabidopsis *LFY* results in loss of floral meristem determinacy and the plants display shoot-like and flower-like organs in a partially spiral phyllotaxy [75–77]. Arabidopsis LFY binds to the regulatory regions of the AP2 domain-encoding genes *AINTEGUMENTA* (*ANT*) and *PLETHORA3* (*PLT3*). Furthermore, in the *plt3*,*ant* double mutant flowers, the whorl pattern is completely disrupted and floral organs with altered identity are irregularly positioned [78], suggesting that LFY regulates floral organ positioning in part by regulating *ANT* and *PLT3* [79, 80]. Similar to *Arabidopsis*, in *Antirrhinum* the whorl arrangement of floral organs is dependent on the action of *FLO* (*LFY* homolog) and *SQUA* (*AP1* homolog) genes. Mutation in either of these genes leads to a near-spiral arrangement of shoot-like floral organs instead of a whorl pattern [81, 82]. Further, mutation in the *Arabidopsis UFO*, the activity of which is dependent on LFY, or mutation in the *Antirrhinum* counter part *FIM*, leads to a tendency toward producing floral organs in a spiral [79, 83]. Remarkably, maize *FLO*/*LFY* homologs *ZFL1* and *ZFL2* control whorled floral organ phyllotaxy. The *zfl1*,*zfl2* double mutant male florets proliferate shoot-like organs in a spiral phyllotaxis instead of whorl [84]. These studies suggest that the type of phyllotaxis and determinacy of the shoot apex may be correlated. However, the differences in the nature of the meristems alone may not fully explain the initiation of two different patterns at the shoot meristem and flower meristem. The *Arabidopsis agamous* mutant flower meristem is indeterminate yet produces floral organs in a whorl pattern [85].

Effects of changes in auxin signaling and meristem size

Auxin accumulation patterns are quite dynamic in different stages of *Arabidopsis* flower primordia before they produce floral organs. Auxin is accumulated throughout the initiating flower primordia, and perhaps such high levels of auxin set up a reduced field of inhibition to begin with [86, 87]. As expected, just before producing a whorl of sepals, auxin accumulation marks the spots in the flower meristem where four sepals will be formed in a whorl pattern [23]. Thus, polar auxin transport acts in the flower meristem as predicted from modeling of the patterns in the shoot meristem [26, 27]. However, it remains largely unknown how an initial ramp up of auxin in the developing flower primordia sets the morphogenetic field for a whorl pattern in *Arabidopsis* flowers. It is interesting to note that several mutants that display abnormal phyllotactic patterns of lateral organs derived from shoot meristem continue to maintain the global whorl pattern of floral organs (Table 2). For example, in the *pin1* mutant occasional flowers maintain the whorl pattern, although the spacing within the whorl is altered [35]. Thus, the strong mutant defective in polar auxin transport cannot completely abolish the whorl pattern. Further, mutants in which the floral meristem is defective in stem cell maintenance and terminates quickly, such as the *wus* mutant or a mutant for which the final meristem size is larger, such as *clv3*, floral organs continue to retain the whorl pattern, suggesting that alteration in the floral meristem size alone may not be sufficient to change the prespecified whorl pattern. On the other hand, there are examples such as *Helianthus annuus* L. (Asteraceae) where, upon transition to reproductive phase, the size of apical meristem increases and the meristem becomes flattened. This exemplifies the circumstantial correlation between change in meristem size/shape and pattern of floral organ arrangement [90].

Further, *Arabidopsis* mutants that change the floral organ number such as *perianthia* (*pan*) retain the whorl pattern [91]. The *ETTIN* gene, which encodes an auxin response factor, together with *PAN* controls spacing between sepal or petal primordia within a whorl. Although the whorl pattern does not change to another pattern in the *ettin*, *pan* double mutant, spacing within the whorl of sepals and petals become

Table 2. Patterns of flower primordia and floral organs in different mutant backgrounds defective in meristem size or hormone signaling

Mutant	Gene	Flower primordia pattern (inflorescence meristem)	Floral organ pattern (floral meristem)	Altered meristem size/growth hormone signaling	Reference
clv3	*CLAVATA3*	Random	Whorl	Large IM, FM[d]	[52]
ckx3, *ckx5*, *ahp6*	*CYTOKININ OXIDASE/ DEHYDROGENASE, ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6*	Random	Whorl	Large IM, FM, and defective in cytokinin signaling	[54]
ant, *ail6*	*AINTEGUMENTA, AINTEGUMENTA-like6*	Spiral	Random[b]	CZ[a]	[78]
wus	*WUSCHEL*	On/off meristem activity	Whorl	CZ[a]	[88]
ett, *pan*	*ETTIN, PERIANTHIA*	Spiral	Whorl[c]	Defective in auxin signaling	[89]

[a] CZ, central zone – defective in stem cell maintenance.
[b] Accompanied with severe outgrowth and identity defects.
[c] Spacing within a whorl is altered.
[d] FM, floral meristem; IM, inflorescence meristem.

irregular [89]. Furthermore, the *ettin* mutant when combined with *clv3* increases floral organ number in each whorl without affecting the whorl pattern [89]. Thus, alteration in auxin response by loss-of-function of *ETTIN* and changes in meristem size by removing the activity of CLV3, together are unable to cause any change in whorl pattern of floral organs in *Arabidopsis*. It is conceivable that once the whorl pattern is initiated, further decrease in the inhibitory field by increasing the meristem size would maintain the whorl pattern instead of changing it. Nevertheless, the question still remains how the sporadic initiation of primordia in the *clv3* mutant shoot apex transits to a well-defined whorl pattern of floral organs. Notably, changes in the mechanical properties of the cell alter the phyllotaxis of flowers but are unable to alter the whorl pattern within the flower [67].

Floral organ positioning in grasses

In another model plant species, *Oryza sativa*, a member of the grass family, flowers (spikelets) are produced from a branched stem. A mature spikelet comprises sets of bract-like structures, a pair of rudimentary glumes and a pair of empty glumes. These bract-like structures enclose florets comprising of lemma and palea (sepal analogs) and three whorls of floral organs: two lodicules (petal analogs), six stamens and a central carpel [92]. Thus, upon transition from the vegetative to the reproductive phase, there is a transition from a distichous to a whorl pattern. A double AP2 domain containing the protein SUPERNUMERARY BRACT (SNB) regulates the transition from the spikelet to floret meristem. The *snb* mutant produces several bract-like structures in an alternate fashion, suggesting a role for SNB in promoting whorled phyllotaxy [93]. Although the experimental studies have begun to yield information on molecules controlling the phyllotactic patterns of floral organs, the mechanisms of switch of the pattern remain largely unknown. It will be interesting to evaluate whether transition of patterns during the plant life cycle utilizes unique mechanistic or shared evolutionary modules.

Search for the mechanism behind the models

An overview of the different modeling efforts is presented in Table 1 and Fig. 2 where the assumptions that go into each model as well as the main conclusions that emerge are summarized. Phyllotaxis is the result of regularly spaced auxin peaks, and can be thought of as a product of directional auxin transport via the dynamic localization of PIN1 proteins. There are two main approaches to modeling the dynamic localization of PIN1 proteins: mechanical, chemical, and a third in which these two are combined. In the chemical models [22, 25–27], PIN1 localization is dependent on the concentrations and fluxes of auxin in neighboring cells. In the concentration-based models [26, 27], PIN1 proteins localize most onto adjacent cells with the highest auxin levels. In flux-based models [25], PIN1 proteins localize most onto adjacent cell membranes that have the highest auxin flow through them. Mechanically based models [24] are based on the premise that auxin affects cell wall mechanics. An increase in the auxin levels causes a decrease in the cell wall's rigidity, and thereby an increase in the amount of force that it exerts onto neighboring cells. In this formulation, PIN1 proteins localize mostly onto cell membranes that are experiencing the highest stress from neighboring cells, and as a result they orient toward the cell with the highest auxin concentration. Among these models, Smith et al. [26] have been successful in recreating the largest range of phyllotactic arrangements. Their de novo concentration-based model generated distichous, decussate, tricussate and spiral patterns, all of which are commonly observed in nature.

The dynamic localization of PIN1 proteins brings about an auxin-PIN feedback loop in the meristem. PIN1 proteins orient themselves toward the region with the highest stress/auxin levels (depending on mechanism) and lead to a local accumulation of auxin in a focused region. This auxin accumulation strengthens the current orientation of PIN molecules, until a new primordium emerges and the orientations begin to change. Models like those of Stoma et al. [25] and Smith et al. [26] incorporate another level of feedback by making the cellular synthesis of PIN1 protein itself dependent on auxin concentration or flow, unlike other models that assume a fixed concentration of PIN1.

Conclusions and perspective

The majority of the experimental studies focus on how the spiral phyllotaxis is propagated in the model dicot species *Arabidopsis*. The question that remains largely unanswered is how diverse patterns arise in nature. There is compelling evidence pointing toward the role of auxin in controlling the organ initiation pattern. Nevertheless, alteration in polar auxin transport alone does not suffice to explain the formation of various patterns, which appear to be more complex [94]. Most of the experimental studies carried out in the dicot model or grass species suggest a role of auxin and cytokinin in controlling phyllotaxis. Other hormones such as gibberellic acid can also bring about chemical changes and influence pattern formation. For example, gibberellic acid treatment of *Xanthium* shoots alters the phyllotactic leaf arrangement [95]. It is likely that cross-regulation between cytokinin or gibberellic acid signaling pathways and auxin signaling control the phyllotaxis. Given the tools available to sense the hormone response and its directional transport, auxin seems to be the best candidate that can be used as a read out of chemical changes during dynamic pattern generation.

From the knowledge on *Arabidopsis*, it is apparent that the pattern formation is determined by chemical and physical change(s) in the meristem. Changes in mechanical properties of cells influence growth heterogeneity in the shoot meristem and thus impact morphogenesis [96]. Accumulating evidence indicates that mechanical signals, auxin accumulation and growth are linked to each other [72]. Transient manipulation of cell mechanics or auxin accumulation, which can be controlled in time and space, together with live imaging in models of plant species should probe how physicochemical changes influence the growth dynamics of meristem and thus the pattern formation in real time. Moreover, changes in patterns during the life cycle of plants should be exploited for an in-depth understanding of mechanisms of pattern formation. The

challenge ahead is to unravel whether the transition from one pattern in the vegetative phase to another in the reproductive phase (flowers) utilizes a common regulatory module and whether evolutionary tinkering with this module played an important role in floral evolution.

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