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Integrating hormones into the floral-transition pathway of *Arabidopsis thaliana*

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ABSTRACT

The transition from vegetative to reproductive growth is a major phase change in angiosperms. In annual plants such as *Arabidopsis thaliana* (Arabidopsis), this change is irreversible, and as such, the regulation of its timing must be tightly controlled. Plant hormone (phytohormone) signalling is known to regulate suites of morphogenic processes in Arabidopsis a role in flowering-time control is starting to emerge as one key-controlling step. This review focuses on experimental evidence in the Arabidopsis that both classical and newly described phytohormones serve within the signal network leading to a reproductive phase transition, as both positive and repressive elements, depending on the phytohormone and growth conditions. Examples of genetic and pharmacological experiments that implicate phytohormones as components of the floral-timing syndrome will be described. I hope that this review will serve as a primer for future research on the mechanisms of action for each respective phytohormone on the floral transition in Arabidopsis, and lead to further experimentation on the crosstalk that likely bridges between them.

Key-words: flowering time; plant hormones – phytohormones.

INTRODUCTION

Plants have evolved with many strategies that allow them to reproduce under continuously changing environmental conditions. One such feature that enables effective competition for essential resources is developmental plasticity. Namely, this is the ability to adjust developmental programs in response to variations in the environment. The interplay between intrinsic signals complements the influence of diverse environmental factors, and convergence of these brings about the plasticity of morphological and physiological responses. One major morphological transition to ensure reproductive survival is that of from vegetative to reproductive growth; in annual plants, this is a terminal choice and must be precisely regulated. This transition occurs at the shoot apical meristem (SAM). During the vegetative phase, the SAM gives rise to lateral meristems that develop into leaves. Various environmental and endogenous signals that promote flowering induce an array of biochemical and cellular changes that alter the developmental fate of SAMs (Zeevaart 2008). Resultant features are that these start initiating floral primordial, which leads to the formation of lateral tissues that generate the floral organs (Sablowski 2007).

The introduction of *Arabidopsis thaliana* (Arabidopsis), a small weed from the family *Brassicaceae*, as a model annual plant has greatly facilitated studying genetic and molecular basis of various physiological processes regulating development. Numerous genetic screens for floral-timing mutants have been performed. These have aimed to find early and late flowering plants perturbed in different physiological processes that affect the timing of flowering, such as in response to varying light regimes, and altered response to known inductive treatments, such as prolonged exposure to cold or stress (Redei 1962; Martinez-Zapater & Somerville 1990; Sheldon *et al.* 2000; Michaels & Amasino 2001). Based on these studies, genetic pathways that regulate floral transition in Arabidopsis are beginning to be defined. Initially, four main genetic pathways were described based upon specific phenotypes of late-flowering mutants. These pathways define the role of the inductive photoperiods, a class of plant hormones [the gibberellins (GAs)], prolonged exposure to cold, and autonomous factors in the control of flowering time (Koornneef, Hanhart & van der Veen 1991). Further genetic analyses increased the complexity of our understanding of the floral promotion by including influence of light quality, ambient temperature and other factors into such models. The net effect towards the transition to flowering is a coordinated response to the convergence of these signals on a small number of developmental-transition genes, called floral-pathways integrators. Three genes are currently proposed to perform this function: *FLOWERING LOCUS T* (*FT*), *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*) and *LEAFY* (*LFY*) (Kardailsky *et al.* 1999; Kobayashi *et al.* 1999; Blazquez & Weigel 2000; Samach *et al.* 2000). Floral-pathway integrators lead to the activation of floral-meristem identity genes, which trigger the transition from vegetative to reproductive phase.

HORMONAL ACTION

Plant hormones (phytohormones) are endogenously occurring compounds that regulate multiple aspects of plant growth and development at low concentrations (Davies 2004). Classic studies have implicated several

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phytohormones in the floral transition as 'textbook' knowledge, but the role of phytohormones in floral timing has not been thoroughly explored in Arabidopsis. Originally, the term phytohormone was defined based on the definition for animal hormones, which are synthesized in one place, and transported to another tissue to exert signalling activity. The degree of the response is regulated by the concentration of the hormone (Davies 2004). Detailed analyses in plants have defined a broader definition, which includes that the phytohormone synthesis and action site need not be separated in time or space. Signalling of phytohormones can occur within the same tissue and even the same cell (Davies 2004). Of course, many plant hormones do undergo short- or long-distance transport, and this transport can be important for the physiological output (Davies 2004). The fact that phytohormones are being transported between plant tissues/organs makes them excellent candidates for messengers for specific physiological processes, such as, for example, a transition to flowering.

A physiological response to a given hormone depends on the concentration of the compound and the sensitivity of the plant tissue to the hormone (Davies 2004). The concentration of a phytohormone in a tissue/cell can be affected by its biosynthesis levels, and by inactivation and transport of the compound. It remains to be established in Arabidopsis, for all of the described phytohormones, the temporal and spatial network that defines the homeostatic mechanisms in the genesis and catabolism of these signalling molecules. What is known is that for many responses, a tissue-specific, developmentally programmed threshold must be exceeded to activate the cognate signal transduction pathway leading to morphogenic alterations, such as at the SAM leading to the hormonally triggered induction of floral primordia (Davies 2004).

Five classical phytohormones were described: GAs; auxins; cytokinins (CKs); ethylene; and abscisic acid (ABA). For decades, each of these molecules have been implicated in the flowering-time pathway (Davies 2004; Taiz & Zeiger 2006). Of particular note is classical evidence that the GA pathway could contribute to the florigenic signal; whether this is true in Arabidopsis has not been established (Zeevaart 2006, 2008). Recently, other compounds working at a low concentration have been additionally classified as phytohormones. A current list of phytohormones typically includes the jasmonates (JAs), salicylic acid (SA) and brassinosteroids (BRs) (Davies 2004). These 'new' phytohormones were also shown to be players in the floral transition. Understanding how the integrative network of 'old' and 'new' phytohormones regulates the floral transition will likely emerge as a robust field of investigation.

Phytohormone effects on flowering time have been performed in many classical experiments on a variety of plants. Depending on the species studied, phytohormones have differential effects on the timing of flowering. The collective knowledge gained from miscellaneous studies on the phytohormonal effects on diverse plant species has lead to confusing understandings of the effects of these molecules on this critical developmental phase transition. Recent progress has been made on examining a number of phytohormone responses on the reproductive transition within Arabidopsis. Studying complex phytohormone effects within a single species should assist a rational model of physiological action. In an effort to unify such a model, this review will focus on the hormonal control of flowering in Arabidopsis.

GA – THE MAJOR PHYTOHORMONE REGULATOR OF FLOWERING?

GAs are a class of phytohormones whose role in the transition to flowering in Arabidopsis is best understood. Further, their importance in flowering-time control seems to be the most pronounced over that of the other phytohormones. Wilson and colleagues were among the first to report that Arabidopsis plants attenuated in endogenous GAs flower late. They showed that a mutation in *GA1* locus results in severely delayed flowering, so much so that the *ga1* mutant can be incapable of flowering under growth under non-inductive short days (Wilson, Heckman & Somerville 1992). Lines impaired in GA signalling display similar phenotypes (Koornneef *et al.* 1991; Wilson *et al.* 1992; Moon *et al.* 2003). These mutant genotypes display only a weak late-flowering phenotype when grown under long-day photoperiods, indicating that this phytohormone promotes flowering under non-inductive conditions (Wilson *et al.* 1992; Reeves & Coupland 2001). Further supporting the positive effect of GAs on flowering time comes from studies on plants with enhanced GA-signalling, for example, *spindly* (*spy*), *gai* and overexpressors of *FLOWERING PROMOTIVE FACTOR1* (*FPF1*); these genotypes all flower early (Wilson *et al.* 1992; Jacobsen & Olszewski 1993; Kania *et al.* 1997). Finally, exogenous GAs or increasing GAs endogenous levels by overexpression of a biosynthetic gene, such as *GA5*, leads to early flowering. This response is most notable under short-day growth (Huang *et al.* 1998; Coles *et al.* 1999). GA is now considered an unquestioned floral-timing promoter.

Double-mutant analysis allowed defining the GA pathway as distinct from other flowering-promotive pathways (Reeves & Coupland 2001; Blazquez, Trenor & Weigel 2002). However, crosstalk between the GA pathway and other flowering-time pathways likely exist as a loss-of-function allele of the autonomous gene *FPA* was identified in a screen for components of GA signalling (Meier *et al.* 2001; Schomburg *et al.* 2001). In addition, contradictory to findings in other plant species (Zeevaart 2006), the vernalization response in Arabidopsis is independent from the GA pathway (Michaels & Amasino 1999b). Collective views that GA is a dominant phytohormone in the floral transition are thus supported by an array of physiological experiments using pharmacological, genetic and transgenic assay tools.

Recent efforts have tried to establish the mechanism of GA promotion of flowering. The GA pathway functions mostly through the upregulation of the floral integrators *LFY*, *SOC1* and *AGL24*, and overexpressing either of these

integrators partially rescues the GA-deficient line *ga1* (Blazquez & Weigel 2000; Moon *et al.* 2003; Liu *et al.* 2008). The molecular basis of the GA-mediated activation of *LFY* is quite well understood. Under short days, *LFY*-promoter activity increases gradually during vegetative development, and GA application, which accelerates flowering, enhances this promoter activity (Blazquez *et al.* 1998). A cis-regulatory element within the *LFY* promoter, distinct from a photoperiodic-response element, was identified. It is sufficient to drive GA-mediated upregulation of *LFY* (Blazquez & Weigel 2000). Thus, GA signals act directly on an integrator gene.

GA-dependent activation of *LFY* is likely mediated by a GAMYB-like transcription factor. This factor, termed MYB33, was shown capable of *in vitro* binding the GA-response motif in the *LFY* promoter (Blazquez & Weigel 2000; Gocal *et al.* 2001). GAMYB-mediated regulation of *LFY* within GA signalling was further investigated by the group of Harberd. They concluded that GAMYB proteins are predicted to target the microRNA miR159 (Achard *et al.* 2004). Additionally, it was shown that miR159 could direct cleavage of *MYB33* transcript, which caused a decrease in the abundance MYB33. miR159 levels were found to be GA-regulated, as their transcript levels were decreased upon the GA deficiency, and applying GA restored miR159 levels to the wild-type levels. GA-regulated abundance of miR159 was found to be mediated by a family of GA-signalling factors, collectively termed DELLA. They observed that miR159 levels were comparable in the wild type with the *ga1* mutant, which also lacked the activity of two DELLA proteins GAI and RGA. Thus, GA positively regulates miR159 by overcoming repression of DELLA proteins. miR159 was shown to be involved in the control of flowering time as transgenic elevation of miR159 led to delayed flowering, particularly under short days. Moreover, the level of the putative miR159 target, the *MYB33* transcript, was reduced in these lines, which was accompanied with lower *LFY* expression. Collective indications were that miR159-dependent regulation of *MYB33* is physiologically relevant and is likely important for the regulation of *LFY* expression.

Eriksson and colleagues demonstrated that in Arabidopsis the naturally occurring GA_4 is the most active GA in the floral induction. Furthermore, they found that GA_4 was the most abundant of all tested GAs in the shoot apex, and the endogenous GA_4 levels increased up to 100-fold just before floral initiation in plants grown under short days (Eriksson *et al.* 2006). This GA was also most efficient in inducing transition to flowering of the GA-deficient mutant *ga1*. The authors provided evidence that the GA_4 found in the shoot apex is derived from tissues outside the apex. It was also shown that transcription of GA-negative feedback-regulated *GA20OX* and *GA3OX* genes were unchanged when the GA_4 levels started to increase, and expression of positively regulated *GA2OX* genes was increased in the shoot apex at this temporal window. Finally, GA_4 application to a single leaf promoted flowering, and radioactively labelled GA could move from a leaf to the shoot apex (Eriksson *et al.* 2006). Thus, the implication is that endogenous GA_4 functions as a critical part of the florigenic signal.

BR – NEWLY DISCOVERED FLORAL PROMOTER

BRs are a described class of steroids in plants originally isolated from pollen as compounds with strong growth-promoting properties. They also effect cell elongation and division (Mitchell *et al.* 1970; Grove *et al.* 1979). This role was confirmed in BR-deficient lines. Steroid-deficient Arabidopsis mutants are dwarfs because of the pleitropic defects in elongation and division effects of this class of phytohormones. Among an array of phenotypes, these mutants were also reported as modestly late flowering (Li *et al.* 1996). As BRs have a role in photomorphogenesis, the positive effect of BRs in the floral-controlling network could be a simple misregulation of photoperiodic genes. For example, as BRs speed up circadian timing (Hanano *et al.* 2006), the trivial explanation for the floral-promotive activity could have been a phase misexpression of the *GIGANTEA* (*GI*) photoperiodic regulon, which detects day length. Genetic analysis of the steroid effect on flowering revealed that this was not the case (see further discussion).

Over the last decade, genetic work has furthered a postulated role for BRs in the promotion of flowering, as BR-deficient mutants *det2* and *dwf4* display a weak late-flowering phenotype (Chory, Nagpal & Peto 1991; Azpiroz *et al.* 1998). Moreover, the *bas1 sob7* double mutant, which is impaired in metabolizing BRs to their inactive forms, flowers slightly earlier, supporting the promoting role of BRs in floral transition (Turk *et al.* 2005). Genetics has thus been proven useful to catalog BR effects on flowering.

How BR signalling contributes within a genetic network leading towards floral induction was defined by recent work. As a result of a genetic screen, two independent alleles of *bri1* were isolated as strong enhancers of late-flowering phenotype of the autonomous mutant *luminidependens* (*ld*) (Domagalska *et al.* 2007). *BRI1* encodes a leucine-rich repeat receptor-like kinase (LRR-RLK) that functions as a receptor for BRs (Li & Chory 1997); thus, the result of the screen indicated that *BRI1* or BRs play a direct role in floral timing. Further work showed that *BRI1* establishes a previously uncharacterized genetic pathway that regulates the timing of the floral transition. The *BRI1* pathway appears to function mostly independent from the GA, the photoperiod and the vernalization pathways. This was derived from analysis of double mutants of *bri1* to mutations in those respective pathways. As a side note, these experiments exclude the photoperiodic modulator *GI* as a trivial explanation of BR effects. In contrast to BRs not fitting with GA, photoperiod or vernalization floral-timing networks, *BRI1* was found to genetically interact with the autonomous pathway. This genetic interaction was found to correlate in the repression of expression of the strong repressor *FLOWERING LOCUS C* (*FLC*) (Domagalska

et al. 2007). *FLC* in combination with *FRIGIDA* (*FRI*) define the winter annual habit of some natural populations (Lee, Bleecker & Amasino 1993; Michaels & Amasino 1999a). Given that the *bri1* single mutant has only a modest late-flowering phenotype, whereas the autonomous mutants or *FRI* plants have much more pronounced phenotypes, *BRI1* probably has an assistive role to the autonomous pathway in repression of *FLC*. This also implies that the *BRI1* pathway does not function to directly promote flowering, but acts to enable repression of strong floral repressor, which introduces the competence in the SAM to respond to floral-inductive signals. In support of this, BR acts as a modifier of autonomous elements to regulate the chromatin status at *FLC* (Domagalska *et al.* 2007). Another interesting observation is that BR signalling, as was seen with GA, is largely independent of the vernalization response. Collectively, this work firmly establishes the nature of BR induction of the floral transition.

Efforts to establish components of the BR-floral pathway came from studies on signalling. A downstream component in this pathway is the transcription factor family bri1-ethylmethane sulphonate suppressor 1/brassinazole-resistant 1 (BES1/BZR). In a protein-interaction screen, these factors were found to interact with EARLY FLOWERING 6 (ELF6) and RELATIVE OF EARLY FLOWERING 6 (REF6) (Yu *et al.* 2008). ELF6 and REF6 are sequence-related proteins involved in chromatin remodeling. Mutations in *elf6* and *ref6* result in early flowering (Noh *et al.* 2004). Interesting, *elf6* and *ref6* display BR-related phenotypes (Yu *et al.* 2008). Therefore it is plausible that the chromatin changes at *FLC* seen in *bri1* combined with autonomous mutants could be caused by defects in BES1/BZR regulation of ELF6/REF6 (Clouse 2008). This waits to be tested.

STRESS HORMONE EFFECTS AND LIMITED MECHANISTIC UNDERSTANDINGS

SA

SA serves as a known signalling molecule in the plant-defence response, and has been linked to leaf senescence (Morris *et al.* 2000; Loake & Grant 2007). A positive role for SA in the floral transition has been clearly demonstrated by a recent report highlighting that ultraviolet-C (UV-C) induces flowering, and does so mostly through SA-dependent processes (Martinez *et al.* 2004). Furthermore, phytochrome signalling intersects with SA signalling (Genoud *et al.* 2002). This correlates to flowering (Martinez *et al.* 2004). Thus, SA participates in flowering.

The physiological induction of flowering by UV-C was mostly found to be SA dependent (Martinez *et al.* 2004). Here, UV-C-irradiated NahG plants, which do not accumulate SA because of rapid catabolism, did not respond with accelerated flowering, as do wild-type plants. UV-C treatment in the wild type was found to induce expression of *FT*, and weakly *CONSTANS* (*CO*). This effect was greatly attenuated in the NahG plants, suggesting SA dependence of the response. Interestingly, both NahG plants and SA-deficient mutants were delayed in flowering in the absence of UV treatment, indicating an additional stress-independent role of SA in the floral transition. The effect of SA on flowering time is complex, as it appears that SA interacts mainly with (sub)-branches of the photoperiod and autonomous pathways. This suggestion was based on double-mutant analyses and related gene-expression studies. In one study, *gi* NahG genotypes flowered comparably with the *gi* single mutant of the photoperiod pathway. However, NahG delayed flowering of the *co* photoperiod mutant, collectively suggesting independence from *CO* and dependence on *GI*. *CO* levels were reduced in the SA-deficient plants, indicating that the SA pathway regulates *CO* expression. Surprisingly, *CO* expression was elevated in the late-flowering SA-deficient lines under non-inductive photoperiodic growth conditions. However, it seemed that SA-mediated regulation of flowering under long days is of less importance, as the phenotype of NahG plants and SA-deficient mutants is much more pronounced when plants are grown under non-inductive short-day conditions (Martinez *et al.* 2004).

The crosstalk of the autonomous pathway to the SA pathway was genetically tested (Martinez *et al.* 2004). When potential genetic interactions with selected autonomous mutants were tested, it was found that NahG enhanced *fca* regardless of the growth photoperiod. Curiously, NahG *fve* flowered similarly to *fve* under a long day growth, and this genotype flowered significantly later than single *fve* under non-inductive conditions. Consistent with the interaction of the autonomous pathway, *FLC* levels were elevated in SA-deficient mutants, as assayed under any photoperiodic regime. Surprisingly, the *flc* mutation was not found to suppress late-flowering of NahG plants under long days, and only partially suppressed under short days, indicating that that the delayed flowering of NahG is independent of *FLC* under long days, and only partially dependent on this floral repressor in short days. Furthermore, SA application reduces *FLC* levels in *fca* and *fve* mutants, but this was not sufficient to accelerate flowering of these lines. Thus, it appears that SA signalling acts to repress *FLC*, a mechanism most relevant under short-day growth, and this does not involve *FCA* nor *FVE*.

Many late-flowering mutants do not exhibit altered flowering time after SA application (Martinez *et al.* 2004). Interestingly, *co* responded to SA treatment by flowering earlier. This indicates the presence of other SA-independent factors required to promote flowering. Notably, other photoperiod and autonomous pathway mutants tested did not respond to SA application. Further, SA signalling does not appear to physiologically interact with the vernalization pathway, as NahG genotypes respond to vernalization treatment. Furthermore, the GA floral-induction pathway was not found to be affected by the decreased SA response, as the double *spy* NahG mutant flowers similarly to *spy* under both long and short photoperiods, and GA application induces earlier flowering in the SA-deficient plants. Collectively, much is to

be learned regarding how SA-derived signals promote the floral transition.

ABA

ABA plays a key role in the plant responses to abiotic stresses such salinity, drought and cold, and also serves as a developmental growth regulator (McCourt & Creelman 2008). This phytohormone therefore is an excellent candidate to integrate environmental inputs of abiotic stimuli with the timing of floral transition. Harberd and colleagues were among the first to study in detail this integrative role of ABA on Arabidopsis. They demonstrated that salt slows plant growth and extends vegetative growth through a DELLA-dependent mechanism. Furthermore, they showed that salt-signalling operates through ABA signalling (and is ABI1-dependent), and application of exogenous ABA results in late flowering. Further, this is attenuated in the quadruple DELLA-mutant (Achard *et al.* 2006, 2007). They also examined the molecular mechanisms of salt repression of flowering. They detected a reduction in *CO* levels, and an induction of *FLC* expression. Curiously, no evident changes upon salt treatment were found for the integrators *FT* and *SOC1*, which are known to be repressed by FLC (Searle *et al.* 2006). Finally, they observed that *LFY* expression in the salt-treated quadruple DELLA mutant was higher than that of the salt-treated wild type, indicating that salt signals inhibit flowering thorough its DELLA-dependent effect on *LFY* (Achard *et al.* 2006). As well, there were interactions with the photoperiod pathway. This is not a surprise, as ABA participates in circadian-clock function (Hanano *et al.* 2006). Connections to *FLC* were also noted (Achard *et al.* 2006). These two latter findings are consistent with the observation that ABA-deficient mutants are early flowering, in line with a genetic interpretation that ABA is an inhibitor on the floral transition (Barrero *et al.* 2005). As part of the salt effect was triggered by increases of ABA levels and by ABA-dependent signalling, and as salt and ABA had the same effect on floral transition, it seems reasonable that the flowering effects observed upon salt treatment could be attributed to ABA (Achard *et al.* 2006).

ABA action was linked to the autonomous floral-promotive pathway. One report demonstrated a connection between ABA and *FLC* expression (Razem *et al.* 2006); it is noted that these authors subsequently retracted their work (Razem *et al.* 2008). The authors reported in their retracted work that the FCA protein, which has been recognized as a regulator of flowering time acting in the autonomous pathway, is an ABA-binding protein. FCA was previously described as a nuclear-localized RNA-binding protein that interacts with FY (another protein that genetically acts within the autonomous pathway). This FCA–FY interaction is crucial for the autoregulation of the *FCA* polyadenylation in the control of *FLC* expression (Simpson *et al.* 2003). In the *fca* and the *fy* loss-of-function mutants, the balance between different *FCA* splice forms is altered, and as a result, the long form of the *FCA* transcript is abundant whereas levels of the truncated form are decreased (Simpson *et al.* 2003). Whereas others have reported evidence that FCA does not bind ABA (Risk, Macknight & Day 2008), and that ABA does not disrupt the FCA–FY interaction (Jang, Lee & Kim 2008), elements of the retracted Razem *et al.* (2006) work deserve consideration. It is particularly noted that the experiments showing that ABA application alters the ratio between the long and short splice forms of *FCA* have not yet been questioned (Razem *et al.* 2006). Similarly, their experiments confirmed that ABA application delays flowering, and this was extended by showing that this effect depends on the genetic activity of *FCA* and *FY*. Moreover, they showed that ABA application increased *FLC* transcript levels in the wild-type and ABA-deficient mutants, but not in *fca*, further supporting that FCA participates in this molecular response. This effect of ABA on *FLC* expression was similar to a previous report (Achard *et al.* 2006). Thus, even though FCA is not an apparent ABA receptor, the role of the autonomous pathway in ABA signalling, and vice versa, still merits investigation. Editorial discussion to the matter (McCourt & Creelman 2008) leads one to be tempted to focus on the canonical ABI1-dependent processes hypothesized by the Harberd group as the mechanism of ABA action on floral timing (Achard *et al.* 2006, 2007). Whether canonical ABI1-dependent processes intersect with autonomous genes, such as *FCA* and *FY*, appears a particularly timely question.

Ethylene

Ethylene is another example of phytohormone that is not only involved in plant-stress responses, but also regulates diverse developmental processes (Benavente & Alonso 2006). Seed germination, senescence, cell elongation and root formation are all described as ethylene-regulated processes. Timing of floral induction is also coordinated by this phytohormone action (Achard *et al.* 2007). Similarly to ABA, ethylene production is induced by salt stress, which results in delayed floral transition (Achard *et al.* 2006, 2007). Furthermore, growing plants in the presence of an ethylene precursor, or in ethylene-enriched air, resulted in late flowering (Achard *et al.* 2006). These initial findings were the basis for more thorough investigation that has lead to quite an in-depth understanding of the ethylene mode-of-action in flowering-time control. It was noted that the loss-of-function mutant *ctr1* (a major negative regulator of ethylene signalling) flowers late under any photoperiod (Achard *et al.* 2007). Further experiments revealed that ethylene signalling interacts with the GA pathway by reducing endogenous levels of GAs (Achard *et al.* 2007). Application of GA promoted flowering of *ctr1* and ACC-treated plants under all tested photoperiods. As well, the levels of endogenous bioactive GAs were found to be lower in *ctr1* compared with the wild type, whereas the levels of intermediate compounds are elevated. This suggested that ethylene inhibits the activity of GA catabolic enzymes. Increased GA signalling, as detected in the *SPINDLY* mutant, accelerated the flowering of *ctr1*. Further, GA application restored defective *LFY* and *SOC1* transcript levels in *ctr1*

to essentially wild-type levels. This effect was DELLA-dependent. The collective hypothesis was that ethylene effects on flowering are through a canonical GA pathway that this is DELLA mediated (Achard *et al.* 2007).

It has been shown that the ethylene effect on flowering time depends on EIN3-dependent signalling. Ethylene causes stabilization of EIN3 and EIN3-like proteins by inhibiting the activity of their protease $SCF^{EBF1/EBF2}$ (Binder *et al.* 2007). Simultaneous reduction of the activity of *EBF1* and *EBF*, by mutations, resulted in a *ctr1*-like delayed flowering (Achard *et al.* 2007). Similarly as *ctr1*, GA application promotes flowering of *ebf1 ebf2*, but the restoration of flowering response is not complete, indicating that this effect cannot be exclusively attributed to the reduced GA signalling. This collectively implicated the existence of an unknown DELLA-independent, ethylene-mediated mechanism of flowering-time control (Achard *et al.* 2007).

JAs

Jasmonic acid (JA) is a phytohormone with described roles in disease resistance to herbivory and in resistance to microbial pathogens (Browse 2005). Other work implicates a role for JA in another dehiscence, and thus implicates this phytohormone in developmental processes (Browse 2005). The studies of the role of this phytohormone in flowering time have been limited and still await in-depth investigation. In one study, where the concerted effect of JA application and lateral shading was examined, it was observed that when combined, the result was slightly late-flowering (Cipollini 2005). Thus, it seems that JA does not have a direct effect on flowering, but could function to modulate other inductive pathways. The descriptions on the role of JA in Arabidopsis flowering must be expanded, and only then can a mechanistic understanding be initiated.

Nitric oxide (NO)

Though NO is not described as a classical phyhormone, it has been shown to function as a biologically active compound that acts as a signalling molecule in plant cell biology (Neill *et al.* 2008). NO has been also reported to act as a growth regulator in plants, where it is involved in regulation of leaf expansion, senescence and de-etiolation (Leshem & Harmaty 1996; Beligni & Lamattina 2001). Importantly, NO production in plants is regulated by environmental factors, in particular by drought, salt stress and pathogen infection (Lamattina *et al.* 2003). Thus, this is another example of bioactive molecule that serves as integrator of environmental conditions with plant growth and development.

Recent studies revealed that NO performs functions in the control of flowering, and that its effects on the floral transition are inhibitory (He *et al.* 2004). In this study, exogenous treatment of Arabidopsis plants with a NO donor resulted in a delay in flowering, in a dose-dependent manner. As well, the *NO overproducer 1* (*nox1*) plants flowered late. At the same time, the NO-deficient mutant *nos1* displayed early flowering, confirming genetically a repressing function of NO in the floral transition. The delayed flowering of the *nox1* mutant was observed under long and short days, which resembles the phenotype of autonomous mutants. Indeed, *nox1* was found to have elevated levels of the *FLC* transcript. The mechanism of this altered *FLC* expression by NO still awaits investigation. However, the late-flowering phenotype cannot be exclusively assigned to the interaction with the autonomous pathway, as altered expression of genes in the photoperiod pathway was also observed. Specifically, the levels, but not the phase of the rhythmic expression of *CO* and *GI* transcripts in *nox1*, were reduced, whereas the expression of the two members of the central oscillator, *TOC1* and *CCA1*, were not changed. Thus, NO was proposed to interact with the photoperiod pathway to regulate *CO* expression through a *GI*-dependent but *CCA1*/*TOC1*-independent mechanism (He *et al.* 2004). It is still unclear how stress conditions alter the flowering-time regulation by the NO-dependent pathway, and it would be interesting to explore this further.

THE CELL DIVISION REGULATORS

CKs

CKs are a class of classical hormones with reported roles to regulate both the division cycle and meristem homeostasis (To & Kieber, 2008; Zhao 2008). CKs are believed to promote the floral transition, but their role in this process needs to be clarified (Bernier & Perilleux 2005). Early studies on Arabidopsis reported that the *amp1* mutant, which has increased CK content, flowers early. This mutant could suppress the late flowering of *gi*, but not *fca* (Chaudhury *et al.* 1993; Bernier & Perilleux 2005). Consistent with this photoperiodic effect, exogenous application of CK accelerates flowering in a light-dependent manner (Bernier & Perilleux 2005). Similarly consistent, Arabidopsis plants deficient in CK or impaired in CK signalling are delayed in the floral transition (Werner *et al.* 2003; Nishimura *et al.* 2004; Bernier & Perilleux 2005). Finally, upon photoperiodic induction, the amount of isopentenyl (iP)-type CKs increase in Arabidopsis leaves, in phloem sap and at the SAM (Corbesier *et al.* 2003). With insight that aspects of CK signalling contribute to flowering, much work is needed to establish the molecular pathways of this input. In part, one can speculate hormone crosstalk at the meristem as one component (see further discussion).

Auxin

Auxins are another classical hormone with well-reported roles as a regulator of both the division cycle and meristem homeostasis (Kepinski 2007; Zhao 2008). It can be hypothesized that auxins have a floral-inductive signalling role, as they regulate multiple aspects of embryonic and postembryonic developments. Topical application of auxin can induce flowering (Shimada, Yamane & Kimura 2005). Furthermore, auxins are known to be transported through the

plant and generate polar fluxes by being actively pumped from the shoot apex down the plant (Vieten *et al.* 2007). As such, auxins are excellent candidates to carry information over long distances. Interestingly, even though this phytohormone could be a major signal in a phase change, publications on the auxin effects on floral timing in Arabidopsis have been limited.

In one study, where day temperature and night temperature differed, there was a correlation between endogenous auxin content and the timing of flowering (Thingnaes *et al.* 2003). It was found that if the day temperature was warmer than night, auxin levels were found to be higher and elevation of temperature at either periodic window increased the timing rate of flowering. This is of interest as studies on hypocotyl elongation implicate signal flux as the capacitor of auxin action at elevated temperatures (Gray *et al.* 1998). Furthermore, this auxin effect appears to be a component of the shade-avoidance response, which is in itself a physiological trigger of flowering (Tao *et al.* 2008). Taken together, auxin can be a floral-promotion factor in Arabidopsis.

How does auxin lead to an induction of flowering? Recently, a clue came from the Michaels group where they showed that a component of the nuclear-pore complex participates in auxin responses and in flowering-time control (Jacob *et al.* 2007). Here, the trafficking of mRNAs was suggested as the basal phenotype. It could be inferred that the SAR3 and HST gene products, isolated in auxin screens, similarly contribute to this molecule process, perhaps via RNA metabolic processes, and this can modulate flowering time. Connected to this, mutations in a *bona fide* RNA polymerase II phosphatase termed C-terminal domain phosphatase-like 2 (CPL2) results in auxin signalling defects and early flowering (Ueda *et al.* 2008). What is clear from these collective studies is that there is a large gap of knowledge must be filled in how auxins could contribute to the floral syndrome.

CONCLUSION AND PERSPECTIVE: MORE QUESTIONS THAN ANSWERS?

Physiological and genetic approaches have revealed that different organ parts of Arabidopsis are important for the transition to flowering. Thus, these different tissues/organs must be able to communicate in order to coordinately regulate floral transition of the plant. Hormonal signals must partially mediate this process. Recent studies in Arabidopsis have successfully started to dissect the leaf-to-shoot apex relation and identified florigen and two other compounds that are transported from leaves to the apex before the floral transition (Zeevaart 2006, 2008). It can be argued that one hormonal compound is the phytohormone GA, which could be a component of the florigenic activity under non-inductive photoperiodic conditions, thus acting redundantly to the protein factor FT. Furthermore, the role of diverse phytohormones needs to be coherently integrated, in a full context of signal convergence. Only then will a view of long-range signals in the promotion of flowering be understood.

Root biology has been neglected as a signal source in Arabidopsis flowering-time research. One can wonder about CKs here, in the context of development, physiology and molecular biology. Root-derived CKs bring information to the shoot. Added to this, it is noted that autonomous genes and *FLC* are strongly expressed in root apical meristem (Noh & Amasino 2003). What is the role of such expression pattern? Do – and how do – CKs modulate this *FLC* root expression pattern? Many hormones could regulate *FLC* expression and interact with the autonomous regulation of flowering, but such regulation has solely focused on studies on aerial tissues. How these interactions and effects function in root-derived signals, and how phytohormones act in this tissue to promote/repress flowering, should be an interesting line of investigation.

Recent genetic data for some phythormones has revealed a conclusive effect on flowering time in Arabidopsis. I propose that this should be further explored to put these findings in the context of the whole plant and its different organs. It seems particularly noteworthy to explore which parts of the plant are necessary for each hormonal mode of action in relation to flowering, all at a spatial and temporal level of resolution. One can also speculate that the biosynthesis/catabolism/sensitivity to a given phytohormone, and a differential regulation at any of these steps, all leads from various environmental factors, providing another layer of complexity in the flowering network. What is collectively clear is that a need exists to integrating physiological approaches with genetics to build a comprehensive model for phytohormone activity during the floral transition in the model plant Arabidopsis.

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