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Just say no: floral repressors help *Arabidopsis* bide the time

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Floral repressors ensure correct reproductive timing by safeguarding against premature flowering. In the past decade, several mechanisms of floral repression have come to light. Discrimination between direct and indirect repressors has been facilitated by increasing the use of chromatin immunoprecipitation assays. Certain MADS-domain transcription factors such as SHORT VEGETATIVE PHASE and FLOWERING LOCUS C bind directly to target euchromatin to repress specific loci including *FLOWERING LOCUS T* (*FT*) and *FD*. The AP2-domain transcription factor TEMPRANILLO 1 has also been shown to directly repress *FT* by binding its 5′ UTR. We highlight emerging systems level approaches, including genome-scale direct binding studies (ChIP-chip and ChIP-Seq), which stand out in their promise to elucidate the complex network underlying the transition to flowering at an unprecedented level.

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Introduction

Rooted in place, flowering plants must sense and respond appropriately to the environment in order to achieve reproductive success. As a result they have evolved versatile genetic networks. Distinct pathways throughout the plant relate environmental and physiological information to modulate the expression of floral integrator genes, allowing their expression only when the time is right for reproduction. Proteins encoded by these genes, the vast majority of them transcription factors, in turn orchestrate the reprogramming of stem cells in the shoot apical meristem (SAM), which lies at the core of this decision making network.

Of these signaling pathways, the photoperiod pathway has seen some of the greatest recent advances in our understanding. Light quantity and quality is perceived in leaves and ultimately controls the production of the FLOWERING LOCUS T (FT) protein, which is thought to serve as a florigen, the long sought mobile signal that travels to the SAM to induce flowering. The functions of flowering-promoting factors — and of FT in particular — have been the subject of excellent recent reviews [1–4]. Here, we focus instead on floral repressors, which are essential to safeguard against the premature activation of the flowering promotion pathways and thereby ensure the correct timing of reproduction. In particular, we concentrate on direct molecular interactions indicating specific mechanistic links between repressors and their targets.

Disentangling repression

Floral repressors act on several levels. At the most system-wide global chromatin remodeling factors have been observed to influence diverse developmental processes (reviewed in [5]). For example, the target repertoires of polycomb group (PcG) proteins and ATP-dependent chromatin remodeling machines are beginning to be elucidated in plants [5]. In fact, direct repression of the floral homeotic gene *AGAMOUS* (*AG*) by EMF1 has recently been described [6]. Interaction of EMF1 with the *AG* locus strictly depended on the presence of the PcG protein EMF2, which is a component of a predicted complex along with CURLY LEAF (CLF), FERTILIZATION-INDEPENDENT ENDOSPERM (FIE), and MULTICOPY SUPPRESSOR OF IRA1 (MSI1). In addition, EMF2 is necessary for trimethylation of histone 3 lysine 27 on the *AG* locus [6]. A dynamic state of affairs is envisioned, replete with a complex interplay of factors governing the accessibility of euchromatic loci. At 'open' loci, the next level of regulation is possible: transcriptional activators and repressors may access particular binding motifs in target loci to induce or prevent their expression.

The recent widespread use of chromatin immunoprecipitation (ChIP) in plant biology is enabling greater insight into the precise mechanisms of transcriptional control and consequently, network architecture. ChIP allows one to observe whether the effect of a transcriptional regulator on a target is direct or indirect. FLOWERING LOCUS C (FLC) is a good example of a direct repressor: it prevents flowering by physically binding to the *FT* promoter to repress transcription [7,8]. FRIGIDA, on the other hand, inhibits *FT* transcription indirectly, through promotion of *FLC* expression (reviewed in [9]). Until recently, such

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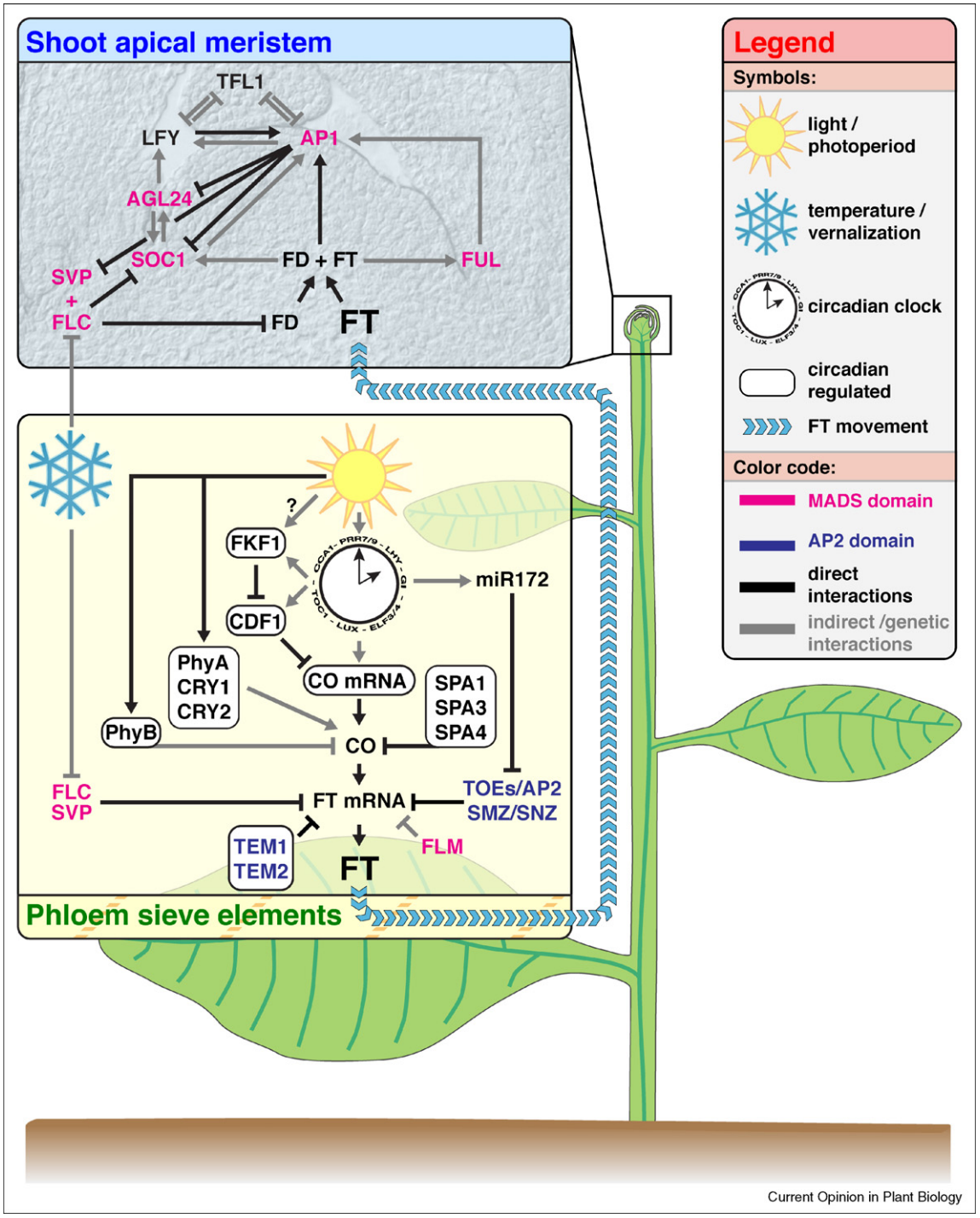
Figure 1



A balance of repressors and activators governs flowering. Light perception occurs in the leaves. Signal from photoreceptors in conjunction with the circadian clock control expression of *CONSTANS* (*CO*) in the vasculature. CO protein accumulates in the light but is rapidly degraded in the dark, a process that depends on the SUPRESSOR OF PHYA-105 proteins [51] and COP1 [52,53]. In addition, phyB has been shown to influence CO protein stability in the light [54]. The major target of *CO* is *FLOWERING LOCUS T* (*FT*) [34], whose expression is negatively regulated by MADS (red) and *AP2* (blue) transcription factors. FLOWERING LOCUS C (FLC) directly binds the *FT* locus and represses its activation. Under the control of the autonomous and vernalization pathways, FLC functions in a complex with SHORT VEGETATIVE PHASE (SVP). *FLOWERING LOCUS M* (*FLM*), a close relative of *FLC*, also represses *FT*, but it is unclear if this is a direct or indirect effect. Two classes of *APETALA2* (*AP2*)-domain proteins repress *FT* in the leaf and counteract its activation. The TEMPRANILLO (TEM) proteins appear to be direct repressors of *FT*, as TEM1 has been shown to bind the *FT* locus. Finally, six miR172-target genes that encode for AP2-domain-containing proteins also repress flowering. At least in the case of SMZ, direct binding to *FT* locus has recently been demonstrated [24••]. miR172 has been reported to be regulated by *GIGANTEA* in a *CO*-independent manner, but is also

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discrimination between direct and indirect repressors was very difficult.

MADS-domain repressors in the leaf

The best-studied direct floral repressor is the MADS-domain transcription factor FLC, which acts both in the leaves and in the shoot apex to integrate the vernalization and autonomous pathways (reviewed in [9,10]). *FLC* expression is under multifactorial regulation exploiting both transcriptional and epigenetic mechanisms [5,9]. ChIP on leaf tissue has revealed that FLC is a direct repressor of *FT* and *SUPRESSOR OF CONSTANS OVEREXPRESSION 1* (*SOC1*), binding CArG boxes in the first intron of *FT* and in the promoter of *SOC1* (Figure 1) [7,8]. Interestingly, FLC is at least partly dependent on another MADS protein, SHORT VEGETATIVE PHASE (SVP) [11•,12••]. FLC and SVP interact in yeast [13], as well as *in planta*, and mutations in one suppress the late flowering of transgenic plants expressing high levels of the other [11•,12••]. ChIPs from both protoplasts and from plants expressing HA-tagged SVP driven by its native promoter indicate strong enrichment for a fragment of the first intron of *FT* containing the FLC binding site [11•,12••], suggesting that FLC and SVP may act in a heteromeric complex to prevent *FT* transcription. SVP was also found to bind a second CArG box in the *FT* promoter region upstream of the transcription start site in an experiment employing constitutive expression in protoplasts [11•]. However, further experiments are necessary to confirm the biological relevance of this binding, as this interaction could not be confirmed in an independent experiment using the endogenous *SVP* promoter *in planta* [12••].

The closest homolog of *FLC*, *FLOWERING LOCUS M* (*FLM*) [14], also acts as a repressor of flowering [15,16] and, as with *FLC*, functionally distinct alleles segregate in natural populations [17]. In contrast to *FLC*, however, *FLM* expression is not responsive to vernalization or elevated *FRI* levels [16]. *FLM* and *SVP* are closely related in function: they suppress each other's misexpression phenotypes, and the double mutant is phenotypically identical to *svp* [15]. The late flowering conferred by constitutive FLM expression does not depend on *FLC*, and *SVP* expression levels do not change in these plants [15]. Given that MADS proteins interact in higher order complexes and that SVP directly represses *FT* and *SOC1* together with FLC, these results hint toward direct repression of one or more floral integrators by a FLM/SVP heterodimer.

AP2-domain repressors chime in at the leaf

Aside from the MADS-domain transcription factors, another major group of transcription factors involved in direct repression of *FT* in leaves consists of proteins containing one or more APETALA 2 (AP2) DNA binding domains. *TEMPRANILLO 1* and *TEMPRANILLO 2* (*TEM1* and *TEM2*) are members of the RAV family and contain one AP2/ERF-DNA and one B3-DNA binding domain. *TEM1* and *TEM2* are partially redundant: neither displays a pronounced loss of function phenotype on its own, but RNAi-mediated simultaneous knockdown of both induces early flowering, while constitutive expression of either gene results in delayed flowering [18•]. Additionally, the rate of leaf production (plastochron) seems severely perturbed in *TEM1* and *TEM2* misexpressors, although this was not analyzed for its own sake [18•]. Genetic analyses place the *TEMs* between *CO* and *FT*, based on the evidence that *35S::FT*, but not *35S::CO*, completely suppresses the late flowering phenotype of *35S::TEM1* [18•]. Further, the *ft-101* loss of function phenotype is not attenuated in *ft-101 tem1-1* double mutants. The suppression of ectopic *CO* expression is dependent on *TEM1* copy number, as the semidominant late flowering of *co*/+ plants is attenuated by RNAi-*TEM1/2*, hinting at a quantitative competition of CO and TEM1 on their downstream targets [18•]. While *FT* mRNA levels are upregulated in RNAi-*TEM1/2*, its circadian expression profile remains unchanged. Constitutive *TEM1* expression almost completely abolishes *FT* expression. Finally, TEM1 can directly bind to a *RAV* binding motif in the 5′ UTR of the *FT* locus as shown by EMSA and by ChIP using *35S::TEM1* [18•], providing additional evidence that TEM1/2 and CO might directly compete for the activation of *FT* at the transcriptional level.

AP2 is the founding member of a clade of six closely related transcription factors that are characterized by the presence of two conserved AP2 DNA binding domains and, importantly, a miR172-target site [19–21,22•]. The first suggestions of an involvement of this protein family in flowering time regulation came from the observation that plants misexpressing miR172 exhibited extremely early flowering [19,23], in addition to having floral defects

(**Figure 1 Legend Continued**) induced at the shoot apex in an FT-dependent and CO-dependent manner [23]. Eventually, FT protein is exported from the leaves and is transported via the phloem sieve elements. Arriving at the shoot apex, it interacts with the bZip transcription factor *FD*. At the shoot apex, *FT* and *FD* induce the expression of floral integrator and flower-specific genes [33,34]. Direct binding of FT/FD to the promoter of *APETALA1* (*AP1*) has been demonstrated [34]. *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFUL* are clear genetic targets of *FD*, but binding of FD to these loci has not been reported. *SOC1* and *FD* are under negative regulation by *FLC*, which binds to and directly represses expression of these key floral integrators. Together with the meristem identity gene *LEAFY* (*LFY*), *AP1* specifies flower primordia at the flanks of the shoot meristem. Expression of *LFY* and *AP1* in the inflorescence center is prevented by *TERMINAL FLOWER 1 (TFL1)*, which is required to maintain the inflorescence meristem in an indeterminate state. More recently, it has been shown that AP1 directly represses the flowering integrators *SVP*, *AGL24*, and *SOC1* in emerging flower primordia [44••]. Thus a situation is established where flowering integrator genes (*SOC1*, *FUL*, and *AGL24*) activate expression of flower meristem genes (*LFY* and *AP1*) at the flanks of the meristem, but are switched off again in a negative feedback loop in the emerging flower.

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that can largely be explained by impaired *AP2* function [20]. Sequence analysis initially revealed the other AP2-like genes, *TARGET OF EAT 1–3 (TOE1–TOE3)* and *AP2* itself as putative miR172-targets [21]; later two additional AP2-like genes, *SCHLAFMÜTZE* (*SMZ)* and *SCHNARCHZAPFEN* (*SNZ*), were also predicted to belong to this target group [23]. With the exception of *TOE3*, misexpression of any of these genes induces late flowering [22•], and the expression of all these *AP2*-like genes — except *TOE3* and *AP2* — has been shown to be downregulated during late vegetative development concomitant with an increase in miR172 expression [22•]. miR172 upregulation has been shown to be dependent on *GIGANTEA*, but independent of *CO* [22•].

Of the miR172-targeted AP2-like genes, only *toe1* has been reported to affect flowering time as a single mutant [19,22•]. *toe1* mutants are mildly early flowering, a phenotype that is enhanced in *toe1 toe2* double mutants, demonstrating functional redundancy among miR172-targets [19]. Because *toe1 toe2* double mutants still flower much later than plants constitutively expressing miR172, further redundancy among other members of the clade seemed likely.

To date, only *TOE1* and *SMZ* have been analyzed in detail for their effects on flowering time. TOE1 seems to act primarily as a repressor of *FT*; while *CO* levels remain unchanged in *toe1* mutants, *FT* mRNA levels are strongly upregulated. *FT* expression is almost completely abolished in plants constitutively expressing *TOE1* [22•]. However, 35S::miR172 *ft*-10 plants still flower earlier than the strong loss of function mutant *ft*-10 alone [22•], suggesting that *FT* is not the only floral-promoting gene regulated by this AP2-like gene family.

A genome-wide view of direct SMZ target binding was recently provided by Mathieu *et al.*, who used ChIP coupled to tiling arrays to show that SMZ binds directly to the *FT* genomic locus [24••]. Repression of flowering was ensured by SMZ also binding genomic loci encoding proteins downstream of FT, including *AP1* and *SOC1*. SMZ binding to all these target loci was accompanied by repression of the transcribed mRNAs. One unexpected finding from this study was that SMZ also binds to its own regulatory sequences and to those of three closely related AP2-like miR172-targets, providing a mechanism for the strong regulatory negative feedback between SMZ and members of this clade [24••]. Interestingly, feedback has also been demonstrated for *AP2* [25]. Given the importance of the miR172-target clade in floral repression, one may speculate that interdependent transcriptional feedback among the miR172-targets would serve as a rheostat in the control of flowering time.

Informational inputs sensed and transduced in the leaf ultimately converge on regulatory regions surrounding the *FT* locus. Once translated, the FT protein, together with its homolog TWIN SISTER OF FT, confer a floral stimulus from the phloem companion cells to the SAM. Despite intense investigation [26–30], *FT* mRNA has never been reported at the SAM in wild-type *Arabidopsis thaliana*. Recently, however, a study described movement of a mutant, nontranslatable *FT* mRNA, independent of FT protein [31]. This study was unique in that a viral vector system was used to express *FT* mRNA fused to heterologous viral or *GFP* sequence [31]. In contrast, another study demonstrated that mutation of *FT* sequence by synonymous substitution in 171 of 175 codons and replacing both UTRs, thus reducing sequence identity to less than 64% compared to wild-type *FT*, does not abolish its long-distance flower-promoting activity [30]. Thus, further investigation is still needed to determine whether there is a role for *FT* mRNA movement in flowering regulation. It may yet be that sections of the *FT* mRNA are sufficient, though perhaps not necessary, to confer movement, but the functional significance of this is unclear. Whatever the outcome, the fact that there is no detectable endogenous *FT* transcription at the apex suggests that, although the above repressors play active roles in the repression of flowering both in the leaf and at the apex, their target sets are likely to differ between the two compartments.

It all comes together at the apex

The transition from vegetative to reproductive development is effected at the shoot apex. Here, incoming signals from the photoperiod, GA, autonomous and vernalization pathways converge. Their outputs modulate the expression of flowering integrators such as *SOC1* and the FT interactor, *FD*. Direct repression of both of these involves FLC, since FLC was detected binding to their promoters using ChIP [8]. However, *FLC* expression in the phloem also blocks flowering-related upregulation of *FD* at the apex, suggesting both direct and indirect effects [8]. More recently, Li *et al.* discovered that FLC and SVP function together as a repressor complex that integrates signals from all four pathways to directly repress *SOC1* by binding adjacent sites on its promoter [12••]. Clear *in vivo* substantiation was provided by data implicating this same SVP-bound motif: mutation of this CArG box caused early flowering and increased SOC1-GUS reporter staining, indicating a clear role for SVP in the repression of SOC1 at the SAM [12••]. These reports begin to illuminate a picture of MADS transcription factor complexes controlling flowering time, quite possibly as they were shown so elegantly to control floral organ identity [32].

But flowering repressors may work in ways other than directly binding *cis*-regulatory elements. In the case of the bZip transcription factor FD, the action of its physical interactor [33,34] FT is counteracted by *TEMINAL FLOWER 1 (TFL1)*, which is very closely related to *FT* [35–37]. In fact, domain swapping and directed

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mutagenesis studies have demonstrated that only one amino acid change is necessary to switch the floral-repressing *TFL1* function to a floral-promoting effect reminiscent of *FT*. These data, combined with evidence from protein interaction studies [33,34], have led to a model in which FT and TFL1 compete to modulate *FD* activity [2,35,38]. Indeed, ChIP with an anti-FT antibody pulled down a bZip motif-containing region on the *AP1* promoter, suggesting that *AP1* is a direct target of an FT/FD transcriptional complex which is thought to be repressed by competitive interaction with FD by TFL1 [33,34]. This regulatory module is conserved as far as wheat: TaFT, the wheat ortholog of *FT*, interacts with TaFDL2, a wheat functional homolog of *FD*, to activate *VRN1*, the wheat *AP1* homolog [39].

Recently, an additional function of *TFL1* as an integrator of photoperiod and thermosensory inputs was revealed. Strasser *et al.* showed that *EARLY FLOWERING 3* and *TFL1* are independently necessary to control flowering time in response to ambient temperature [40]. The precise mode of *TFL1* function, however, remains an enigma. One report showed TFL1 localization to endomembrane compartments and found evidence that *TFL1* plays a role in directing protein trafficking [41]. This seems at odds with the prevailing model that TFL1 acts by competing with FT for protein partners. Whatever the mechanism — or mechanisms — of *TFL1* function, its general role as a floral repressor has been recently found to be conserved in species as divergent as *Capsicum* spp. (wild pepper) and *Malus* × *domestica Borkh.* (apple) [42,43].

An emerging model envisions the vegetative state at the SAM to be one of constant floral repression, only broken when the balance of the floral-promoting pathways outweighs the negative sway of the floral repressors. Eventually, though, derepression is achieved and reproductive identity is conferred to the SAM. Expression of the MADS gene *AP1* is generally considered a cardinal indicator of this developmental transition. In this context, it is particularly interesting to note that ChIP and expression data have been employed to show that even AP1 can function as a master direct repressor of inflorescence identity by binding the promoters of, and directly repressing, *AGAMOUS-LIKE 24*, *SOC1*, and *SVP* [44••]. It has been recognized for some time that repression of *AGL24* by *AP1* is a crucial step in maintaining floral meristem fate and avoiding floral reversion [45], but only recently have studies employing ChIP been able to show a direct mechanistic link [44••].

Conclusion

Transcription factors are generally thought to operate in multimeric complexes, the composition of which may modulate direct target repertoires. All subgenera of the MADS-domain family have recently been shown to participate in such higher order complexes [46]. As we have highlighted, the MADS-domain multimer SVP/FLC repressor complex is an important integrator of flowering signals [12••]. Indeed, FLC was shown to be present in high molecular weight complexes in gel filtration experiments using native protein extracts [7]. What other partners take part in this and other transcriptional complexes is an active area of investigation.

How interactions among other transcription factors affect their binding to target genes remains a mystery, but several groups are embarking on ambitious comprehensive analyses of these interactions. These emerging systems level approaches, including genome-scale direct binding studies coupled with inducible expression systems, stand out in their promise to elucidate the complex network underlying flowering at an unprecedented level. Proof that these approaches can be deployed effectively in plants has been provided by several groups working on diverse biological questions from flowering to trichome initiation [46–49]. Very recently, Gerco Angenent's group has taken advantage of the improved binding site resolution of ChIP-Seq (ChIP coupled to deep sequencing of chromatin) in *Arabidopsis* to present the first genome-wide analysis of a MADS-domain protein, SEPALLATA 3 [50•]. Their results suggest multiple links to auxin singaling pathways for this central floral homeotic protein.

Because of its complexity, understanding the regulatory network architecture controlling the transition to flowering will surely require coordinated genome-scale studies, but a good start has been made at observing direct molecular interactions at its core.

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References and recommended reading

Papers of particular interest, published within the past two years, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Corbesier L, Coupland G: **The quest for florigen: a review of recent progress**. *J Exp Bot* 2006, **57**:3395-3403.
2. Giakountis A, Coupland G: **Phloem transport of flowering signals**. *Curr Opin Plant Biol* 2008, **11**:687-694.
3. Kobayashi Y, Weigel D: **Move on up, it's time for change — mobile signals controlling photoperiod-dependent flowering**. *Genes Dev* 2007, **21**:2371-2384.
4. Michaels SD: **Flowering time regulation produces much fruit**. *Curr Opin Plant Biol* 2009, **12**:75-80.
5. Farrona S, Coupland G, Turck F: **The impact of chromatin regulation on the floral transition**. *Semin Cell Dev Biol* 2008, **19**:560-573.

Please cite this article in press as: Yant L, et al. Just say no: floral repressors help *Arabidopsis* bide the time, Curr Opin Plant Biol (2009), doi:10.1016/j.pbi.2009.07.006

6. Calonje M, Sanchez R, Chen L, Sung ZR: **EMBRYONIC FLOWER1 participates in polycomb group-mediated AG gene silencing in *Arabidopsis***. *Plant Cell* 2008, **20**:277-291.

7. Helliwell CA, Wood CC, Robertson M, James Peacock W, Dennis ES: **The *Arabidopsis* FLC protein interacts directly in vivo with SOC1 and FT chromatin and is part of a high-molecular-weight protein complex**. *Plant J* 2006, **46**:183-192.

8. Searle I, He Y, Turck F, Vincent C, Fornara F, Krober S, Amasino RA, Coupland G: **The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis***. *Genes Dev* 2006, **20**:898-912.

9. Schmitz RJ, Amasino RM: **Vernalization: a model for investigating epigenetics and eukaryotic gene regulation in plants**. *Biochim Biophys Acta* 2007, **1769**:269-275.

10. Simpson GG: **The autonomous pathway: epigenetic and post-transcriptional gene regulation in the control of *Arabidopsis* flowering time**. *Curr Opin Plant Biol* 2004, **7**:570-574.

11. • Lee JH, Yoo SJ, Park SH, Hwang I, Lee JS, Ahn JH: **Role of SVP in the control of flowering time by ambient temperature in *Arabidopsis***. *Genes Dev* 2007, **21**:397-402.
The authors present the first genetic evidence of a functional link between SVP and FLC. SVP binding to CArG boxes in the *FT* locus is demonstrated by ChIP assays on protoplasts misexpressing a SVP:HA fusion protein.

12. •• Li D, Liu C, Shen L, Wu Y, Chen H, Robertson M, Helliwell CA, Ito T, Meyerowitz E, Yu H: **A repressor complex governs the integration of flowering signals in *Arabidopsis***. *Dev Cell* 2008, **15**:110-120.
The mechanistic basis of the SVP/FLC interaction initially reported in [11] is elucidated in detail. SVP:6HA fusion proteins expressed under the native *SVP* promoter are used to demonstrate *in vivo* binding of SVP to a CArG box directly neighboring a *FLC* binding site in the *SOC1* promoter region. This interaction is confirmed using gel shift and reporter assays. Furthermore, SVP binding to *FT* promotor elements reported in [11] is confirmed in this system. Direct interaction of SVP and FLC proteins is demonstrated by coimmunoprecipitation *in vitro* and *in vivo*.

13. Fujiwara S, Oda A, Yoshida R, Niinuma K, Miyata K, Tomozoe Y, Tajima T, Nakagawa M, Hayashi K, Coupland G *et al.*: **Circadian clock proteins LHY and CCA1 regulate SVP protein accumulation to control flowering in *Arabidopsis***. *Plant Cell* 2008, **20**:2960-2971.

14. Alvarez-Buylla ER, Pelaz S, Liljegren SJ, Gold SE, Burgeff C, Ditta GS, Ribas de Pouplana L, Martinez-Castilla L, Yanofsky MF: **An ancestral MADS-box gene duplication occurred before the divergence of plants and animals**. *Proc Natl Acad Sci U S A* 2000, **97**:5328-5333.

15. Scortecci K, Michaels SD, Amasino RM: **Genetic interactions between FLM and other flowering-time genes in *Arabidopsis thaliana***. *Plant Mol Biol* 2003, **52**:915-922.

16. Scortecci KC, Michaels SD, Amasino RM: **Identification of a MADS-box gene, FLOWERING LOCUS M, that represses flowering**. *Plant J* 2001, **26**:229-236.

17. Werner JD, Borevitz JO, Warthmann N, Trainer GT, Ecker JR, Chory J, Weigel D: **Quantitative trait locus mapping and DNA array hybridization identify an FLM deletion as a cause for natural flowering-time variation**. *Proc Natl Acad Sci U S A* 2005, **102**:2460-2465.

18. • Castillejo C, Pelaz S: **The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering**. *Curr Biol* 2008, **18**:1338-1343.
The authors introduce TEM1 and TEM2 as novel direct repressors of flowering upstream of *FT*. Genetic experiments suggest a competition of CO and the TEM proteins for *FT* activation; direct binding of TEM1 to the 5′ UTR of *FT* is shown by EMSA and ChIP.

19. Aukerman MJ, Sakai H: **Regulation of flowering time and floral organ identity by a MicroRNA and its APETALA2-like target genes**. *Plant Cell* 2003, **15**:2730-2741.

20. Chen X: **A microRNA as a translational repressor of APETALA2 in *Arabidopsis* flower development**. *Science* 2004, **303**:2022-2025.

21. Park W, Li J, Song R, Messing J, Chen X: **CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana***. *Curr Biol* 2002, **12**:1484-1495.

22. • Jung JH, Seo YH, Seo PJ, Reyes JL, Yun J, Chua NH, Park CM: **The GIGANTEA-regulated microRNA172 mediates photoperiodic flowering independent of CONSTANS in *Arabidopsis***. *Plant Cell* 2007, **19**:2736-2748.
This paper demonstrates control of flowering time by GI-dependent regulation of miR172 expression. The analysis is extended to the targets of miR172, for which misexpression data are presented, with a focus on *TOE1* as the only member of this group with a single mutant phenotype.

23. Schmid M, Uhlenhaut NH, Godard F, Demar M, Bressan R, Weigel D, Lohmann JU: **Dissection of floral induction pathways using global expression analysis**. *Development* 2003, **130**:6001-6012.

24. •• Mathieu J, Yant LJ, Mürdter F, Küttner F, Schmid M: **Repression of flowering by the miR172 target SMZ**. *PLoS Biol* 2009, **7**:e1000148.
Using chromatin immunoprecipitation coupled to genome tiling arrays, the authors demonstrate that SMZ, a repressor of flowering, binds directly to the *FT* genomic locus and to several other key flowering-related loci. Unexpectedly, the ability of this AP2-like miR172-target to repress flowering strictly depends on the presence of the MADS-domain transcription factor FLM, thus demonstrating a novel link between these classes of transcription factors. They further show that SMZ binds specifically to its own regulatory sequences and to those of three closely related genes, providing a mechanism for the strong negative feedback regulation among this clade of AP2-like miRNA172 targets.

25. Schwab R, Palatnik JF, Riester M, Schommer C, Schmid M, Weigel D: **Specific effects of microRNAs on the plant transcriptome**. *Dev Cell* 2005, **8**:517-527.

26. Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C *et al.*: **FT protein movement contributes to long-distance signaling in floral induction of *Arabidopsis***. *Science* 2007, **316**:1030-1033.

27. Lifschitz E, Eviatar T, Rozman A, Shalit A, Goldshmidt A, Amsellem Z, Alvarez JP, Eshed Y: **The tomato FT ortholog triggers systemic signals that regulate growth and flowering and substitute for diverse environmental stimuli**. *Proc Natl Acad Sci U S A* 2006, **103**:6398-6403.

28. Lin MK, Belanger H, Lee YJ, Varkonyi-Gasic E, Taoka K, Miura E, Xoconostle-Cazares B, Gendler K, Jorgensen RA, Phinney B *et al.*: **FLOWERING LOCUS T protein may act as the long-distance florigenic signal in the cucurbits**. *Plant Cell* 2007, **19**:1488-1506.

29. Mathieu J, Warthmann N, Kuttner F, Schmid M: **Export of FT protein from phloem companion cells is sufficient for floral induction in *Arabidopsis***. *Curr Biol* 2007, **17**:1055-1060.

30. Notaguchi M, Abe M, Kimura T, Daimon Y, Kobayashi T, Yamaguchi A, Tomita Y, Dohi K, Mori M, Araki T: **Long-distance, graft-transmissible action of *Arabidopsis* FLOWERING LOCUS T protein to promote flowering**. *Plant Cell Physiol* 2008, **49**:1645-1658.

31. Li C, Zhang K, Zeng X, Jackson S, Zhou Y, Hong Y: **A *cis*-element within FLOWERING LOCUS T mRNA determines its mobility and facilitates trafficking of heterologous viral RNA**. *J Virol* 2009, **83**:3540-3548.

32. Honma T, Goto K: **Complexes of MADS-box proteins are sufficient to convert leaves into floral organs**. *Nature* 2001, **409**:525-529.

33. Abe M, Kobayashi Y, Yamamoto S, Daimon Y, Yamaguchi A, Ikeda Y, Ichinoki H, Notaguchi M, Goto K, Araki T: **FD, a bZIP protein mediating signals from the floral pathway integrator FT at the shoot apex**. *Science* 2005, **309**:1052-1056.

34. Wigge PA, Kim MC, Jaeger KE, Busch W, Schmid M, Lohmann JU, Weigel D: **Integration of spatial and temporal information during floral induction in *Arabidopsis***. *Science* 2005, **309**:1056-1059.

35. Ahn JH, Miller D, Winter VJ, Banfield MJ, Lee JH, Yoo SY, Henz SR, Brady RL, Weigel D: **A divergent external loop confers antagonistic activity on floral regulators FT and TFL1**. *EMBO J* 2006, **25**:605-614.

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36. Kardailsky I, Shukla VK, Ahn JH, Dagenais N, Christensen SK, Nguyen JT, Chory J, Harrison MJ, Weigel D: **Activation tagging of the floral inducer FT**. *Science* 1999, **286**:1962-1965.

37. Kobayashi Y, Kaya H, Goto K, Iwabuchi M, Araki T: **A pair of related genes with antagonistic roles in mediating flowering signals**. *Science* 1999, **286**:1960-1962.

38. Hanzawa Y, Money T, Bradley D: **A single amino acid converts a repressor to an activator of flowering**. *Proc Natl Acad Sci U S A* 2005, **102**:7748-7753.

39. Li C, Dubcovsky J: **Wheat FT protein regulates VRN1 transcription through interactions with FDL2**. *Plant J* 2008, **55**:543-554.

40. Strasser B, Alvarez MJ, Califano A, Cerdan PD: **A complementary role for ELF3 and TFL1 in the regulation of flowering time by ambient temperature**. *Plant J* 2009, **58**:629-640.

41. Sohn EJ, Rojas-Pierce M, Pan S, Carter C, Serrano-Mislata A, Madueno F, Rojo E, Surpin M, Raikhel NV: **The shoot meristem identity gene TFL1 is involved in flower development and trafficking to the protein storage vacuole**. *Proc Natl Acad Sci U S A* 2007, **104**:18801-18806.

42. Elitzur T, Nahum H, Borovsky Y, Pekker I, Eshed Y, Paran I: **Co-ordinated regulation of flowering time, plant architecture and growth by FASCICULATE: the pepper orthologue of SELF PRUNING**. *J Exp Bot* 2009, **60**:869-880.

43. Mimida N, Kotoda N, Ueda T, Igarashi M, Hatsuyama Y, Iwanami H, Moriya S, Abe K: **Four TFL1/CEN-like genes on distinct linkage groups show different expression patterns to regulate vegetative and reproductive development in apple (*Malus* × *domestica* Borkh)**. *Plant Cell Physiol* 2009, **50**:394-412.

44. •• Liu C, Zhou J, Bracha-Drori K, Yalovsky S, Ito T, Yu H: **Specification of *Arabidopsis* floral meristem identity by repression of flowering time genes**. *Development* 2007, **134**:1901-1910.

A convincing array of complementary evidence is marshalled to show how AP1 acts as a master repressor of *SVP*, *AGL24*, and *SOC1* to shut down the shoot developmental program. ChIP, GR induction experiments and reporter lines implicate particular CArG boxes as direct targets of AP1 mediating a potent repressive effect on flowering time genes. Thus, one of the canonical A-class homeotic genes, AP1, also functions as a potent transcriptional repressor, preventing the reversion of floral to shoot meristems.

45. Yu H, Ito T, Wellmer F, Meyerowitz EM: **Repression of AGAMOUS-LIKE 24 is a crucial step in promoting flower development**. *Nat Genet* 2004, **36**:157-161.

46. Immink RG, Nougalli Tonaco IA, de Folter S, Shchennikova A, van Dijk AD, Busscher-Lange J, Borst JW, Angenent GC: **SEPALLATA3: The ''glue'' for MADS box transcription factor complex formation**. *Genome Biol* 2009, **10**:R24.

47. Lee J, He K, Stolc V, Lee H, Figueroa P, Gao Y, Tongprasit W, Zhao H, Lee I, Deng XW: **Analysis of transcription factor HY5 genomic binding sites revealed its hierarchical role in light regulation of development**. *Plant Cell* 2007, **19**:731-749.

48. Morohashi K, Grotewold E: **A systems approach reveals regulatory circuitry for *Arabidopsis* trichome initiation by the GL3 and GL1 selectors**. *PLoS Genet* 2009, **5**:e1000396.

49. Oh E, Kang H, Yamaguchi S, Park J, Lee D, Kamiya Y, Choi G: **Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in *Arabidopsis***. *Plant Cell* 2009, **21**:403-419.

50. • Kaufmann K, Muino JM, Jauregui R, Airoldi CA, Smaczniak C, Krajewski P, Angenent GC: **Target genes of the MADS transcription factor SEPALLATA3: integration of developmental and hormonal pathways in the *Arabidopsis* flower**. *PLoS Biol* 2009, **7**:e1000090.

The authors present the first genome-wide analysis of binding sites of a MADS-domain transcription factor in plants and also the first ChIP-Seq experiment on a flowering-related protein. They show that the central MADS-domain transcription factor SEPALLATA3 binds to thousands of loci throughout the genome, many of which encode transcription factors. The authors provide evidence that SEP3 binds to, and modulates, the transcription of many target genes involved in hormonal signaling pathways.

51. Laubinger S, Marchal V, Le Gourrierec J, Wenkel S, Adrian J, Jang S, Kulajta C, Braun H, Coupland G, Hoecker U: ***Arabidopsis* SPA proteins regulate photoperiodic flowering and interact with the floral inducer CONSTANS to regulate its stability**. *Development* 2006, **133**:3213-3222.

52. Jang S, Marchal V, Panigrahi KC, Wenkel S, Soppe W, Deng XW, Valverde F, Coupland G: ***Arabidopsis* COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response**. *EMBO J* 2008, **27**:1277-1288.

53. Liu LJ, Zhang YC, Li QH, Sang Y, Mao J, Lian HL, Wang L, Yang HQ: **COP1-mediated ubiquitination of CONSTANS is implicated in cryptochrome regulation of flowering in *Arabidopsis***. *Plant Cell* 2008, **20**:292-306.

54. Valverde F, Mouradov A, Soppe W, Ravenscroft D, Samach A, Coupland G: **Photoreceptor regulation of CONSTANS protein in photoperiodic flowering**. *Science* 2004, **303**:1003-1006.

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