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REVIEW

Organogenesis from stem cells *in planta*: multiple feedback loops integrating molecular and mechanical signals

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Abstract In multicellular organisms, the coordination of cell behaviors largely relies on biochemical and biophysical signals. Understanding how such signals control development is often challenging, because their distribution relies on the activity of individual cells and, in a feedback loop, on tissue behavior and geometry. This review focuses on one of the best-studied structures in biology, the shoot apical meristem (SAM). This tissue is responsible for the production of all the aerial parts of a plant. In the SAM, a population of stem cells continuously produces new cells that are incorporated in lateral organs, such as leaves, branches, and flowers. Organogenesis from stem cells involves a tight regulation of cell identity and patterning as well as large-scale morphogenetic events. The gene regulatory network controlling these processes is highly coordinated in space by various signals, such as plant hormones, peptides, intracellular mobile factors, and mechanical stresses. Many crosstalks and feedback loops interconnecting these pathways have emerged in the past 10 years. The plant hormone auxin and mechanical forces have received more attention recently and their role is more particularly detailed here. An integrated view of these signaling networks is also presented in order to help understanding how robust shape and patterning can emerge from these networks.

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Introduction

The transformation of a little globular embryo into a complex organism composed of thousands or millions of cells, with a specific architecture, polarity, and a stereotyped morphology is one of the most fascinating processes in biology. In multicellular organisms, this requires the coordinated behavior of individual cells, which in turn relies on signals of both biochemical and biophysical nature [82]. The molecular genetics behind these processes has made considerable progress, notably through the development of high-throughput protocols. How thousands of genes interact together, how gene networks are regulated in space and time, and how they impact the final shape are among today's most thriving questions in development. The complexity behind the presence of a dynamic signaling network in a growing structure—an issue that had been underestimated by molecular reductionism for a long time—is now beginning to be addressed using integrative and multidisciplinary approaches [175]. Our current view of the signaling pathways acting in the shoot apical meristem (SAM) of higher plants illustrates this perfectly. Located at the tip of branches, the SAM contains a population of self-renewing stem cells whose activity is responsible for the indeterminate shoot growth and the production of all aerial parts of the plant. During its entire life, new organs such as leaves, flowers, and branches are continually generated at specific sites on the stem, producing a regular arrangement of organs. Also known as

phyllotaxis, this is one of the most striking examples of biological patterning in nature. Organogenesis at the plant shoot apex thus raises two challenging questions: how can the shape of the SAM maintains itself despite permanent organ formation? What mechanisms of positional information account for organogenesis and phyllotaxis?

Early studies demonstrated that cell–cell communications and non-cell-autonomous processes are at the basis of SAM function. Now, a growing number of molecular players have been identified. The crucial role of plant hormones, such as auxin and cytokinin, in growth and development was established decades ago, but considerable advances have been made more recently and their contribution to meristem development is currently being extended and integrated with other factors. In particular, signaling peptides and mobile factors, such as transcription factors or small RNAs playing key roles in specific meristem functions have been described, and the role of mechanical signaling within the SAM is raising exciting perspectives. The mechanisms of action of these signals have been extensively reviewed [5, 48, 70, 78, 174], particularly in the case of stem cells in the central zone [37, 66, 159]. Here we focused specifically on the signals involved in organogenesis at the SAM. After a brief description of the structure of the SAM and its genetic regulation, we describe the cellular parameters controlling the global shape as well as the various signaling pathways wiring the tissue. The role of two signals during organogenesis, the plant hormone auxin and mechanical forces, are described in more detail. Finally, we present recent findings showing that proper organogenesis and patterning in the SAM rely on complex crosstalks and feedback loops between these signaling pathways.

Morphogenesis at the SAM: highly dynamic cells produce a stable structure

The structure and function of the SAM have been extensively described elsewhere [111, 160, 168]. We will simply summarize its main features from a morphogenetic perspective.

Different species produce leaves or flowers of very different shape. Even within a given species, the SAM can produce very different organs, depending on the phase of its life cycle, from leaves with various shapes during the vegetative phase, to flowers during the reproductive phase. However, all SAMs in higher plants share a stereotypical common structure: a central, often dome-shaped domain surrounded by several emerging organs (Fig. 1a). Cells in the meristem proper are considered as undifferentiated, because of their smaller size, a reduced vacuole, and an active mitotic cycle. The tissue structure is nonetheless highly organized in the SAM: the outermost cells define one or several continuous surface layers, called the tunica, covering internal tissue, referred as the corpus. These external layers, numbered from the periphery L1 and L2, maintained themselves as sheets thanks to preferential anticlinal cell divisions (Fig. 1b). Chimera analyses have shown that daughter cells generally remain within the same layer as their mother. Notably, the L1 layer never loses its organization and generates the epidermis of all the aerial parts of the plant. The L2 layer usually loses its planar organization at sites of organogenesis due to random directions in cell division planes. In the corpus (also called L3), cell division planes are more random as well.

Mapping the cell division rates in the meristem revealed that a partitioning into functional zones is superimposed to this layered organization (Fig. 1b, c). First, at the meristem summit, a group of slowly dividing cells defines the central zone (CZ) and ensures meristem maintenance. The CZ contains the stable long-lived stem cells from which all shoot tissues are ultimately derived after germination, and extends through the tunica layers down to the upper part of the corpus. The CZ is surrounded by stem cell daughters, which are progressively advected away from the center by the continuous growth of the structure. Consecutively, these cells enter the peripheral zone (PZ) where organogenesis occurs. In that domain, a restricted number of founder cells considerably increase their proliferation and elongation rate, allowing the formation of the organ primordium. Cells surrounding the primordium adopt a much lower growth rate and a strong anisotropy: they will establish a boundary between the new organ and the meristem. Note that in the ring-shape PZ, only a small subset of cells becomes founder cells. Thus, only a defined number of primordia can arise at the same time. Interestingly, the angle between two successive organs is fixed and depends on the species (Fig. 3a). This will ultimately account for the distribution of lateral organs along the main axis of the stem, also called “phyllotaxis”. Finally, the rib zone is located below the CZ and cells entering this zone produce all the tissues of the stem. Therefore, despite the dynamics of cell behavior within the SAM, this structure exhibits a remarkably stable organization with well-defined positional information and thus represents a model of choice to understand developmental homeostasis.

Patterning of the SAM is under genetic control

To investigate the genes underlying the meristem activity, mutants of various model species (mainly *Arabidopsis thaliana*) have been screened for defects in stem cell maintenance, organ initiation, outgrowth, and polarity. Most of these mutants are impaired in transcriptional regulators that are highly conserved in angiosperms. Their

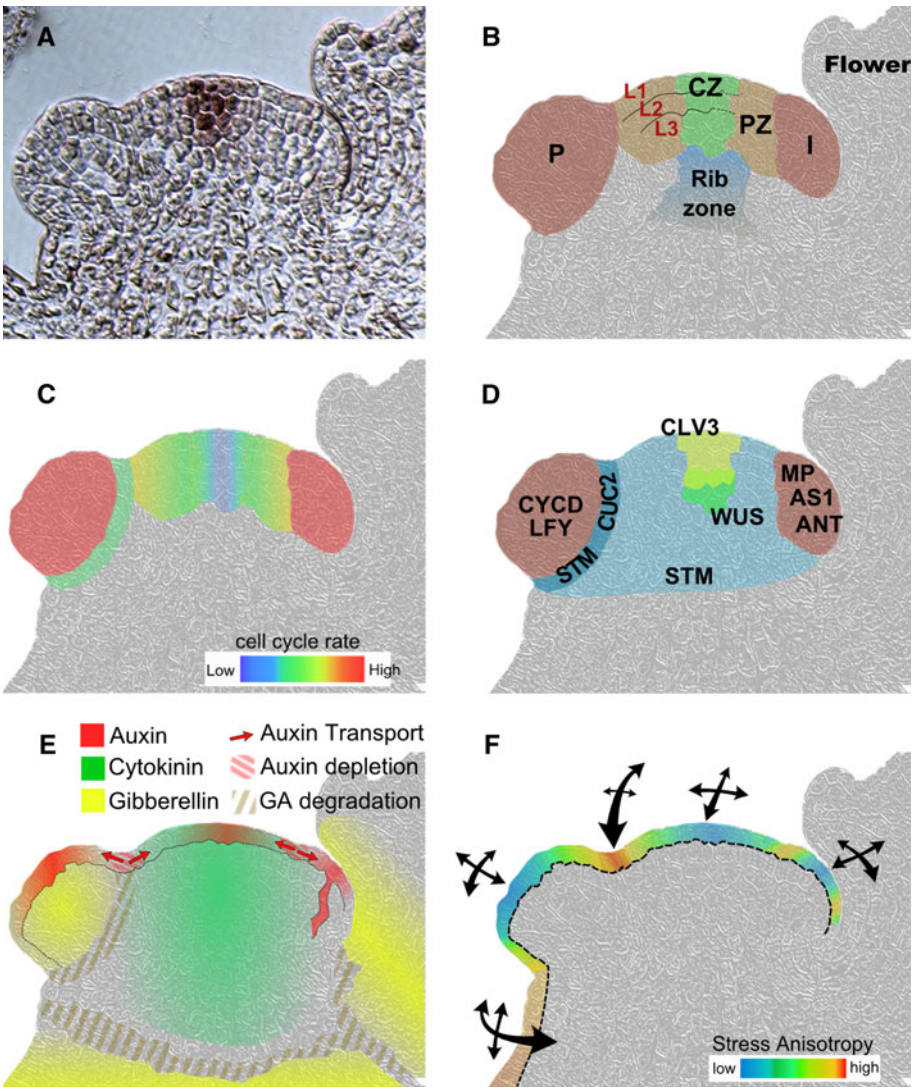


Fig. 1 Structural and functional organization of the SAM in *Arabidopsis thaliana*. **a** Longitudinal section of a paraffin-embedded WT SAM of the plant model *Arabidopsis thaliana*, showing an in situ hybridization of the *CLV3* gene. **b** Structural zonation of the SAM, *CZ* central zone, *PZ* peripheral zone, *P* primordium, *I* Initium, L1–L3 cellular layers. **c** Spatial variations of cell cycle rate in the SAM. **d** Genetic zonation of the SAM. **e** Putative distribution for the three main hormones auxin, cytokinin, and gibberellins in the SAM. **f** Mechanical stresses in the SAM. The models predict that the L1 is under tension and internal tissues are under compression. Anisotropy of stress varies across the L1 layer; *arrows* indicate relative strength (by size) and direction of stress anisotropy in different subdomains (see text for details)

expression patterns are often correlated with the histological zonation presented above. In addition, some also define several important functional subdomains. Here, we only describe the main genes characterized in the model plant *Arabidopsis thaliana* (Fig. 1d).

The integrity of the stem niche relies on an organizing center, defined by cells expressing the homeobox transcription factor WUSCHEL [100]. The activity of *WUSCHEL* (*WUS*) promotes stem cell identity in the cells above [114]. Its precise targets are still unknown, even though a recent study demonstrates that it acts as a transcriptional repressor in this function [79]. The stem cell subdomain is associated with the expression of the *CLAVATA3* (*CLV3*) gene [139] (Fig. 1a), whose transcription is dependent on *WUS* activity. CLV3 is a secreted peptide, which acts as a non-cell autonomous signal repressing the transcription of *WUS* [43, 93]. This creates a dynamic feedback loop that regulates stem cell number and is essential for meristem homeostasis [19, 148]. Here, stem cells are thus not defined by a positive regulator but rather by the transcription of a signaling molecule that eventually represses stem cell identity and its own expression. Other markers for stem cell identity not involved in the *WUS–CLV3* loop have recently been identified [1, 187] and could lead to the identification of such a positive regulator. Note also that the core *CLV3–WUS* loop is connected to a complex gene regulatory network [37, 66, 159] involving hormone signaling and particularly cytokinin [57, 103], nutrients availability, transcription regulation, or chromatin remodeling. The elucidation of the connections between these different regulatory levels is only beginning and will certainly fuel further research on stem cell homeostasis in the coming years.

Surrounding the stem cell pool, two antagonistic pathways control cell differentiation in the PZ. First, the maintenance of a meristematic undifferentiated identity requires the action of the homeodomain protein SHOOTMERISTEMLESS (STM) [39, 50, 104, 110] in combination

with several members of the CUP-SHAPED COTYLEDON (CUC) family of transcription factors [3]. However, as soon as cells are recruited in an organ, these meristematic identity genes are switched off by an unknown factor and maintained in a repressed state by organ-specific regulators like the MYB transcription factor ASYMMETRIC LEAVES 1 (AS1) [21]. The recruitment of founder cells depends on transcription factors belonging to the auxin signaling pathway, like the AUXIN RESPONSE FACTOR 5/MONOPTEROS [4, 71, 130, 137, 149].

As an initium starts to emerge, its size, polarity and identity are progressively determined by a complex network of genetic regulation. The organ identity mainly depends on the presence of the transcription factor LEAFY (LFY), which acts as a master switch controlling the organ fate, typically between a leaf and a flower [180]. Many genes are expressed specifically in boundaries where they are necessary for proper organ separation like *CUC* genes [2, 75]. Some boundary genes like *JAGGED LATERAL ORGANS* both interact with genes of the *STM* gene family (the *KNOTTED*-like Homeobox or *KNOX* genes) and auxin transport, and could coordinate the drastic changes in gene expressions occurring between the meristem and developing lateral organs [15]. Organ polarity relies on the antagonistic activity of HD-ZIP class III transcription factors (PHAVOLUTA/REVOLUTA/PHABULOSA) promoting adaxial polarity (i.e., adjacent to the meristem, the dorsal side of the organ) and *KANADI* and *YABBY* genes promoting the abaxial polarity (i.e., away from the meristem, the ventral side of the organ) [18].

Finally, transcription factors like AINTEGUMENTA (ANT) control organ outgrowth and its final size, notably by promoting the expression of the cell cycle gene *CYCD3* [118]. The expression patterns of some of these genes are further defined by miRNAs. For example, HD-ZIP class III transcription factors and *CUC* mRNA are known to be targeted by miRNAS [131].

Many other genes have been identified as regulators of shoot meristem activity and we refer the reader to recent reviews describing these in further detail [8, 66, 78, 132].

The cellular level: relevant parameters controlling shape in a growing meristem

Thanks to the qualitative description of mutants, many important master regulators of meristem functions have been isolated. A key challenge is now to explain how shape changes, homeostasis, and cell fate can emerge from this genetic network. To address this question, a quantitative description of cell behavior must be undertaken in order to establish a causal link between the gene activity and the meristem biology.

Being a key marker of SAM organization, patterns of cell proliferation rate have been extensively studied in the SAM [20, 59, 81, 98, 99, 133] (Fig. 1c). It was notably thought that this parameter is essential to allow organ bulging or crease formation. Surprisingly, SAM morphology is resilient to a wide range of proliferation rate modifications, in contrast to organs with determinate growth like leaves [185]. Too much proliferation may disorganize cell arrangements in the tunica [16] and too few impair meristem maintenance or promote cell differentiation [6, 47]. Generally, however, cells can compensate a decrease in cell proliferation by increasing their size and higher proliferation rate by reducing their size. This suggests that the frequency at which cells divide is not essential for morphogenesis in the SAM.

It seems therefore appropriate to consider shape changes in meristems in terms of growth rate and anisotropy [32]. Two different non-invasive techniques are now available to retrieve these parameters from sequential in vivo meristem observations. In the sequential replica method, moulds of the surface of apex are taken at different time points. These moulds are then used to prepare rigid casts that can be examined in scanning electron microscopy, giving an indirect time-course imaging of the meristem [182]. A 3-D shape of the surface is obtained thanks to stereoscopic reconstructions and quantitative data of its changing geometry, like the meristem curvature, can be computed [38]. The cell shapes at the surface of the meristem can be compared for different time points, allowing a rigorous analysis of growth (rate and anisotropy) at the cellular level. An alternative method is based on the observation of living meristems by confocal laser-scanning microscopy (CLSM, reviewed in [152]). This time, internal tissue can also be observed and growth analysis can be directly coupled with in vivo gene expression reporters. A pipeline method has recently been developed to reconstruct a 4-D meristem at cellular resolution, with cell lineages tracking [41]. The digitized virtual meristems can serve as a template to provide any growth-related data (growth rates, anisotropy, or volume) for any cell in the tissue.

If shape changes are driven by the modification of growth rate and anisotropy, then how are these parameters controlled at the molecular level? In plants, they depend on the cell wall properties. Cell growth is driven by internal turgor pressure and is limited by the stiffness of the wall. The presence of rigid cellulose microfibrils is the main determinant of wall stiffness, which can be modulated by the extent of crosslinking in the wall matrix (mainly containing pectins, hemicellulose, and proteins). By regulating the mechanical properties of their walls, the cells can control their growth rate. Anisotropy is determined by the structure of the wall: the orientation of stiff cellulose microfibrils limits cell wall extension parallel to the

microfibrils, but is permissive to wall extension in the direction perpendicular to the microfibrils. Regulators of growth rate and growth direction have been identified: several proteins, like expansins, which can increase the spacing between cellulose microfibrils, modulate growth rate [33, 115]; cortical microtubules, by guiding the movement of the cellulose synthase complex, control the orientation of cellulose microfibrils in the wall, and thus control anisotropy [65, 125].

At this stage of the review, we are drawing a picture in which an array of master regulators of meristem functions (mainly transcription factors) directly or indirectly control the properties of the cell wall, and thus generate domains with specific growth parameters and identity. This of course raises many questions. First, what is responsible for the coordination of growth between the different domains? How can we explain the stereotyped gene expressions and growth patterns in the meristems? How is cell–cell communication mediated? Is there feedback from multicellular growth on cell fate?

To what extent are the functional domains of the SAM interconnected?

Exploring cell–cell communications by microsurgery

To address cell–cell communication within the SAM, several creative micromanipulations protocols have been developed. For obvious technical reasons, more attention has been historically paid to species harboring bigger meristems. A long time before the molecular biology revolution, princeps experiments of microsurgery on meristems [116], later complemented by modern laser ablation [134, 135], revealed the extent of intercellular communications in many aspects of meristem function.

First, after needle pricking in the center of the SAM, a meristem usually regenerates [109, 128, 165]. Depending on the size and position of the wound, a new functional meristem is formed. Similarly, when a meristem is cut and reduced to a small cluster of cells, a new entire dome can be regenerated [160]. This demonstrates the robust homeostasis of stem cells. After laser ablations of the center of tomato meristem, analysis of gene expression indicates that the establishment of a new meristem center is associated with the ectopic induction of the *WUSCHEL* gene in the peripheral zone [134]. Early WUS activation is also required during in vitro shoot regeneration from undifferentiated cells in culture [58]. These experiments identify WUS activation as a first step during meristem recovery or formation, and suggest that a robust cell–cell communication network senses WUS loss and ensures its reactivation. Modeling approaches can give some insight into the mechanisms allowing such behaviors. Two works were able to reproduce the capacity of meristem to recover from ablation [76, 85]. Conceptually based on reaction–diffusion systems, they incorporate interactions between stem cells and the organizing center through the CLV3–WUS signaling (Fig. 2a). However, this feedback loop alone is not sufficient to provide self-organizing properties to the meristem: both models have to introduce spatial components within the meristem to correctly anchor the organizing center and the stem cells at their right locations. The authors suggest that the competence of each cell within a meristem is influenced by a combination of different positional cues: the CLV3–WUS feedback loop and other signals, which could derive from the epidermis or from the vasculature (Fig. 2a).

Conversely, ablations can be used to investigate the extent of autonomy that each zone displays. For instance, organ initiation can go on without a lag after the destruction of the central zone [134]. Therefore, organogenesis does not require the stem cells in the short term, even though in the long term, stem cells constantly supply new founder cells. Each zone is of course not totally autonomous from one another. In ferns, if a primordium is physically separated from the meristem center, its development switches from a determinate to an indeterminate growth, forming a new meristem [179]. This suggests that cell fate and differentiation in the peripheral zone depend on signaling from the center. In angiosperm, such a drastic change in cell fate cannot be reproduced. Nonetheless, a similar surgical separation of a primordium from the central zone alters the polarity of the developing organ, supporting a view in which a signal from the meristem is required to determine dorsal or ventral identities of the emerging organ [135, 164] (Fig. 2b).

Microsurgery experiments also gave important insight on the control of phyllotaxis. Organ positioning around the PZ is highly stereotyped, generating intriguing regular patterns with mathematical precision, as seen for instance in a sunflower capitulum (see also Fig. 3a in *Arabidopsis*). The phyllotactic arrangement at a given time point is mainly the result of the number of organ initiated at the same time around the PZ and of the divergence angle between successive primordia [94]. Snow and Snow [156, 157] performed a series of pioneering experiments on *Lupinus albus* meristems. Primordia were isolated from the meristem by tangential incisions and they observed that the next leaves to form near the incisions were displaced, showing that organ position is not predetermined. The authors proposed that preexisting organs help to position the younger organs by defining a pattern of geometrical constraints: an organ would emerge wherever a free surface area is available in the PZ. Further experiments proved that the control of the organ position did not simply involve

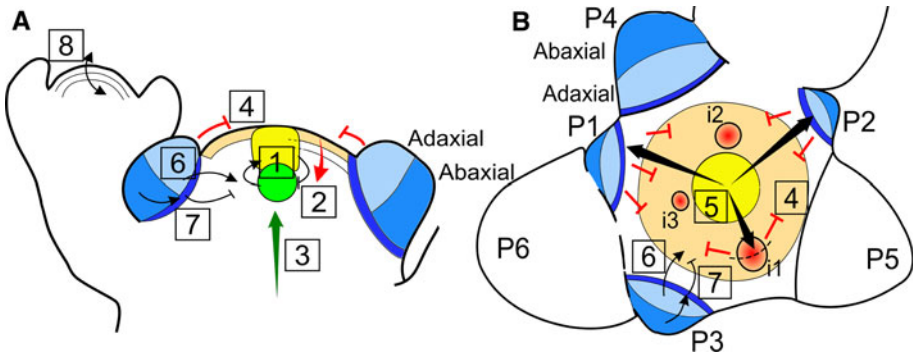


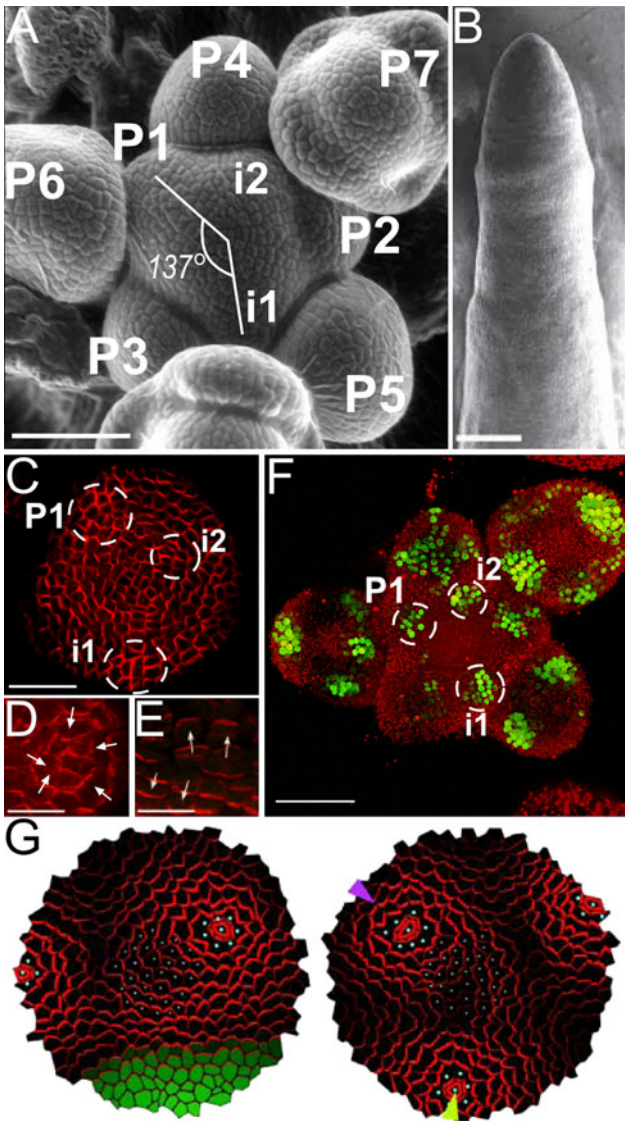
Fig. 2 Schematic representation of some non-cell autonomous processes in the SAM. Longitudinal view (**a**) and top view (**b**) of an *Arabidopsis* inflorescence meristem. Primordia (P) are numbered from the youngest P1 to the oldest P6 and initia (*bright red circles*) are numbered in the order of emergence. *1* stem-cell homeostasis relying on a regulatory loop between the organizing center (*green*) and the stem cell pool (*yellow*). *2* Organogenesis in the peripheral zone (*light red*) controlled by the L1. L1 could also provide putative positional cues for anchoring stem cells. *3* Putative positional cues coming from the stem and or the vascular system. *4* Phyllotaxis relying on lateral inhibition of primordia. *5* Organ polarity controlled by a signal coming from the meristem. *6* Meristem activity controlled by an adaxial signal coming from the organ. *7* Meristem activity antagonized by an abaxial signal, in a two-step signaling module with a relay in the boundary domain (*dark blue*). *8* Identity and shapes of the organ in flowers depending on complex communications between clonal cell layers

steric constraints: using bigger meristems (from a fern, *Dryopteris*) in which no physical contacts between primordial exist, Wardlaw [178] observed the same displacement of phyllotaxis. More recently, Reinhardt et al. [134] show that isolated tomato meristems, only displaying the smallest visible primordium, continue their ontogenic spiral. These results suggest that phyllotaxis do rely on interactions between organs, and that the nature of this interaction involves chemical and/or mechanical signaling in tissue rather than simple steric contacts between primordium (Fig. 2).

Exploring cell–cell communications by genetics

In addition to microsurgical approaches, cell–cell communications in the SAM have also been historically analyzed using genetic chimeras. In plant chimeras, cells of two different genotypes, i.e., coming from two different plants, coexist. A genetic mosaic can be generated by specific mutations affecting a subset of cells in a plant. These genetic differences in the same plant offer an attractive way to question the mechanisms of growth coordination between cell layers and to discriminate cell-autonomous versus non cell-autonomous behaviors. In particular, the L1, L2, and L3 layers separately or in combination, were shown to impact the behavior of other layers, conferring a particular shape or fate to the whole organ [146, 166]. For instance, the analysis of chimeric flowers in camellias suggests that the identity of floral organs, like petals, stamens, and carpels, could be given by the genotype of the L1 layer. Furthermore, the analysis of chimera in *solanacea* species with different leaf shapes revealed that only the L2 layer specifies whether the final leaf will be simple or compound. Other morphological traits of leaves like dorsiventral polarity or blade extension are controlled by both L2 and L3 in tobacco, or by both L1 and L2 in cotton. Finally, the deepest layer L3, but neither L1 nor L2, dictates floral meristem size and the total number of carpel produced in tomato chimera [146, 166] (Fig. 2a).

In parallel to these ancient genetic studies, the recent analysis of mutants has further demonstrated that the functional zones of the meristem are highly coordinated. We saw earlier that a meristematic signal can control organ polarity. In turn, analyses of several mutants in *Arabidopsis* and *Petunia* reveal that the developing organs also emit signals that impact meristem activity. In *Petunia*, the *HAIRY MERISTEM* (*HAM*) gene, is expressed in the nascent vasculature of primordia and prevents the differentiation of meristem cells in a non-cell autonomous way [163]. The *HAM* gene encodes a GRAS transcription factor that could move between cells to exert signaling at a distance, as seen for other GRAS transcription factors in the roots. However, this hypothesis has not been confirmed yet. *Arabidopsis* HAM homologues have been characterized recently, and they redundantly promote meristem maintenance [40]. However, their broad expression patterns, extending in the meristem, indicate that the non-cell autonomous signaling present in *Petunia* may not be necessarily conserved in *Arabidopsis*. A second signal from organs to shoot further links organ polarity with meristem maintenance: ectopic expression of abaxial genes or repression of adaxial genes causes meristem arrest; conversely, overexpression of adaxial genes produces bigger meristem (reviewed in [55]). Recently, Goldshmidt et al. [53] demonstrated that the abaxially expressed *YABBY* gene non-autonomously repress



◀ **Fig. 3** Polar auxin transport controls organogenesis and phyllotaxis in the SAM. **a** Top view (scanning electron microscopy) of the SAM of *Arabidopsis thaliana* showing the regular spacing of organs with a mean divergence angle of 137°. Primordia (P) are numbered from the youngest P1 to the oldest P7. Initium position (I1, then I2 in the sequence of appearance) is determined although initia are not bulging out. **b** Meristem from the *pin1* mutant shown by electron microscopy: stem cells are maintained while organogenesis is abolished in the periphery (taken from Vernoux et al. 2000). **c** Immunodetection of PIN1 efflux carrier in the L1. Note the subcellular polarized localization of the auxin pump that is thought to direct auxin fluxes out of cells and distribute auxin in the whole meristem. **d** Concentric PIN1 patterns around predicted new organs are responsible for auxin accumulation in founder cells. Note that PIN1 is up-regulated in these organs, suggesting a positive feedback loop of auxin on the quantity of PIN1 proteins. **e** Divergent PIN1 orientations in the boundary domain deplete these areas in auxin (c, d and e are taken from de Reuille et al. [34]). **f** Activity of a nuclear DR5 reporter in a WT SAM, imaged from the top by a confocal microscope. DR5 reports high auxin signaling mediated by ARF transcription factors. Clusters of *green* nuclei indicate primordium (P1) and allows the identification of early initium (I1 and I2). Plant tissue is colored in *red* by autofluorescence. **g** Computer simulation of auxin transport by a model of self-regulatory PIN1 pump network (taken from Stoma et al. [162]). This model reproduces PIN1 patterns and is able to generate a correct phyllotaxis: *arrows* indicate the sequential emergence of two new initium (*yellow* then *magenta*). Putative auxin concentrations are indicated in *green*. *Scale bar* 50 μm, excepted **b** (200 μm) and **e**, **f** (10 μm)

meristem size. Using a suppressor screen they discovered that this signal is mediated by another GRAS transcription factor, LATERAL SUPPRESSOR (LAS), which is expressed at the boundary between the meristem and the organ. They propose a two-step signaling event: YABBYs genes would emit a first short-range signal from the abaxial side to the boundary, promoting *LAS* expression. Then LAS would emit a second signal controlling meristem size and/or maintenance. The nature of those two signals remains to be identified (Fig. 2).

Finally, recent studies suggest non-cell autonomous communications between the meristem and the underlying nascent vascular system, which could both affect stem cell regulation and organ polarity. This involves a member of the *Arabidopsis* ARGONAUTE gene family, *ZWILLE* (*ZLL*, also known as *ARGONAUTE10* or *PINHEAD*). Like its animal homologues, the ZWILLE protein is a core-member of the RNA-induced gene silencing complex (RISC), which controls post-transcriptional gene silencing of specific target genes. During embryogenesis, *ZLL* promotes meristem maintenance, notably through *STM* expression [112, 120, 171]. Interestingly, *ZLL* is expressed in the vascular primordium and on the adaxial side of cotyledons, but excluded from the SAM proper. Tucker [171] and colleagues elegantly showed that ZLL acts non-cell-autonomously from the provascular strand, and not from the cotyledons, to sustain meristem maintenance, notably by potentiating WUS signaling. Moreover, *zll* mutants also exhibit polarity defects due to misexpression of the adaxially expressed HD-ZIP class III genes [112, 120, 122]. Recently, Liu et al. [108] showed that both SAM and leaf polarity defects of *zll* mutant seedlings were due to an increase in the abundance of the micro-RNAs *miR165* and *miR166*, which specifically target HD-ZIP class III degradation. Thus, a pro-vascular ZLL-dependant signaling is responsible for a repression of miR165/miR166 levels in the upper shoot tissue and, together with the activity of ZLL in the adaxial side of cotyledons, it controls the level of HD-ZIP class III transcription factors in the SAM. During post-embryonic shoot development, ZLL still affects meristem regulation and organ polarity, but its role is more complex. Consistent with the embryonic phenotype, ZLL promotes axillary meristem formation [112], but, unexpectedly, *zll* loss-of-function leads to an enlargement of inflorescence and floral meristems [112, 120], as does overexpression of miR166 [181] or some

Table 1 Signals involved in cell–cell communication and growth coordination in the SAM (see text for details)

Nature of signals	Molecules involved	Distribution within the SAM	Perception	Cellular effects	Meristem effects
Phytohormones	Auxin	Polar transport due to PIN efflux carriers	Plasma membrane and cell wall: ABP1 receptor	Cell wall loosening and cell expansion inhibition of endocytosis, promotes PIN membrane localization and its auxin efflux	Organ founder cell commitment (instructive signal)
		Diffusion through the apoplast and symplasm	Nucleus: F-box TIR1/AFB receptors	Transcriptional regulation by ARF transcription factors, permissive role on cell division	Regulation of organ spacing (phyllotaxis)
	Cytokinin	Diffusion through the apoplast and symplasm	Plasma membrane: transmembrane histidine-kinase receptor AHK1, AHK2, AHK3	Cell division, transcriptional regulation by B-type response regulators and CRF transcription factors	Stem Cell maintenance role in early differentiation?
	Gibberellic acid	Diffusion through the apoplast and symplasm	Nucleus: GID1 receptors	Cell expansion, cell differentiation, transcriptional regulation	Organ differentiation restrict stem cell activity
	Ethylene	Diffusion (gas)	Plasma membrane and endoplasmic reticulum: transmembranes histidine-kinase receptors Histidine-kinase receptors ETR1/2,ERS1/2,EIN4	Cell elongation, cell differentiation, transcriptional regulation, e.g., EIN3	Organ differentiation Restrict stem cell activity see [68]
	Brassino-steroids	Diffusion through the apoplast and symplasm	Plasma membrane: transmembrane receptor BRI1 and coreceptor BAK1	Cell expansion transcriptional regulation, e.g., BIN2	Control of plant stature and growth see [146]
	Strigo-lactone	Diffusion through the apoplast and symplasm	Nucleus: not identified yet, sensing requires the F-box protein MAX2	Not established	Regulation of shoot branching: inhibits axillary meristem outgrowth see [56, 173]
Molecule exchange through plasmodesmata	Transcription factors, e.g., LFY, STM, KNAT1, MP/ARF5…	Diffusion through the symplasm possible targeted transport	Association with DNA cis-regulatory sequences in the nucleus	Control of gene expression	Possible non cell-autonomous signaling: “fall-safe” mechanism (e.g., KNOX genes) or spatial control of stem-cell/differentiation balance for MP
	Small RNAs, e.g., ta-siARF from the TAS3 gene	Diffusion through the symplasm	Association with target RNA in the cytoplasm within the RISC complex	Degradation and/for translational inhibition of target RNAs	Acts as a morphogen gradient to set ARF-3 expression domain and the dorsi-ventral polarity of organs
Small diffusing peptide	CLV3	Diffusion through the apoplast	Plasma membrane transmembrane receptor CLV1 and CLV2/CORYNE complex	Inhibition of *WUS* transcription	Control of stem cell number, WUS-CLV regulatory loop

Table 1 continued

Nature of signals	Molecules involved	Distribution within the SAM	Perception	Cellular effects	Meristem effects
Mechanical stress	Cell wall components, remodeling proteins, cytoskeleton or transmembrane proteins	Tension across tissues	Not identified yet wall receptors? Stretch-activated channels? cytoskeleton?	Impact cortical microtubule orientation and PIN1 polarity, Cell expansion, cell anisotropy	Growth coordination control of morphogenesis robustness in patterning

References for the table [56, 173]

multiple loss-of-function mutants of HD-ZIP class III genes [129]. These data show that ZLL, miR165/166 and HD-ZIP class III genes regulate differently embryonic and post-embryonic shoots. This may be due to different gene-expression patterns in these different contexts, and also to additional regulators. For example, HANABA TARANU, a GATA transcription factor expressed in organ boundaries and also in the vasculature of embryo and inflorescence meristems, has been shown to negatively regulate meristem activity [121, 190]. Thus, many regulators could operate from the vascular tissue, providing various signal combinations to control meristem activity (Fig. 2a).

In summary, these experiments support a view in which most of the functions of the SAM emerge from dynamic communications between cells located in different subdomains (Fig. 2). This dynamics certainly accounts for the astonishing plasticity of plant cells, illustrated for example by the ability of various plant tissues to regenerate shoots. Conversely, these data are absolutely required to understand the mechanisms behind the homeostasis of the ever-active, yet stable SAM structure. One limit of these approaches, however, is that they are not sufficient to reveal the nature of the signals involved, nor the mechanisms through which cells emit, exchange, or perceive these signals. The development of molecular and biochemical approaches has provided a number of important candidates that could coordinate the growth and fate of different regions of the SAM.

Signals behind cell–cell communication and growth coordination in the SAM

A great diversity of molecules, including phytohormones, small peptides, mobile transcription factors, or small RNAs has been involved in SAM function. In addition to these biochemical signals, the role of mechanical signals is emerging as an important contributing factor to the control of meristem biology. A list of those signals is provided in Table 1. Thanks to molecular and genetic studies, we begin to understand the role of these signals in the SAM.

First, it appears that nearly all signals are produced and act locally in the SAM, as it is now well established for auxin and cytokinin (reviewed in [189]). This accounts for the considerable autonomy of SAM development: even isolated on artificial media, stem cell homeostasis and organogenesis still go on in a very robust and stereotypic manner (reviewed [160]). In parallel, long-range effects of phytohormones circulating through the vascular system are also well documented: for example, an acropetal cytokinin flow from the roots is important to sustain shoot growth and development. But in the meristem proper, where no

mature vascular system is formed, the local production of cytokinins could be critical to generate differential concentrations, and thus biochemical patterns. It should be noted here that the term "hormones" in plant maybe misleading. If some may have an action at a distance as their animal counterparts, most of them are also characterized by a paracrine action and context-dependent effects. In this respect, they rather resemble animals growth and developmental factors like, e.g., Wnt.

Despite their local production, one major bottleneck for the study of signals in plants is the difficulty to precisely assess where they act and to quantify the strength of the signal. For example, no antibody is available for the CLV3 peptide. Its localization is currently inferred from the expression domains of the *CLV3* gene itself and of the CLV1/CLV2 receptors. This issue is even more acute for plant hormones. Indeed, plant hormones usually are small molecules, deriving from simple metabolites (like auxin, which is a derivative of tryptophan) and it is hard to define methods to identify the active hormone from the widespread precursor. Furthermore, each hormone is in fact represented by a group of natural chemical variants of the same molecule, with possibly subtle differences in activity. In addition, many enzymes catalyze reactions that activate or deactivate hormones. For example, in rice and *Arabidopsis*, the local activation of cytokinins by the LOG enzymes in the central zone is essential for stem cell maintenance [95, 96]. In general, the exact sites of hormone biosynthesis, activation, deactivation, and degradation are still poorly documented. As a result, we still ignore the precise localization and concentration of plant hormones at a cellular level. Finally, no measurements of the physical properties throughout the SAM have been reported yet, even though techniques like atomic force microscopy could provide valuable inputs in the future. More generally, the development of new tools to acquire quantitative data on signal localization and intensity will certainly help in unraveling the precise mechanisms through which signals coordinate multicellular growth in the SAM. Despite these shortcomings, signal intensity and localization has been predicted from the indirect description of their production and distribution in the SAM (Fig. 1e, f).

Beyond the molecular control of signal production and decay in the SAM, the histological structure of the SAM itself adds another layer of complexity: the extracellular matrix and the presence of an extensive symplasm channel also control the distribution of signals in the tissue.

Extracellular signaling molecules like hormones and small peptides are able to travel throughout the cell wall, by the so-called apoplastic way. They are then specifically recognized by membrane-embedded or nuclear receptors and their transduction pathway is rather well documented [30]. Besides, because each cell is mechanically glued to its neighbors, the presence of cell walls provides a simple mean of cell growth coordination. Sharing common walls can also generate physical stress between neighboring cells, and in theory act as a coordinating factor [17, 69]. Last, plant cells can be connected by tubular channels called plasmodesmata. This complex structure establishes continuity between the cytoplasm of neighboring cells, but also between their plasma membranes and their endoplasmic reticulum membranes. Like gap junctions in animals, these channels allow the passage of ions and metabolites between cells, as well as molecules of various size including hormones, peptides, proteins, or RNAs. The movement of these molecules can obey simple diffusion rules or follow a targeted path (reviewed in [49]). The maximal size of a molecule that flows through plasmodesmata defines its size of exclusion limit (SEL). Interestingly, the SEL can change in space and time and is likely to be developmentally controlled, as shown during embryo development for instance [88] or flowering [51] in *Arabidopsis*. Sometimes, protein movement can even be restricted to a one-way direction [158]. All these properties define different symplasmic domains, the size of which depends on the molecules considered.

In meristems, microinjections [22] and targeted expression of diffusible fluorescent reporters [90, 91] has demonstrated extensive communications between cell layers. In contrast, the diffusion from the meristem proper into developing organs was found to be rather limited. As proposed by Kim et al. [89], a restriction of the SEL may be a general feature accompanying cell differentiation in plants [22]. Many transcription factors important for meristem functioning, like LFY, STM and KNAT1/BP (a STM paralogue) have been shown to move through plasmodesmata [91, 150, 184], albeit with different range. Notably, the movement of the maize orthologue of STM, KNOTTED1, is clearly restricted in a developing leaf, since the movement is only possible from the inner tissues to the L1 layer but is impossible the other way around. This suggests that the meristem, as an immature tissue, is generally more permissive to protein movements between cells than other tissues in differentiating organs. In view of these data, all transcription factors with partial cytoplasmic localization, unless specifically retained in cells, could thus extend their activity outside the domain where their RNA is transcribed [184]. This idea was recently further supported by the observation that the transcription factor AUXIN RESPONSE FACTOR 5/MONOPTEROS (ARF5/MP), a key regulator of auxin signaling (see below for further details on auxin signaling), establishes a gradient from its site of transcription in the PZ towards the CZ [191]. Are these movements required for normal development? A LFY fusion protein that is impaired in its diffusion is not

as efficient as the wild-type LFY to rescue *lfy* loss of function mutants, suggesting that LFY movement in flower is critical for its function [184]. The wide expression domain of STM would suggest that the role of STM movement is secondary. Nevertheless, some authors have proposed that STM mobility could homogenize the concentrations of these meristematic identity genes and acts as a "fail-safe" mechanism to control cell fate in the SAM [91]. In the case of ARF5/MP, Zhao et al. [191] convincingly showed that MP regulates transcription in the central zone non-cell autonomously, with a functional impact on stem cell homeostasis.

Very exciting results concerning a putative role of plasmodesmata in growth coordination came from recent reports on developmental patterning by mobile small RNAs, miRNAs, or plant-specific ta-siRNA [24, 28]. In particular, during leaf polarity establishment, a ta-siRNA moves from its site of biogenesis in the adaxial side of the organ to the abaxial side and creates a gradient that patterns the expression of an abaxial determinant [28]. These pioneering data pave the way for the discovery of other important mobile small RNAs in the SAM. More generally, it highlights the importance of plasmodesmata for the mobility of various cytoplasmic molecules, which could behave as plant non-conventional morphogens.

To conclude, the meristem can be viewed as a multicellular object with interconnected functional domains, under the control of known molecular switches (Fig. 1b–d). The homeostasis and coordination of growth between these different functional domains is controlled by the distribution of various signals in the tissue (Figs. 1e, f, 2). To go beyond these general concepts, we further detail one example, the control of organ formation in the SAM. In the light of recent experimental and modeling data, we will discuss how auxin cell–cell signaling can generate positional information and patterning and how mechanical constraints and hormonal crosstalks cooperate with auxin to determine organ shape and position in the meristem.

Auxin is a polarly transported signal that controls organogenesis and phyllotaxis

Auxin is polarly transported from cell to cell

Auxin is both diffusible and transported throughout tissues in defined directions. Early experiments showed that auxin travels in stem from the ontogenic top to the base and towards the tip of roots, following a global shoot-to-root flux. These oriented fluxes are explained by the action of specific auxin efflux carriers. Auxins, like the indo-acetic acid IAA, are weak acids. Depending on the pH of the solution, they can be protonated (IAAH) or ionic (IAA−). This property has fundamental biological consequences, formulated in the "chemio-osmotic model" [54]. In the acidic environment of the cell wall, auxin is found under its neutral form (IAAH): it can cross biological membranes and enter the cell. This entry is also facilitated by influx carriers, like the *Arabidopsis* AUX1 and its paralogues LAX1, LAX2, LAX3, as well as PGP4, a member of the multidrug resistance/*p*-glycoprotein (MDR/PGP) ATP-binding cassette transporters [7, 12, 113, 137, 167]. In the cytoplasm, whose pH is around 7, IAAH loses a proton and becomes ionized (IAA−). This change has two effects. First, it maintains a gradient of IAAH between the extra- and intra-cellular compartments, and thus maintains the entry of IAAH into the cell. Second, as it becomes charged in the cytoplasm, anionic auxin loses its ability to cross membranes. It thus absolutely requires efflux carriers to exit the cell, a role played by the members of the PIN-FORMED (PIN) family, as well as PGP1 and PGP19 [127].

Interestingly, the PIN proteins generally exhibit a polar localization in each cell, and the predicted direction of auxin efflux at the cell level can be correlated with macroscopic auxin fluxes in tissues like stems and roots. PIN polar localization thus likely dictate the direction of the net flux of IAA− outside the cell [45]. PIN polarities show specific supracellular patterns in the SAM (see below, Fig. 3c) and in various organs [13]. The auxin signaling reporter DR5 has been used to evaluate whether such patterns could generate local peaks of auxin. Gene transcription in response to auxin is controlled by members of the ARF transcription factors family that are themselves regulated by interaction with the Aux/IAA transcriptional repressors, a class of protein targeted to the proteasome upon perception of auxin [25, 106]. The DR5 reporter drives the expression of a reporter gene (GFP or β-glucuronidase) under the control of a synthetic promoter, consisting of several auxin response elements (AuxRE) bound by the AUXIN RESPONSE FACTORS (ARF) fused to a minimal CaMV35S promoter [172]. Activation of the DR5 reporter thus corresponds to an induction of auxin transduction that could be expected upon local accumulation of auxin [142, 172]. Overall, the orientations of PIN proteins correlate most of the time with patterns of DR5 activation and simple and multiple *pin* mutants as well as transgenic lines with modified PIN polar localizations consistently display disrupted DR5 expression patterns [11, 34, 142, 155, 183]. Altogether, this demonstrates the key role of PIN proteins for auxin distribution in plants.

Auxin transport controls organogenesis and phyllotaxis at the shoot apical meristem

Evidence supports a role for auxin transport in organogenesis in the SAM. First, mutants in the PIN1 efflux

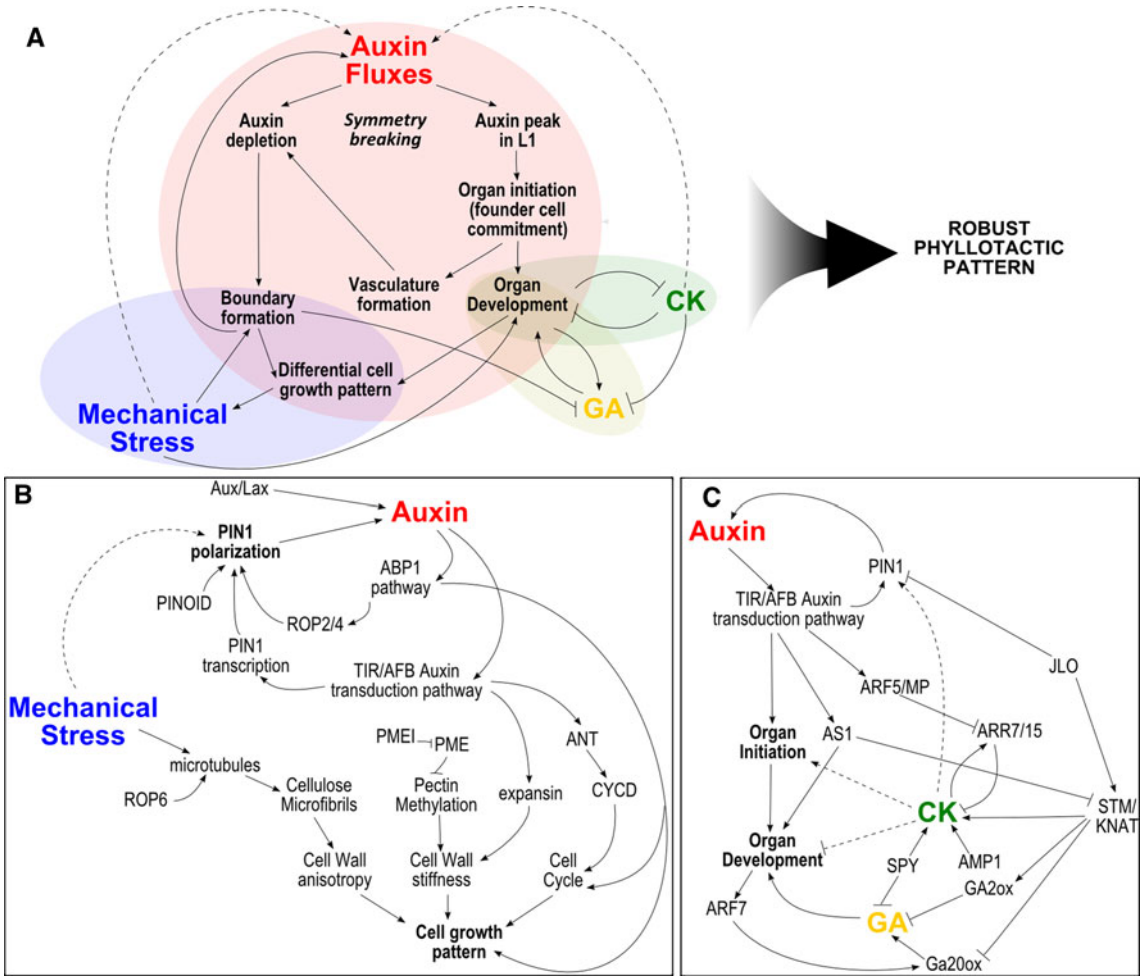


Fig. 4 Signaling network controlling organogenesis in the SAM of *Arabidopsis thaliana*. **a** Intersection of auxin signaling with cytokinin, gibberellins, and mechanical signaling during organogenesis. The numerous positive and negative feedbacks existing between these pathways may be essential for the robustness of organ development and the control of phyllotaxis. **b** Details of the molecular crosstalks between auxin signaling and mechanical signaling controlling cell growth. **c** Details of the hormonal crosstalk between auxin, cytokinin, and gibberellin signaling controlling organogenesis

carrier are unable to produce organs, and generate naked pin-shaped inflorescences [123] (Fig. 3b). This indicates a limiting role for the PIN1 efflux carrier in polar auxin transport at the SAM. In line with this idea, inhibition of auxin transport by drugs like NPA phenocopies the *pin1* phenotype [136]. Reinhardt et al. [137] further showed that a local application of auxin in the PZ of naked *pin1* meristems or NPA-treated plants can rescue the formation of organs, suggesting that auxin accumulation is necessary and sufficient to trigger organogenesis.

What would then be the mechanism responsible for such an accumulation of auxin?

First, auxin carriers of the AUX/LAX family are thought to concentrate auxin in the L1 layer of the SAM [7, 137]. This is consistent with the specific role played by the L1 layer in controlling organogenesis, as evidenced by inhibition of organogenesis following laser ablation of cells in the epidermis ([135]). Second, PIN1 is expressed in the L1 layer throughout the meristem and exhibits concentric and centripetal patterns at sites of emerging organs [34, 137] (Fig. 3c–e). Analysis of the putative auxin distribution using computer simulations based on observed PIN1 orientations confirm that this cellular transport network likely directs auxin to the sites of organogenesis [34]. Expression of the DR5 reporter in the SAM supports this hypothesis (Fig. 3f) and live imaging of the SAM identified the induction of DR5 expression as one of the earliest landmarks for organogenesis [74]. In addition, local biosynthesis of auxin is important to supply the hormone to the pump network: loss-of-function of YUCCA enzymes, a family of flavin-monooxygenases catalyzing a rate-limiting step in auxin synthesis, further enhances the inhibition of organogenesis in a *pin1* or *aux1* background [27]. The different YUCCA genes are expressed in discrete groups of cells in the SAM [26], but it is not clear how this localized auxin production participates to the spatial variation in

auxin signaling. Consequently, patterns of auxin accumulation in the SAM are thought to be mainly the results of auxin distribution through the PIN1 network (Fig. 4a). Note that this original mechanism differs from traditional reaction–diffusion models, where the formation of the pattern requires both the movement of molecules (through diffusion) and local self-enhanced production [154].

As auxin is predicted to accumulate at sites of organ emergence, this also suggests that auxin could get depleted in the vicinity of primordia. In addition to its expression in the L1 layer, PIN1 is also expressed in internal layers below incipient organs, in the developing vasculature [9, 137]. Thus, if auxin is driven into inner tissues through these provascular strands, organs likely behave as sinks. Patterns of auxin depletion around organs would then be equally important because they would define zones where new primordia cannot be produced. The PIN1 carriers network would thus be able not only to control the sites of auxin accumulation but also to determine their relative position, and thus phyllotaxis [137] (Fig. 4a). Note that, this time, the auxin transport-based mechanism is somewhat comparable to reaction–diffusion models because it can generate inhibitory fields. Indeed, in the reaction–diffusion models, two molecules, an activator and an inhibitor, interact and diffuse. Inhibitory fields result from the diffusion of the inhibitor. In the auxin-transport model, only one activator is present: the active transport of auxin generates activation by local accumulation as well as lateral inhibition by depletion around the site of accumulation.

A self-regulatory network of auxin carriers provides a plausible model for the control of phyllotaxis

The major role of auxin transport for both organ formation and organ spacing raises the question of whether such an auxin distribution network can function in a self-regulatory manner, without external factors or inputs. Such a property would require feedback between auxin signaling and transport, and accumulating evidences supports such links.

Several studies demonstrated that auxin stimulates its own efflux out of cells, by a quantitative control on PIN proteins: it both promotes PIN transcription and triggers the delivery to the plasmic membrane of internalized PIN [124]. Up-regulation of PIN can be clearly seen in the incipient primordium in the SAM [74] (Fig. 3c). Thus auxin increases the intensity of its own in feedback. However, patterns of auxin accumulation and depletion more rely on PIN1 polarity rather than PIN1 quantity. Does auxin also feedback on PIN1 polarity? Two concurrent mechanisms have been proposed and their plausibility was explored using computer modeling approaches. In the first one, PIN1 orients according to the direction of the main auxin fluxes. This flux-based mechanism, first proposed by Sachs [143], is known as the canalization model because of its ability to progressively drive auxin flows in discrete merging channels between auxin sources and sinks. Experimental evidence indicates that this mechanism could explain vascular strand formation in leaves [147] or in stems after wounding [145]. In the alternative mechanism, the authors proposed that PIN1 orients towards the cell with the highest auxin concentration, pumping auxin up the gradient. This concentration-based mechanism appears to better fit with the observations in the SAM. It could explain the presence of PIN1 concentric patterns towards the incipient primordium, where auxin concentration is thought to be the highest (Fig. 3d).

Based on this simple mechanistic framework (auxin increases its own efflux from cells and controls PIN polarization), several computer models have recently been elaborated to test whether such feedback could confer self-regulatory properties and explain phyllotaxis [9, 86, 155, 162]. All the tested models were able to reproduce plausible PIN1 polarity patterns as well as various phyllotactic arrangements (Fig. 3g). The two concurrent hypotheses explaining the influence of auxin on PIN1 polarity, canalization [162] or concentration-based [86, 155], are thus equally plausible, according to these computer simulations. Bayer [9] and colleagues even developed a model combining the two hypotheses, with a concentration-based mechanism and canalization governing PIN1 polarities in the L1 and internal tissues of the SAM, respectively. Taken together, these models provide a demonstration that a simple self-regulatory mechanism for polar auxin transport would be sufficient to explain phyllotaxis. They strongly support a scenario in which phyllotaxis is an emerging property of local cell–cell interactions driving PIN1 polarity. While these computer models give conceptual insights into the possible mechanisms operating in a complex system, they also predict that the elucidation of the biological basis for PIN1 polarization likely holds the key to the identification of the actual mechanisms involved in phyllotaxis.

How do cells perceive auxin and polarize in a multicellular context?

As discussed above, models of PIN1 polarization postulate the existence of two unknown molecular mechanisms: canalization models require a “flux sensor” while up-the-gradient models incorporate a “concentration sensor” of the neighboring cell. Which molecules could play this role in the SAM?

Coen et al. [32] speculate a molecular mechanism to explain how a cell can interpret the direction of a molecular flow: the translocation of the signaling molecule across the membrane could generate a molecular by-product, which would thus accumulate asymmetrically on one side of the

membrane. This molecule would keep a record of the net morphogen flow. Differences in its concentration between outer membranes would polarize the cell according to the morphogen's main stream. Sensing a flow rather than concentrations is conceptually attractive, because a concentration is measured at a particular time point, whereas a flux measurement integrates morphogen flow over time, giving robustness to patterning. However, in the context of the SAM, where the auxin transport network is changing each time a new initium is created, the requirement of such a mechanism remains to be investigated. Furthermore, to date, no molecular players have been reported to act as a tally molecule, although pH changes associated with auxin transport across membranes could provide some elements of cell polarization.

On the contrary, very recent results may provide a mechanism by which a cell senses auxin concentrations in its neighbors, or more precisely in the surrounding apoplast.

Many studies have proposed a particular role for one of the first identified receptor of auxin, AUXIN BINDING PROTEIN 1 (ABP1), which is involved in non-transcriptional responses to auxin. ABP1 is present at the plasma membranes and in the cell wall [84], where it is able to functionally bind auxin, at least in protoplasts [101, 161]. Robert [140] and colleagues have now identified an interesting functional link between ABP1 signaling and PIN1 trafficking. They showed that ABP1 promotes clathrin-dependent endocytosis of PIN1 in the absence of auxin, and that binding of the hormone inhibits this function, thus triggering the recycling of the transporters back to the membrane. This is of particular importance, because PIN proteins have been proposed to be primarily delivered evenly to the plasma membrane and subsequently polarized to one cell side through a continuous dynamic endocytic recycling [36]. Furthermore, the effect of ABP1 on endocytosis may account for the positive feedback of auxin on its own flux [124]. ABP1 likely associates with other partners to provide an asymmetry to signaling, as evidenced by the work of Xu and coworkers that show that cell–cell communication could occur via the rho of plants (ROP) protein family. In leaves, epidermal pavement cells exhibit interdigitated shapes. ROP2 and ROP6 have been shown to promote the formation of lobes and indentations, respectively, and in those cells, PIN1 is polarly localized at the lobe's tip. Interestingly, the activation of ROP2 and ROP6 is auxin-dependent via the ABP1 protein. Assuming differences in sensitivity to auxin between the different ROPs, a cell could sense auxin concentration in its neighbor through very sensitive ABP1/ROP molecular modules [186]. Altogether, these results support a model in which cell polarization depends on auxin perception in the cell wall. However, the precise role of the ABP1, ROP signaling pathway, and their link with PIN polar targeting to membranes, still needs to be clarified. Indeed, ABP1 is unlikely setting PIN polarities alone: ABP1 loss-of-function does not change PIN1 or PIN2 polarities or auxin gradients in roots, as suggested by DR5 reporter data [170].

These new findings raise the question of whether a mechanism involving auxin sensing at membranes and local cues for polarization could support either the canalization or the concentration-based model.

A recent computational study has made a significant contribution to this issue: Wabnik [177] and collaborators tested whether the modulation of PIN trafficking by an extracellular auxin receptor could lead to tissue polarization in the frame of the canalization hypothesis. In this model, the authors postulated that auxin binding to its receptor in the apoplast (ABP1 being an obvious candidate for such a receptor) immobilizes the receptor and triggers inhibition of PIN internalization in the nearest cell, increasing the efflux locally. The strength of auxin signaling is proportional to the amount of bound receptor. Since two neighboring cells compete for auxin receptors in their common walls, this model can lead to an asymmetric PIN internalization in the two facing membranes. In addition, the authors implemented other important biological data, including the effect of auxin on PIN transcription through nuclear signaling and auxin transport through the influx and efflux carriers. Interestingly, this mechanistic framework has the capacity to polarize cells in a tissue with discrete auxin source and sink, which is the typical context of canalization. The authors successfully applied their model to the biological situations of vascular formation in leaves and regeneration in stem, where simulations accurately reproduce observed patterns of PIN polarization as well as auxin distribution.

It is interesting to note that instead of a flux-sensor, it integrates concentration sensors, which in turn modulate PIN endorecyling. This is a call for elucidating the molecular basis underlying PIN polarization to understand the coordination of this process at the tissue level. Also, it remains to be shown whether this model could explain organogenesis and phyllotaxis in the SAM, where the context is different. For example, there is no localized auxin sources and multiple organs act as sinks. Stoma et al. [162] already demonstrated that the canalization-based model could generate symmetry breaking and reproduce robust phyllotactic patterns at the SAM. It would be then interesting to determine whether the proposed mechanistic framework could formally behave as the hypothetical flux sensor of canalization model and test whether it also functions for phyllotaxis.

The precise mechanism through which auxin allows cell–cell communication and polarization is still an open question. In the coming years, this exciting role for auxin perception in the apoplast will certainly inspire further

investigations, and may answer whether there is a unifying molecular mechanism explaining auxin-related patterning, from vascular formation to phyllotaxis.

A possible role for PIN1 efflux carrier phosphorylation in phyllotaxis?

Downstream of the cell-polarizing cues acting in the SAM, several intracellular effectors targeting PIN proteins to correct membranes have been identified involving transcytosis, endocyclic recycling, and the cytoskeleton (for review [64]). A striking role for PIN1 phosphorylation has been observed. In particular, mutants of the serine/threonine kinase PINOID produce *pin*-like inflorescences [31] and in this mutant PIN1 switches from apical to basal membranes [46]. Further studies have shown that the kinase PID and the phosphatase PP2A antagonistically control a similar apical to basal binary switch in many cell types [117]. Recently, Huang [77] and coworkers even identified three direct sites of PINOID-phosphorylation in the intracellular loop of PIN1. Interestingly, a phosphomimetic version of PIN1 in those three sites has a restored apical localization in shoot epidermis. However, this PIN1 phosphomimetic line, as well as loss-of-phosphorylation PIN1 lines are unable to rescue organogenesis in *pin1* inflorescences. These experiments highlight the importance of reversible phosphorylation of PIN1 for proper organogenesis in the shoot apex. Interestingly, in the boundaries between the meristem and the emerging organ, PIN1 was reported to undergo polar switches [74] and PID is also expressed at higher levels in boundaries [31]. PID may thus play a direct role in these polar switches and contribute to organ separation.

Are organogenesis and phyllotaxis only regulated by polar auxin transport? The wealth of data and auxin-centered models could lead to such an opinion. However, accumulating data that we discuss below now challenge this vision and suggest that auxin cooperates with other signals to control organogenesis in the SAM (Fig. 4a).

Mechanical signaling in the SAM

A role of mechanical signals in plant patterns has been explored notably by Paul Green [60, 61]. Assuming that the epidermis has a dominant role in morphogenesis, tissue folding associated with the initiation of a primordium will inevitably affect the mechanical constraints in the meristem, and thus could impact the position of the subsequent initiation. This theoretical view has later been supported by a series of experiments in the meristem. In particular, the local application of expansins, a cell wall protein that causes stress relaxation on the meristem has been shown to induce a local outgrowth, followed by a modification of the subsequent phyllotaxis [42]. Knowing that expansins have a very local impact on the mechanical properties of the wall, the global impact on architecture would be due to a modification of the pattern of mechanical stress within the meristem [42]. More recently, modification of pectins, and thus cell wall stiffness, by pectin methyl esterases was also shown to impact phyllotaxis, consistent with a crucial role of the mechanical properties of the wall in organ formation and positioning [126].

Interestingly, auxin is known to promote cell expansion by influencing the mechanical properties of the cell wall. First, auxin can directly or indirectly trigger the expression of cell-wall remodeling proteins like expansins [29], consistent with the preferential expression of expansins in incipient primordia in tomato SAM [138]. Second, auxin signaling stimulates an active transport of hydrogen ions in the apoplast, lowering the pH of the cell wall. This acidification activates the remodeling proteins (e.g., expansins), allowing the cellulose microfibrils to creep and the cell wall to expand (for review, see [107].

As seen above, polar auxin transport generates local accumulation of auxin. Then, auxin modifies locally the mechanical properties of the cell wall to allow primordium bulging: could these patterns of differential growth and cell wall stiffness in the SAM produce a mechanical signaling that could have a feedback on auxin transport and SAM patterning? To confirm this hypothesis, we need to know exactly how those constraints are distributed in the SAM and what the molecular targets of such signals in the cell could be.

Because plant cells display high turgor pressure and are encased in a stiff cell wall, they can be viewed as thin shells with internal pressure, a bit like a balloon. Turgor pressure, by pushing on the wall, allows cell expansion, and is thus considered as the motor of growth. It is also the source of mechanical signals: regional differences in wall stiffness or turgor pressure can generate gradients of constraints that the cell could in principle interpret as positional information. In particular, several microsurgical experiments, mainly cuts in the epidermis, strongly suggest that tension builds up in the epidermis, and that the inner tissues are under compression (Fig. 1f) [97]. In this framework, plant tissues can be seen as thin shells, too, but this time at a supracellular scale. With these assumptions, it is now possible to calculate the principal directions of mechanical stress. For instance, on a cylinder-shaped structure, like a stem, mechanical stress is predicted to be twice as high in the circumferential direction than in the axial direction. These calculations on a meristem-like shape predict the presence of a strong tangential stress at the boundary between the emerging organ and the meristem (Fig. 1f). Interestingly, this direction is parallel to the

PIN1 signal on the anticlinal wall, as viewed from the top. The orientation of the microtubular cytoskeleton at the cell cortex facing the outer wall is also parallel to this direction. It has thus been proposed that both PIN1 and the microtubules are able to sense, indirectly, the direction of the main principal stress direction (Fig. 4b). Cell-based modeling, using mass-spring networks as well as the finite element method show that this assumption is plausible. An array of experiments, involving cell ablation, tissue compression and pharmacological treatments, further support the conclusions of the models [67, 73].

Based on these data, one could imagine that mechanical forces cooperate with auxin to establish a supracellular pattern, for instance by reinforcing the directionality of auxin transport (e.g., by targeting PIN1 on the membrane experiencing the highest tensile stress) and by impacting tissue morphology and thus on the direction of mechanical forces (Fig. 4a, b). For this last point, as cortical microtubules control the deposition of the cellulose microfibrils in the cell wall and thus cell anisotropy, the reinforcement of the wall at the boundary could help in defining the position of the physical separation between the emerging organ and the meristem in the boundary domain. If the boundary is better defined, the pattern of stress is also reinforced and PIN1 could then acquire its polarity more rapidly or more accurately.

Nevertheless, the contribution of mechanics to patterning in the SAM is still largely debated, notably because mechanical forces cannot be observed like a morphogen, and because, in contrast to auxin, we currently do not know how a mechanical signal could be perceived and transduced in plant cells. Both pathways could actually be intertwined: a number of effectors of the auxin-dependent cell polarity are potentially involved in the transduction of mechanical stress. For instance, the mechanosensitive Ca^{2+} channel TOUCH3 has been shown to interact with PINOID [10]. Similarly, in yeast, the Rho proteins have been involved in the wall integrity pathway, and thus it is possible that the ROP proteins could actually be activated by both biochemical and biophysical signals. Last, the importance of the vesicle trafficking machinery for PIN1 polarity could be largely under mechanical control, knowing that membrane tension prevents endocytosis and stimulates exocytosis in animal systems [119].

Integrating complex hormonal crosstalks during organogenesis

In addition to the dominant role of auxin in organ patterning, and its interplays with mechanics, many other signaling pathways, particularly hormones, were also shown to impact organogenesis, providing a more complex (but certainly more realistic) view of the developmental control of organ initiation [48, 66, 151, 174] (Fig. 4).

Hormonal crosstalk has been known to be crucial for plant development and physiology for many years. In angiosperms, changing the ratio between auxin and cytokinin for tissue explants cultured in vitro can switch the developmental program to shoot formation if cytokinin activity prevails, to root if auxin signaling is dominant, and to undifferentiated callus if none dominates. The molecular basis for such interactions is only beginning to be understood (Fig. 4c). Note that most of the recent findings in this field have been obtained from studies on the root, and shall only be extrapolated with care to the SAM.

The classical view of hormone balance in the shoot states that high cytokinin (CK) signaling promotes stem cell identity and maintenance in the central zone of the SAM, while high auxin and gibberellic acid (GA) signaling are required for cell differentiation and elongation in organs [174] (Fig. 1e). This hormone pattern and its homeostasis are directly coordinated by master regulators of the SAM identity. Indeed, in the meristem proper, high CK signaling is promoted by the transcription factors of the *KNOX* family, like *KNAT1* or *STM* (Fig. 1c). They were shown to promote CK synthesis in the meristem [83, 188]. Conversely, they were also shown to promote the expression of GAs deactivating enzyme (GA2ox) at the base of the meristem, either indirectly through CK signaling, or directly by regulating transcription, as recently shown in maize [14]. The barrier-shaped expression domain of GA2ox likely protects the meristem from the influence of external GA (Figs. 1e, 4c). KNOX proteins have also been shown to repress directly GA20ox, a key enzyme of GA synthesis [72, 144]. Consequently, KNOX action creates a high CK/GA ratio within the meristem. Conversely, overproduction of CK induces the expression of the *KNOX* genes and can rescue the *stm* phenotype [83, 141]. Application of GA can suppress the phenotype of several *KNOX* overexpressor lines [72]. Other hormones promote the same effect, like ethylene, which is able to restrict the expression pattern of the *KNAT2* gene and partially rescue the *KNAT2* overexpressor phenotype [68].

Other pathways redundantly function along with *KNOX* genes like SPINDLY, that both inhibit GA signaling and promote CK responses [62, 83]. Now considering the growing organ, auxin and GA act synergistically this time to promote cell elongation and differentiation. First, auxin controls the activation of proteins like AS1, which repress *KNOX* expression in organs. Second, auxin, through its own signaling pathway (notably ARF7), activates GA biosynthesis genes (*GA20ox*) in leaves [44]. Interestingly, it also promotes the activation of GA2ox enzymes, which catabolize GA, at the boundary of the meristem. Thus, in principle, the spatialization of auxin transduction allows

auxin to synergize with KNOX action at meristem boundaries and with GA in developing organs. Altogether, these results suggest that multiple crosstalks between auxin, cytokinin, GA, or ethylene are necessary to precisely control the switch of hormonal balance to allow organ development (Fig. 4c).

Recently, studies have brought some elements that refine the binary vision of organ emergence opposed to meristem maintenance (high CK in meristem versus high auxin and GA in organs). They support a view in which CK would also be required for organ initiation and development (Fig. 4c).

First, Carabelli et al. [23] showed that CK are necessary for leaf primordium growth because catabolism of CK by CKX6 can be responsible for primordium growth arrest when plants are shaded by neighbors. A minimal CK activity must then be necessary to sustain leaf development and the activation of *CKX6* must require auxin signaling. Second, the maize *abphyl1* mutant exhibits a stunning defect in the definition of organ position as the mutant produces two leaves per node instead of one [80]. *ABPHYL1* encodes a cytokinin RESPONSE REGULATOR, a primary cytokinin response protein that inhibits cytokinin signaling. Consistently, *abphyl1* meristems are bigger [52] and have a higher CK content [102]. However, ABPHYL1 may also have a function in the PZ, in relation to auxin. Its expression is localized in the incipient leaf primordium and requires auxin or its polar transport, as treatment with NPA, an inhibitor of polar auxin transport strongly reduced its transcription. *Abphyl1* SAM also have lower *ZmPIN1* expression and reduced auxin content. Furthermore, a short exogenous treatment with CK rapidly induces *ZmPIN1* expression, showing a complex crosstalk between auxin and cytokinin. An interesting model proposed by Lee [102] and collaborators is that lower auxin and PIN1 contents delay leaf initiation: this would lead to an extra growth of the meristem, leaving enough space for the concomitant formation of two organs, thus producing a decussate phyllotaxis. This further underlines the crucial role of hormonal balance in the control of the transition from stem cells to organ founder cells.

In *Arabidopsis*, several *spy* mutant alleles exhibit phyllotactic defects, also suggesting a role for the GA/CK balance in organ positioning [153]. Furthermore, the *amp1* mutant, known to have higher CK content, can rescue the loss of organogenesis of the *mp* single mutant [176]. Consistently, Zhao et al. [191] partially restored organ formation in a *mp* mutant by silencing the *Arabidopsis* RESPONSE REGULATORS 7 and 15. Similarly to their maize ortholog ABPHYL1, these genes inhibit cytokinin signaling in a feedback loop, suggesting that increased cytokinin signaling promotes organogenesis. The authors also demonstrated a mechanistic framework for this auxin/cytokinin synergy. They found that ARF5/MP directly inhibits ARR15 and excludes its expression from primordia, likely potentiating CK signaling in the PZ. Altogether, these recent findings challenge the former models where CK were important only for stem cell maintenance in the central zone. Consistent with this, a role for CK in cell differentiation has been recently shown in roots [35].

Altogether, these new data suggest that more attention must be paid to hormone interactions and specific competence of target cells to understand the diversity and specificity of developmental responses to a limited number of signals.

Conclusions

Understanding complex biological processes like stem cell maintenance or organogenesis requires the comprehensive integration of several functional aspects, including gene regulatory networks, cell–cell signaling, and cell expansion and division. Beyond the individual impact of these parameters on growth, how their action is coordinated at a multicellular level is a central question to fully understand morphogenesis. Here we detailed the known networks regulating organogenesis and phyllotaxis in the SAM of higher plants, notably including biochemical and biophysical signals, as well as the structural features of the tissue in which they act (e.g., the symplastic domains).

At this stage, understanding the complexity of such an intricate network begins to exceed the ability of the human brain. The dynamics of signaling in SAM development is actually hard to grasp, for at least three reasons. First, our understanding of the action of the gene regulatory network is cellular, while the morphogenetic output is multicellular. Second, while the cellular parameters are either biochemical or biophysical, the output is always geometrical. Last, because gradients of morphogens are limited by the geometry of the tissue, the geometry itself defines the extent and intensity of morphogens in individual cells, thus feeding back onto the local behavior of cells. Supracellular patterns of forces are also defined by the geometry of the tissue and can feed back on the growth of individual cells.

For these reasons, the SAM can be viewed as a typical complex system [169]. In such a framework, a robust morphogenesis can in principle emerge from the interactions of individual cells. Combining experimental data from traditional cell and molecular biology with mathematical and computational modeling, provides new insights on how various intercellular signals contribute to shapes [87, 92, 105]. This approach has notably uncovered some essential properties of living organisms, such as bistability, homeostasis, or oscillatory phenomena [63]. Furthermore, because it is a quantitative approach, the formalization of

growth and cell behavior in simulations help compare very different systems like animals and plants, revealing in some cases developmental similarities based on divergent molecular players. The generation of virtual organisms has actually started in various model species, like zebra fish, *drosophila*, *Caenorhabditis* or *Arabidopsis* and there is no doubt that these tools will help in deciphering the mechanisms behind complex notions like shape changes, self-organization, or robustness in development in the near future.

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