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A flower is born: an update on Arabidopsis floral meristem formation

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In Arabidopsis, floral meristems appear on the flanks of the inflorescence meristem. Their stereotypic development, ultimately producing the four whorls of floral organs, is essentially controlled by a network coordinating growth and cell-fate determination. This network integrates hormonal signals, transcriptional regulators, and mechanical constraints. Mechanisms regulating floral meristem formation have been studied at many different scales, from protein structure to tissue modeling. In this paper, we review recent findings related to the emergence of the floral meristem and floral fate determination and examine how this field has been impacted by recent technological developments.

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Introduction

Since the first genes controlling flower development in Arabidopsis and snapdragon were cloned, enormous progress has been accomplished and regularly reviewed [1,2]. For this review, we chose to focus on the early stages of floral meristem (FM) development in Arabidopsis, highlighting recent advances in the field. We also examine how new technologies and methods have improved our understanding of floral development.

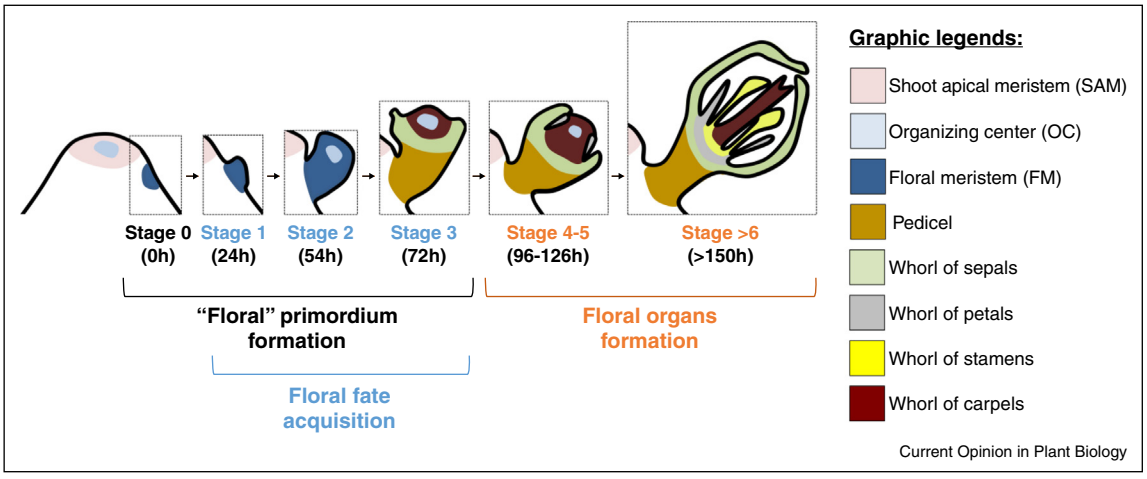
Emergence of floral primordia

In Arabidopsis, the emergence of flowers on the flanks of the shoot apical meristem (SAM) follows a precise pattern (Figure 1). The reason for this positioning of flower primordia (phyllotaxis) can largely be explained by a localized accumulation of auxin due to its polarized transport in the L1 layer through the PIN-FORMED1 (PIN1) efflux carrier [3]. This transient auxin peak releases the AUXIN RESPONSE FACTOR5/MONOPTEROS (ARF5/MP) from Aux/IAA repression [4], which is essential for FM emergence as attested by the pin-shaped inflorescence of the *mp-S319* mutant [5] (Figure 2). The MP protein acts at several levels: it first upregulates the *MACCHI-BOU 4* (*MAB4*) family genes that control PIN basipetal relocalization [6]; it also induces *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN6* (*AHP6*), a negative regulator of cytokinin (CK) signaling. AHP6 diffuses to neighboring initium positions where it inhibits meristem initiation, thus reinforcing the auxin phyllotactic pattern [7•,8]. Finally, MP directly induces the expression of the FM identity gene, *LEAFY* (*LFY*) [5], which acts in concert with *AINTEGUMENTA* (*ANT*), *AINTEGUMENTA-LIKE6* (*AIL6*) and *FILAMENTOUS FLOWER* (*FIL*) in this process [5,9••]. Feedback and feed-forward loops come into action as ANT and AIL6 both induce *LFY*, while LFY in turn reinforces auxin transport [10–12]. LFY also activates *REGULATOR OF AXILLARY MERISTEMS1* (*RAX1* — a Myb-like transcription factor (TF)), which, as its name suggests, regulates meristem formation ([13] and references therein) but also cytokinin signaling through inhibition of *ARABIDOPSIS RESPONSE REGULATOR7* (*ARR7*) [13].

At the molecular level, MP was shown to recruit the SWI/SNF ATPases SPLAYED (SYD) and BRAHMA (BRM) to target loci where they increase chromatin accessibility [9••]. Interestingly, constitutive expression of a fusion protein composed of the MP DNA-binding domain and BUSHI (a member of the SWI/SNF ATPase complex that should allow the recruitment of SYD and BRM) is sufficient to restore a wild-type inflorescence architecture in *mp* mutants. The fusion protein is constitutively expressed and lacks the PB1 domain mediating Aux/IAA repression, therefore this result suggests the existence of an additional pathway which can pattern the SAM to determine the site of floral emergence [9••]. This pathway has, as yet, remained elusive, and no other information on the components involved is available.

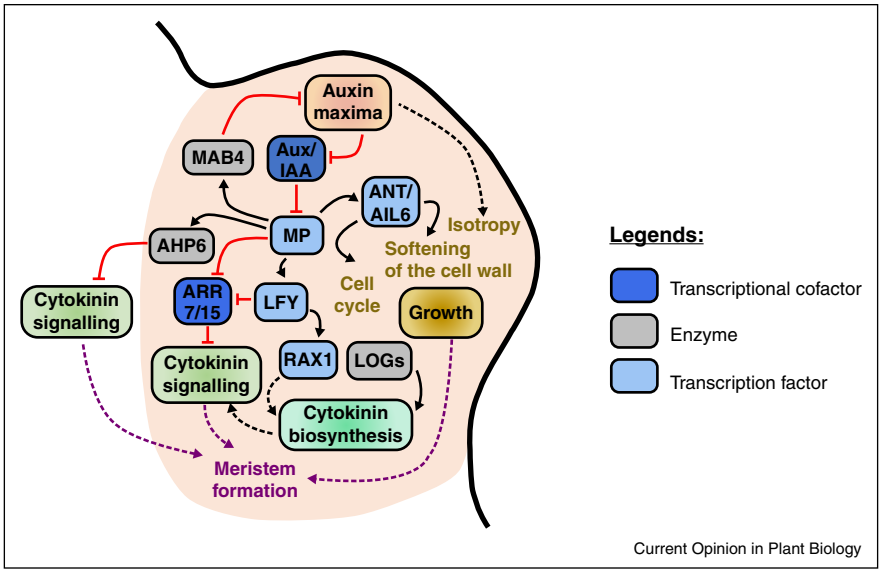
After reaching its peak level, auxin is channeled away from the primordium. This is achieved by PIN1 relocalization to the internal pole of the cell combined with the local action of auxin-influx carriers. This combination effectively creates an auxin sink in the inner tissues that contributes to vascularization and phyllotaxis [6,14].

Figure 1



Schematic representation of flower development in *Arabidopsis*. The floral bud emerges on the flanks of the shoot apical meristem (stage 0). Bud outgrowth during stages 1–3 allows a meristem-organizing center to be established at stage 2. During these early stages floral identity is acquired, leading to definition of the floral organ whorls, starting with sepal outgrowth at stage 3. The meristem-organizing center finally disappears at stage 6, once all floral organs have been initiated and the pedicel has begun to expand. Hours indicate approximately the age of the flower at the end of a given stage as defined in Ref. [63].

Figure 2



Genetic network involved in floral meristem formation. In the nascent floral bud, the size of the primordium and the presence of a cytokinin-induced signal combine to control the emergence of a stem-cell niche. This process is largely initiated by the auxin response factor, MP, which is activated in response to a local auxin maximum after the release of Aux/IAA inhibition. Induction of MAB4 triggers relocalization of the PIN1 auxin efflux carrier to channel auxin away from the primordium. By inducing ANT and AIL6, MP stimulates the cell cycle and cell wall softening to allow meristem growth. Plasticity is reinforced by the effect of auxin on cell wall stiffness and isotropy. Cytokinin biosynthesis is triggered by *LOG* genes, and probably by the action of MP through LFY and RAX1. MP and LFY also stimulate cytokinin signaling by inhibiting ARR7/15. Finally, MP induces *AHP6* which diffuses from the primordium to inhibit cytokinin signaling in surrounding areas, and thus control the emergence of new primordia.

Floral bud outgrowth

Once its position has been established, the floral meristem enters its growth phase. This involves the ANT and AIL6 [15,16] proteins, cell cycle regulators which also control pectin dimethyl-esterification of SAM cell walls [16], a process which is thought to facilitate organ outgrowth [17]. Cell-wall-forming enzymes play a specific role in floral outgrowth, as indicated by their localized expression patterns in the inflorescence [18]. Moreover, it has also been shown that auxin can alter tissue plasticity

through its effect on microtubule reorganization and dynamics, controlling both the stiffness and isotropy of cell walls [19•].

CK establishes FM meristematic competence

After an initial growth stage, the FM acquires meristematic features (Figure 2). Around stage 2, activation of the CK signaling reporter pTCS [20] is closely followed by the expression of *WUSCHEL* (*WUS*) and *CLAVATA3* (*CLV3*) which mark the establishment of an organizing center and a stem cell niche, respectively. Inter-regulations of WUS and CLV3, CK and its receptors, and a hypothetical diffusible signal from the L1, have been integrated in a model recapitulating gene expression and stem cell organization in the SAM in various conditions [21,22••]. Remarkably, this model can also explain how *WUS* and *CLV3* expression are triggered in the early flower, as soon as the primordium has attained a size threshold [22••]. The central role played by CK in this process is illustrated by a flurry of recent results: (i) MP represses the negative regulators of the CK response, *ARR7* and *15* [23], while LFY represses *ARR7* [13], thereby locally increasing the CK response in FMs. In support of this, ARR7/15 inhibition by amiRNA was also shown to compensate for the loss of primordia initiation in *mp* mutants [23]. (ii) RAX1 may also act on CK levels in the SAM as overexpression of a CK biosynthesis enzyme can compensate for loss of axillary meristems in *rax1* mutants [24]. (iii) As described in the SAM network model mentioned above, cytokinin biosynthesis is thought to occur mainly in the L1, as suggested by the expression of the *LONELY GUY4* and 7 (*LOG4*/7) reporters [22••]. Although few data are available regarding their involvement in flower formation and their regulation, *LOG7* is specifically expressed in FM, while *LOG4* is found throughout the inflorescence, strongly hinting at a role in primordia development [22••]. In addition to the CK receptor AHK4 [21,22••,25], the HECATE family (bHLH TFs) [26] and REVOLUTA (a HD-ZIPIII TF) [27,28] were also shown to stimulate meristem activity and are expressed in young flower primordia.

Floral fate acquisition

Acquisition of the floral fate is determined by a gene regulatory network (Figure 3) based on the central players LEAFY, the MADS-domain TF APETALA1 (AP1), CAULIFLOWER (CAL), AGAMOUS-LIKE 24 (AGL24), and SHORT VEGETATIVE PHASE (SVP). Other MADS-domain TFs — SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), FRUITFULL (FUL), and XAANTAL2/AGL14 (XAL2) — might be involved or at least can compensate for AGL24 and SVP function in various mutant backgrounds [29,30]. AP1 and LFY positively regulate each other and share partially overlapping functions. Genome-wide analyses revealed that these TFs act as activators and repressors [31–33]. AP1 for example activates *LFY* but represses a myriad of other genes expressed in the SAM and in early floral stages [32]. The analysis of common AP1 and LFY targets identified multiple genes (including EUI-LIKE P450 A1 (*ELA1*), genes involved in peptide signaling, nitrate transport, UV-B signaling and carbohydrate homeostasis) paving the way for future functional analysis [31,34]. Several gene network models describe the regulatory relationships and predict steady states corresponding to organ-identity gene expression in all four floral whorls [30,35,36]. The mechanistic basis for whorl-specific activation of ABC floral organ homeotic genes through the combined actions of TF and their cofactors remains unclear and this field has progressed relatively slowly. Recent data suggest that ubiquitination could be involved as SVP upregulates the *WDR55 DCAP* gene, which is involved in restricting the expression pattern of *AG* and potentially targets proteins for ubiquitination [37].

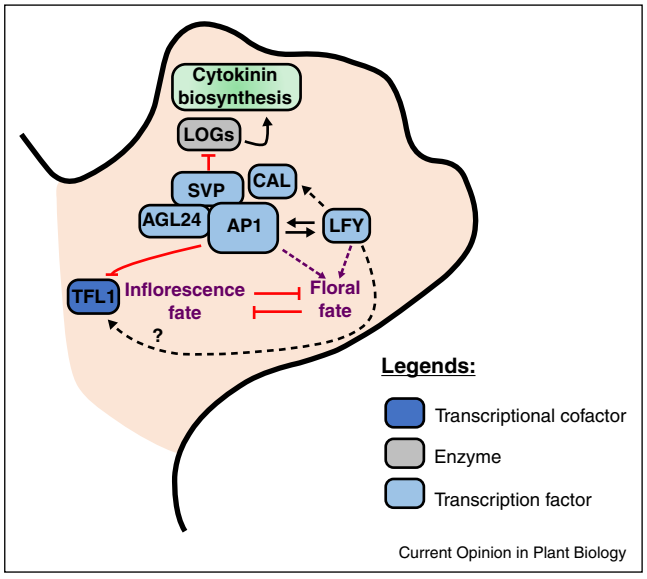
Repression of the inflorescence identity gene *TERMINAL FLOWER1 (TFL1)* is also essential for proper floral development [38] (Figure 3). This repression involves LFY, AP1, CAL, SVP, a variety of remote regulatory elements in the 3′ region of *TFL1* and DNA looping [38,39]. LFY was initially thought to directly repress *TFL1*, a hypothesis supported by ChIP-Seq assays showing LFY binding to the 3′ region of *TFL1*, mediated by the LFY oligomerization domain [31,40•] (Figure 4). However, when analyzing raw data from Ref. [31], we realized that LFY-GR appears to induce *TFL1* expression in seedlings and it was recently found that deletion of a region encompassing the LFY binding sites resulted in reduced *TFL1* expression in the stem [39]. Thus, LFY could actually be a direct *activator* of *TFL1* in the stem, and its repressive capacity in the flower might be indirect, through activation of *AP1* and *CAL*. AP1 also suppresses another inflorescence trait, the production of axillary buds which grow in the axil of first-whorl organs in *ap1* mutants. To achieve this inhibition, AP1 represses *LOG1*, a gene encoding a CK biosynthetic enzyme, and induces the expression of the CYTOKININ OXIDASE/DEHYDROGENASE3 CK catabolic enzyme [41]. The resultant reduction in CK levels prevents the sepal axil from acquiring meristematic competence [41].

It is interesting to note that meristem emergence and floral fate acquisition may be coupled by the continuous action of LFY, acting in these two processes throughout flower development.

Impact of novel technologies on our understanding of flower development

Floral development will only be understood once it can be fully modeled. The formation of flowers is a robust process, indicating that founder-cells interpret information they possess (genome and other molecules inherited from cell division) as well as external cues (such as hormonal cues or mechanical constraints imposed by their

Figure 3



Genetic network involved in floral identity. Inflorescence and floral identity are mutually exclusive statuses in the nascent meristem. In the young floral meristem, expression of LFY induces the *AP1* and indirectly *CAL* MADS-box genes. These three genes work together with other MADS-box genes, *SVP* and *AGL24*, to specify the floral identity. A feed-forward regulatory loop between LFY and AP1 stabilizes the floral identity. At this stage, the inflorescence identity gene, *TFL1*, is repressed — mostly by AP1 and other MADS-domain TFs. As discussed in the text, repression of *TFL1* by LFY might be only indirect through activation of AP1 and LFY might be a direct activator of *TFL1* in the inflorescence stem. AP1 also inhibits cytokinin biosynthesis through its effect on the *LOG* genes, thus preventing meristem formation at the sepal axil.

neighbors) to determine their development. However, for us it is extremely difficult to understand the rules that these cells so easily follow every time a flower is formed.

Over the last 30 years, genetics has extremely efficiently identified a number of regulators affecting floral development in a fairly specific way [1,2]. Here, we will analyze how the advent of new technological developments has generated new information which should help us to understand and model this process at multiple levels: atomic, genomic, cellular, and organ.

Structural advances

Although the technique is far from new, protein crystallography has recently been applied to several key floral regulators such as MP, LFY, and SEPALLATA3 (SEP3) MADS-domain TF [40•,42–44] (Figure 4). Beyond the satisfaction of obtaining a picture of these important proteins, the results obtained offer the possibility to understand how the atomic features of major proteins (for example elements mediating DNA binding, dimerization, tetramerization or oligo-merization) contribute to their functions. In contrast to genetics, structural data make targeted modifications of protein properties possible in the search to unravel new functions, as in the case of LFY [13,40•]. Structural advances also revealed unexpected similarities in the overall organization of LFY, ARF and MADS-domain TFs (Figure 4): they all harbor a dimeric face-to-face DNA-binding domain associated with a higher-order oligomerization domain. The shared organization of these 3 TFs, which form an activation cascade, might confer them common functional properties (such as the capacity to loop DNA, to bind closed chromatin, or to interact with chromatin remodelers SYD and BRM). These properties could never have been inferred from their primary amino-acid sequences [9••,40•,42,44,45].

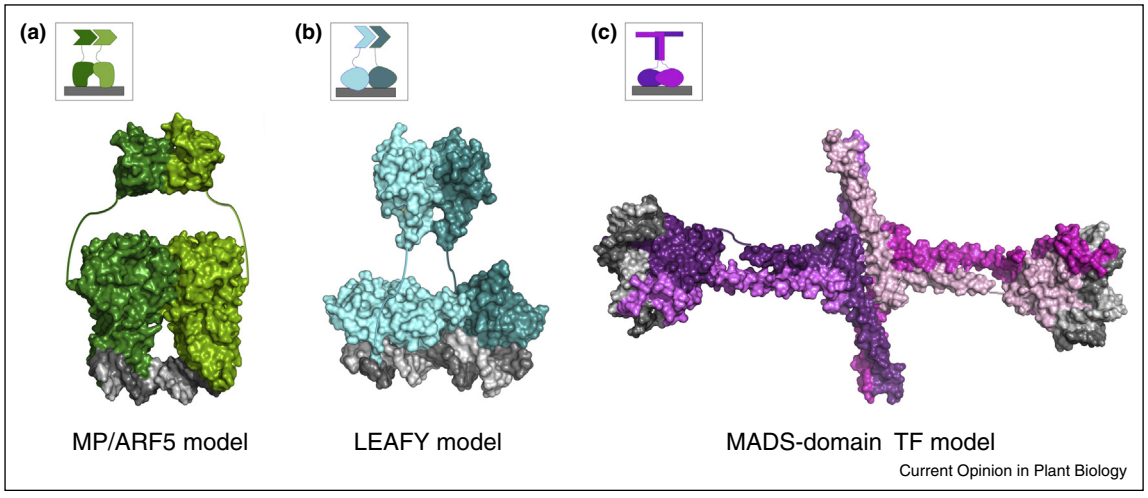
Impact of genome-wide techniques

When applied to floral development, Next-Generation Sequencing (NGS) based genome-wide studies (describing gene expression, TF binding, chromatin compaction, histone marks, nucleosome positioning or 3D genome architecture) hold great promise as they can be used to infer general rules, thus extending beyond the bounds of individual case-studies. These types of study also represent important data collections that can easily be reused by the broader community. Genome browsers can be utilized as 'NGS microscopes' to help generate hypotheses for genome-wide testing. The diversity of the floral cell types present in the inflorescence is a clear hindrance to genome-wide studies, but several technical advances have made it possible to sort tissues or cells [46,47], even if more specific promoters are needed to dissect floral meristems over both space and time.

In some cases, large-scale studies identified new actors (such as with AHP6 or ELA1) which could then be investigated by classical genetics techniques [7,34]. In others, they highlighted novel or understudied features: genes involved in pathogen defense are overrepresented in the genes expressed in the epidermis [46]; this group of genes is shut off by LFY and ANT/AIL6 [16,31]; expression of cell cycle genes displays a burst during the floral transition [48]. One of the most fascinating questions that can be addressed through genome-wide analysis is the target-specificity of MADS-domain TF complexes. This specificity was elegantly analyzed for SVP and FUL, and their roles in regulating common or specific target genes was identified [49]. However, for organ-identity MADS-domain TF tetramers, ChIP-Seq data suggest that they bind to similar genomic regions ([1] and references therein), indicating that their specific capacity to trigger separate developmental programs lies in elusive-sequence specific — co-regulators that have yet to be identified. Exhaustive proteomics-based studies of protein interactions might help to identify these co-regulators [45,50].

A fertile area that will certainly be more exploited in the future is the comparison of various types of genome-wide

Figure 4



Structural models of complexes between transcription factors and DNA. **(a)** MONOPTEROS (MP) model based on the complex between ARF1 and DNA (pdb 4LDX) and the MP PB1 oligomerization domain (4CHK). **(b)** LFY model based on the complex between LFY, DNA (2VY1) and the GbLFY SAM oligomerization domain (4UDE). **(c)** MADS-domain TF model involving the MEF2 (1TQE) DBD and the SEP3 tetramerization domain (4OX0). Arbitrarily-shaped linkers (narrow lines) have been added between domains for which the structures are known. The C-terminal domain of MADS-domain TFs is not represented. The insets aim at showing the similarity between the three proteins that share a face-to-face dimeric DNA-binding domain and another domain that allows higher-order complex formation (oligomer for LFY and MP and tetramer for MADS-domain TF) with the potential to form DNA loops.

data. Two such analyses, from very different angles, proposed that LFY and the MADS-domain TFs AP1 and SEP3 could act as pioneer TFs with the capacity to bind closed chromatin regions allowing the subsequent recruitment of other TFs [40•,51,52•]. This attractive hypothesis remains to be confirmed, but is consistent with earlier reports showing that some floral genes are controlled by Polycomb Group proteins repression and that their activation require chromatin remodelers such as SYD and BRM [53]. The cross-talk between transcriptional and chromatin regulation appears particularly prominent during the early stages of flower development [45,54,55].

Modeling regulatory networks

Built with information relating to key regulators and their relationships, models of gene regulatory networks (GRN) can be used to understand whether known relationships explain gene expression patterns or morphogenesis, or if additional actors or connections need to be identified. Models are becoming increasingly complex [22••,30,36], but there is still a gap between what can be modeled and the wealth of information available from genome-wide studies. Gene expression cannot yet be modeled based only on genome sequences plus some information about chromatin, but predictive tools are improving, in particular with regard to the location of TF binding sites [56,57]. A major challenge is to understand when TF binding leads to gene regulation. New CRISPR-Cas9 tools which can directly modify regulatory elements at their genomic locations [58] will provide a novel means to test GRN models and better understand complex gene regulation such as the control of *TFL1*'s expression [39].

Ultimately, GRN will have to be integrated into cellular models such as those modeling growth and integrating mechanical constraints [59–61]. Novel techniques such as Atomic Force Microscopy or Fluorescence emission-Brillouin imaging are also being developed or adapted to provide access to the mechanical properties of living cells within developing tissues [62].

Conclusion

Over the last 30 years, genetic approaches have identified the main regulators of FM development. We must now build multi-scale models that integrate other types of information such as tissue mechanics, morphology, hormone transport and signaling. Although they may remain incomplete, these models will play an essential role in the search to identify missing elements, inconsistencies or network behavior that cannot be simply apprehended. While data on gene expression, cell growth and mechanics will soon coexist in individual models, in the future we hope they can be included in multi-scale models bridging atomic and genomic resolutions.

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- •• of outstanding interest

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