Tissue specific action of Gibberellin in *Arabidopsis* flowering and development

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Aimone Porri

aus Pisa, Italien

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Berichterstatter: Prof. Dr. George Coupland

Prof. Dr. Martin Hülskamp

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Ich möchte meinem besten Freund Alfredo diese Arbeit widmen, der immer an mich geglaubt hat und mir die letzten fünfzehn Jahre geholfen hat mich als eine bessere Person zu entwickeln.

I want to dedicate this Ph.D thesis to my best friend Alfredo, who helped me during the last 15 years to grow up as a better person, and always believed in me.

Desidero dedicare questa tesi di dottorato al mio caro amico Alfredo che negli ultimi quindici anni ha saputo valorizzare al massimo le mie capacità umane, stimolandomi a diventare una persona migliore, e che non ha mai smesso di credere in me.

SUMMARY

This work focuses on understanding the roles of the plant hormone gibberellin (GA) in controlling the initiation of flowering in *Arabidopsis thaliana*. GA is essential to promote the transition to flowering under non inductive short-day (SD) photoperiods by activating transcription of the floral integrator *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and of the meristem identity gene *LEAFY (LFY)*. However, mutations in GA receptors also prevent flowering under inductive long days (LDs), suggesting that this hormone also has crucial functions in the initiation of flowering under these conditions. Here by overexpressing the GA catabolic enzyme GIBBERELLIN 2 OXIDASE 7 (GA2ox7) in specific plant organs, we show that GAs play important regulatory roles in the leaves and shoot apical meristem (SAM) to promote flowering under LDs. Our results suggest that GAs are required in the leaf to increase levels of *FT* mRNA, which encodes a protein that is part of the systemic florigen signal of *Arabidopsis*. At the SAM GAs promote expression of *SQUAMOSA PROMOTER BINDING PROMOTER LIKE (SPL)* genes downstream of the floral integrator *SOC1*. In addition, we characterised a novel function of the MADS box transcription factor SHORT VEGETATIVE PHASE (SVP) and demonstrated its link to the GA biosynthetic pathway at the SAM. Mutation of *SVP* results in a significant accumulation of active GA_4 through the upregulation of *GIBBERELLIN 20-OXIDASE 2 (GA20ox2)*, which encodes an enzyme involved in GA biosynthesis. Conversely overexpression of *SVP* from the *35S* promoter causes phenotypes characteristic of GA deficiency plants. We demonstrate that the SVP/GA20ox2 module is controlled by photoperiod through *FT, TSF* and *SOC1* at the SAM. Wild-type plants shifted from SDs to LDs showed downregulation of *SVP* in the centre of the SAM and increased levels of *GA20ox2* transcripts in the rib meristem region. These expression patterns are significantly compromised in plants lacking *FT, TSF* or *SOC1* functions. We propose that in response to LDs, *FT, TSF* and *SOC1* act to repress expression of *SVP* leading to upregulation of *GA20ox2*. The activation of *GA20ox2* expression causes increased GA content, which promotes flowering by activating transcription of *SPL* genes. Finally, we identified a link between a core subunit of chromatin remodelling complexes (CRCs) SWI3C and the GA signalling and biosynthesis pathways. The *swi3c* mutant displayed several developmental impairments, which

resembled those of GA deficient plants. In agreement with the phenotypic characterization, *swi3c* mutants showed lower levels of active GAs and reduced mRNA abundance of the GA receptor *GIDa*, suggesting that *SWI3C* is required to control development by modulating GA biosynthesis and perception. Moreover we demonstrate that SWI3C binds *in vivo* to some of the DELLA repressors (RGA, RGL1, RGL2, RGL3) and SPY O-GlcNAc transferase, two components of the GA signalling pathway. Our results indicate that CRCs control plant development at least in part by promoting GA biosynthesis, and by regulating expression of some GA responsive genes. Overall this work increases our understanding of the regulation of GA biosynthesis and signalling, as well as demonstrating new functions for these processes in the control of the floral transition.

ZUSAMMENFASSUNG

Die vorliegende Arbeit beschäftig sich mit der Aufklärung der Rolle des Pflanzenhormons Gibberellin (GA) bei der Kontrolle der Blühinduktion von *Arabidopsis thaliana*. GA ist essentiell für den Übergang von vegetativer zu reproduktiver Phase unter nicht-induktiven Kurztagbedingungen, da das Hormon die Transkription des Blühsignalintegrators *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) sowie des Meristemidentitätsgens *LEAFY (LFY)* aktiviert. Mutationen in GA-Rezeptoren verhindern jedoch auch eine Blüte unter induktiven Langtagbedingungen, so dass angenommen werden kann, dass das Hormon auch unter diesen Bedingungen wichtige Funktionen bei der Steuerung des Übergangs zur Blüte hat. Durch die Überexpression des am GA-Katabolismus beteiligten Gens GIBBERELLIN 2 OXIDASE 7 (GA2ox7) in spezifischen Pflanzenorganen konnte gezeigt werden, dass Gibberelline in Blättern und dem apikalen Sprossmeristem wichtige Rollen bei der Förderung der Blüte unter Langtagbedingungen spielen. Die gewonnenen Ergebnisse legen nahe, dass Gibberelline im Blatt benötigt werden, um die Menge der *FT* mRNA zu steigern, die für ein Protein kodiert, welches Teil des systemischen Florigensignals in *Arabidopsis* ist. Im apikalen Sprossmeristem hingegen fördern Gibberelline die Expression von *SQUAMOSA PROMOTER BINDING PROMOTER LIKE (SPL)* Genen im Signalweg nach dem Blühintegrator *SOC1*. Darüber hinaus haben wir eine neue Funktion des MADS box Transkriptionsfaktors SHORT VEGETATIVE PHASE (SVP) charakterisiert und seine Verbindung zum GA Biosyntheseweg im apikalen Sprossmeristem gezeigt. Mutationen von *SVP* führen zu einer signifikanten Anreicherung von aktivem GA_4 durch die Hochregulierung des Gens *GIBBERELLIN 20-OXIDASE 2 (GA20ox2)*, welches für ein Enzym kodiert, das an der GA Biosynthese beteiligt ist. Umgekehrt führt die Überexpression von *SVP* durch den *35S* Promoter zu Pflanzen mit einem Phänotyp, welcher für GA-Mangel typisch ist. Wir zeigen, dass das SVP/GA20ox2 Modul von *FT*, *TSF* und *SOC1* im apikalen Sprossmeristem kontrolliert wird. Wildtyppflanzen, welche von Kurztag- zu Langtagbedingungen transferiert werden, zeigen eine verringerte Expression von SVP im Zentrum des apikalen Sprossmeristems und eine gesteigerte Menge von *GA20ox2* Transkript im Rippenmeristem. Diese Expressionsmuster konnten in Pflanzen ohne *FT*, *TSF* oder *SOC1* Funktion so nicht gefunden werden. Wir postulieren, dass *FT, TSF* und

SOC1 unter Langtagbedingungen die Expression von *SVP* unterdrücken, was wiederum zu einer höheren Expression des *GA20ox2* Gens führt. Die Aktivierung der *GA20ox2* Expression führt zu einer gesteigerten GA Konzentration, welche die Blüte durch Transaktivierung der SPL Gene fördert.

Weiter haben wir eine Verbindung zwischen einer Kern-Untereinheit des Chromatin Remodelling Komplex (CRCs) SWI3C, des GA-Signals und dem GA Biosyntheseweg identifiziert. Die *swi3c* Mutante zeigt mehrere Entwicklungsstörungen, die denen der GA-Mangelmutanten ähneln. In Übereinstimmung mit der phänotypischen Charakterisierung weisen *swi3c* Mutanten eine niedrigere Konzentration von aktiven Gibberellinen sowie ein geringeres Vorkommen von mRNA auf, welche für den GA Rezeptor *GIDa* kodiert, was nahelegt, dass *SWI3C* benötigt wird, um die Entwicklung durch Modulierung der GA-Biosynthese und Perzeption zu kontrollieren. Weiterhin zeigen wir, dass SWI3C *in vivo* einige der DELLA Repressoren (RGA, RGL1, RGL2, RGL3) und die SPY O-GlcNAc Transferase bindet, welche zwei Komponenten des GA Signaltransduktionsweges darstellen. Unsere Ergebnisse weisen darauf hin, dass CRCs die pflanzliche Entwicklung zumindest teilweise durch Förderung der GA-Biosynthese sowie Regulierung der Expression einiger auf GA reagierender Gene kontrollieren. Die vorliegende Arbeit trägt zum Verständnis der Regulation der GA-Biosynthese und der GA-abhängigen Signalweiterleitung bei und zeigt neue Funktionen für diese Prozesse in der Kontrolle des Übergangs zur Blüte auf.

Chapter 1: GENERAL INTRODUCTION

Flowering is a crucial step in the life cycle of most plant species, which ensures a successful reproduction, determines the adaptation to a certain environment, and contributes to the yield. In the model plant *Arabidopsis thaliana* several pathways that promote the transition to flowering have been elucidated and deeply studied in the last 20 years (Turck et al., 2008; Andres and Coupland, 2012). These genetic pathways respond to environmental stimuli such as day length or winter low temperature, as well as endogenous signals, and converge on the regulation of a cluster of floral integrator genes whose functions have been characterised by forward genetic, molecular, biochemical and transgenic approaches.

Impressive progress has been made in understanding the genetics and molecular mechanisms that induce flowering, which reveals the complexity and the dynamics of this trait, and highlights its importance for plant evolution.

Induction of flowering by changing day length

Day length or photoperiod can be defined as the duration of the light period in the 24 hour light/dark cycle. This differs in Nature as changing seasons occur, with short photoperiods (SDs) in winter, and long photoperiod (LDs) in the spring-summer seasons. Plants were divided into 3 major groups, based on their responses to day length (Andres and Coupland, 2012). Long day plants flower in response to long photoperiods when the number of sunlight hours exceeds a critical day length. In short day plants, flowering is activated when the day period is shorter that a critical day length, in day neutral plants flowering occurs independently of photoperiod.

Arabidopsis thaliana lives in Nature as a summer or winter annual plant and switches from the vegetative to reproductive phase in response to LDs. *Arabidopsis* seeds germinate characteristically in autumn and seedlings stay vegetative during winter when short photoperiods block flowering. In spring, the number of sunlight hours progressively increases reaching a threshold that triggers reprogramming of the shoot apical meristem (SAM) to produce flowers instead of leaves.

Changing day lengths are perceived in the leaf where important regulators of flowering act to increase the transcription of *FLOWERING LOCUS T (FT),* a major regulator of *Arabidopsis* floral transition (Kobayashi et al., 1999; Samach et al., 2000). These regulators include the genes *GIGANTEA (GI), FLAVIN KELCH F BOX 1 (FKF1),* and *COSTANS (CO)* (Kobayashi and Weigel, 2007; Turck et al., 2008). Genetic and molecular analysis suggested that these genes are expressed in the leaf where CO protein is stabilised by exposure to LDs, and activate transcription of *FT*, presumably directly (An et al., 2004; Adrian et al., 2010). The circadian clock and light condition regulates *CO* transcript through GI and FKF1, two proteins that belong to the circadian clock system of *Arabidopsis* (de Montaigu et al., 2010). FKF1 and GI interact at the protein level in LD, leading to the degradation of *CO* transcriptional repressors CYCLING DOF FACTORs (DOFs) (Sawa et al., 2007; Fornara et al., 2009) allowing *CO* mRNA to increase in abundance. Interaction between FKF1 and GI occurs specifically under long photoperiods, ensuring high levels of *CO* mRNA (Sawa et al., 2007) (Fig. 1). *CO* mRNA increases in abundance at the end of the day allowing CO protein to be translated. In dark conditions, CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) and SUPPRESSOR OF

PHYTOCHROME (SPA) form a complex that leads to degradation of CO protein by the 26S proteasome (Valverde et al., 2004; Laubinger et al., 2006; Jang et al., 2008). The complex SPA-COP1 is then inactivated in the light through the activity of PHYTOCHROME A (PHYA) and CRYPTOCHROME 2 (CRY2) enabling CO protein to accumulate in abundance (Fig. 1). Therefore *CO* is regulated at the transcriptional and at the protein levels with CO protein only accumulating under LDs.

Activation of CO protein is a crucial step to promote transcription of *FT* (Turck et al., 2008) in the companion cell of the leaf (CC). FT protein is therefore produced in the leaf but flowering occurs at the SAM.

FT has been proposed to be a part of the florigen (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007; Tamaki et al., 2007) for its ability to move through the phloem system to the SAM where it induces flowering. The movement of FT protein is likely to occur through the plasmodesmata of the CC, where an FT integrating protein (FTIP1) helps FT to be uploaded to the sieve elements (Liu et al., 2012). FT is a small protein that belongs to the CETS protein family and shares homology with RAF Kinase Inhibitor Protein (RKIPs) in bacteria and mammals (Kardailsky et al., 1999; Kobayashi et al., 1999). Once FT has reached the SAM it forms a complex with the bZIP transcription factor FLOWERING LOCUS D (FD). In rice, this interaction was shown to be mediated by the 14-3-3 protein that functions as a bridge between FT and FD (Taoka et al., 2011). The heterodimer complex FT/FD is proposed to activate transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS (SOC1),* which encodes a MADS box transcription factor that plays an important function in promoting flowering under LDs and SDs (Borner et al., 2000; Samach and Coupland, 2000; Searle et al., 2006; Lee et al., 2008). In later events, SOC1 protein interacts with another MADS box transcription factor AGAMOUS-LIKE 24 (AGL24) and the resulting complex activates the floral meristem identity gene *LEAFY (LFY)* (Lee et al., 2008), which initiates floral development at the flanks of the SAM. Therefore, activation of *SOC1* by FT/FD complex is a crucial event of floral transition and for the onset of floral development. In addition, FD/FT activates expression of *APETALA 1 (AP1),* another important floral meristem identity gene that like *LFY*, induces floral formation at the flanks of the SAM (Wigge et al., 2005). Recently, it was shown that other important floral activators, *SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPLs)* genes are also target of the FT/FD and SOC1/AGL24 complexes.

These activate *SPLs* at the transcriptional level by binding directly to the promoter region (Wang et al., 2009; Yamaguchi et al., 2009). Overall, induction of *FT* transcription in the CC of the leaves and transport to the SAM is an important aspect of floral induction, which enables *Arabidopsis* plants to switch from the vegetative to the floral stage at the appropriate time of the year when environmental condition are suitable for reproduction.

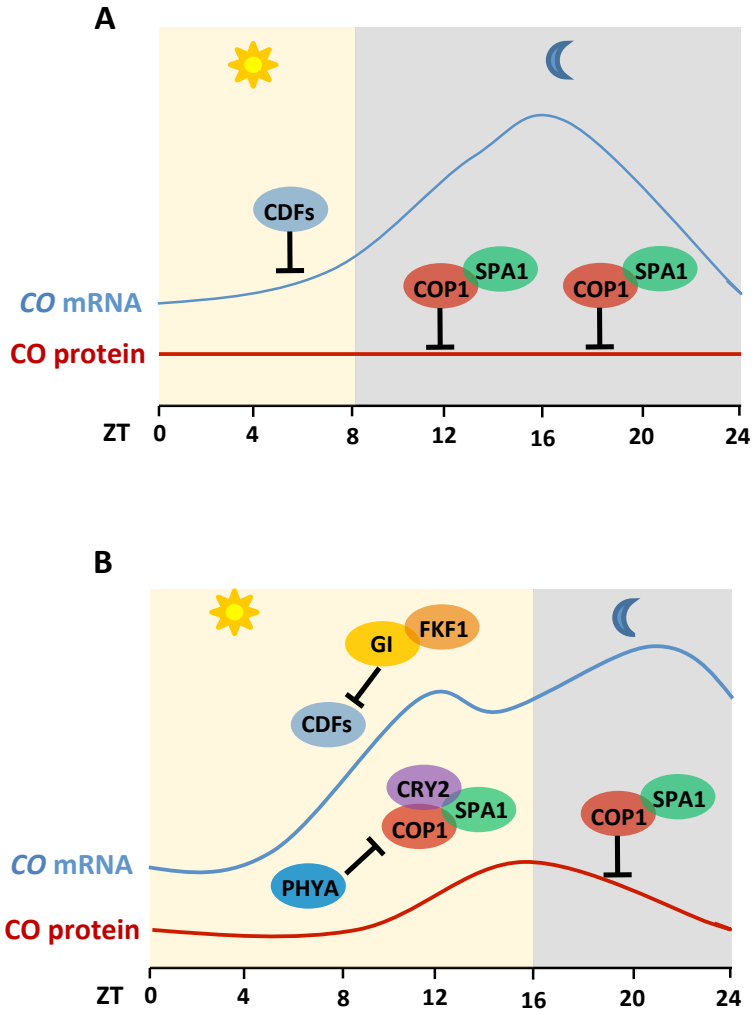

Fig 1. Mechanisms of *CO* mRNA and CO protein regulation in LDs and SDs.

Under SDs (A) CDFs repress *CO* transcription during the day, and the COP1/SPA1 complex leads to the degradation of CO protein in the dark, so that it never accumulates. Under LDs (B) GI/FKF1 complex promotes the degradation of CDFs enabling *CO* mRNA transcript to increase at the end of the day. The complex COP1/SPA1 is inhibited by CRY2 and PHYA resulting in increased levels of CO protein. ZT= Zeitgeber = hours after the beginning of the day period.

Induction of flowering by low temperature

Winter annual *Arabidopsis thaliana* plants experience a cold period (vernalization) in Nature during the entire winter season. The Vernalization event slowly induces flowering by provoking a steady repression of the floral repressor *FLOWERING LOCUS C (FLC)* (Michaels and Amasino, 1999). *FLC* encodes a MADS box transcription factor that inhibits flowering prior to vernalization (Sheldon et al., 2000). The expression of *FLC* decreases during cold treatments and remains stably repressed when plants are returned to normal temperature. The mechanisms that underlie *FLC* downregulation during winter involve changes in chromatin structure at the *FLC* locus (Bastow et al., 2004; Sung et al., 2006). The beginning of *FLC* transcriptional repression coincides with the rise in expression of two non-coding RNAs, COOLAIR and COLDAIR. Expression of *COOLAIR* is controlled from a promoter located within the 3´end of the *FLC* locus and COOLAIR is transcribed as antisense transcript (Swiezewski et al., 2009). COLDAIR is a non-coding transcript produced from the first intron of *FLC*, and is required to repress *FLC* expression (Heo et al., 2011). Vernalization induces *COOLAIR* expression, which reaches its maximum at the beginning of *FLC* downregulation. The decrease in *FLC* transcript is associated to the expression of *COLDAIR*, which is transcribed after *COOLAIR* has reached its peak of expression (Fig. 2). It has been proposed that COOLAIR acts to create a suitable chromatin structure that allows *COLDAIR* to be transcribed (Heo and Sung, 2011). Specific proteins that are required to repress *FLC* bind to COLDAIR, suggesting that this non-coding RNA might be implicated in recruiting protein complexes that represses *FLC* expression. The CURLY LEAF protein, a component of the polycomb repressive complex 2 (PRC2), interacts with COLDAIR, and induces histone H3 lysine 27 methylation required to steadily repress *FLC* transcription (Gendall et al., 2001; Heo and Sung, 2011). The PRC2 complex introduces tri-methylation at the amino terminus of Histone H3 at the *FLC* gene and later, this is recognised by the PRC1 complex, which sets a stable repression of the locus. After methylation of the histones, expression of *COOLAIR* and *COLDAIR* is not required. In addition, when both *COOLAIR* and *COLDAIR* fall in expression, *VERNALIZATION INSENSITIVE 3 (VIN3)* becomes expressed, and encodes a protein required for silencing *FLC* by interacting with PRC2 (Fig.2).

FLC exerts its function by repressing expression of *FT* in the leaf and of *SOC1* in the SAM (Searle et al., 2006). Therefore the vernalization period plays an important function in repressing *FLC* enabling the downstream targets *FT* and *SOC1* to activate flowering when the plant is later exposed to LDs. The role of *FLC* in flowering is linked to the function of *SHORT VEGETATIVE PHASE (SVP)*, which encodes another MADS box protein involved in the repression of flowering. SVP and FLC act together as a heterodimer complex to represses the expression of several flowering genes including *SOC1* and *FT*. Furthermore, genetic and molecular analysis showed that *FLC* function is dependent on *SVP* to fully repress flowering (Lee et al., 2007). Like *FLC*, *SVP* is also expressed in the leaf and in the SAM and *SVP* transcription falls when plants are exposed to inductive LDs, suggesting that its expression is regulated by changing day length (Jang et al., 2009). Therefore, *SVP* and *FLC* are important regulators of *Arabidopsis* floral transition, which act by repressing major flowering pathways activated under LDs. However, when important *SVP* downstream targets such as *FT* and *SOC1* are mutated in the triple mutant *svp ft soc1,* plants flowered much earlier than *ft soc1* double mutant (Jang et al., 2009; Torti et al., 2012). This suggests that *SVP* probably acts together with *FLC* to regulate important factor/s other than *FT* and *SOC1*.

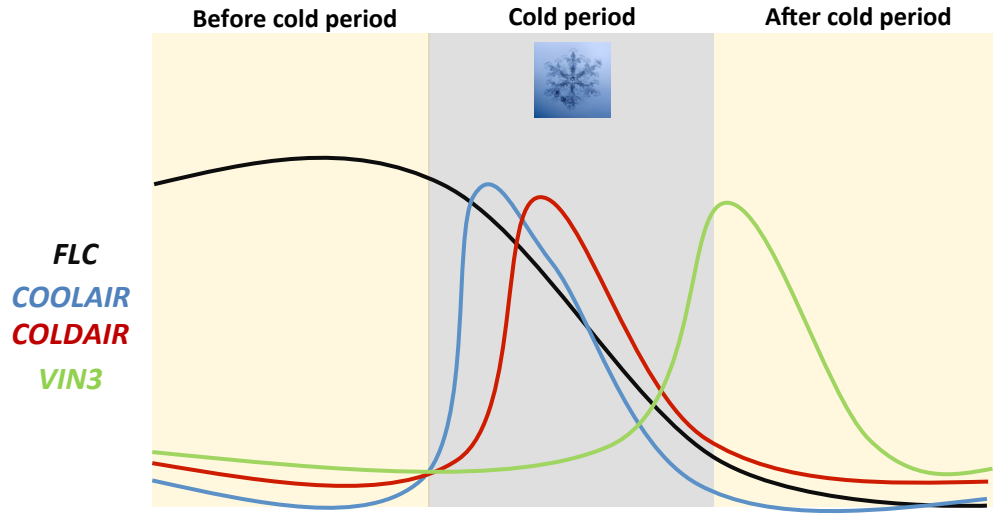

Fig 2. Cold treatments repress the expression of *FLC*.

The transcriptional repression of *FLC* occurs around the time of expression of two non-coding RNAs, COOLAIR and COLDAIR. COOLAIR may indirectly induce *COLDAIR*, which silences *FLC* by recruiting the PRC2 complex. At the end of the vernalization period *VIN3* leads to the steady repression of *FLC* by interacting with the PRC2 complex. The downregulation of *FLC* results in the activation of flowering by upregulation of *FT* and *SOC1* expression.

Induction of flowering by Gibberellins

Arabidopsis flowering is also controlled by endogenous signals that act independently of environmental cues such as day length or temperature. For instance, the plant growth regulator Gibberellin (GA) promotes the transition to flowering.

GAs are small organic molecules belonging to the family of diterpenoids, and are biosynthesized from geranylgeranyldiphosphate (GGDP) through several enzymatic reactions that involve three distinct classes of enzymes: terpene synthases (TPSs), cytochrome P450 monooxygenases (P450s), and 2-oxoglutarate-dependent dioxygenases (2ODDs) (Yamaguchi, 2008). TPSs are located in the plastid membrane (Helliwell et al., 2001) and catalyse the transformation of GGDP into *ent*-Kaurene intermediate. The latter is used as substrate from P450 enzymes to produce GA_{12}, which is a common precursor for most of the active GAs (Nelson et al., 2004). GA_{12} is further converted to active GA_1 and GA_4 through the activity of 2ODD enzymes, including GA20-oxidase (GA20ox) and GA3-oxidase (GA3ox) (Fig. 3). GA20ox introduces oxidations at the C-20 of GA_{12} to form GA_9, a C-19 y-lactone that is converted to active GA_4 or GA_1 by GA3ox enzymes. GA20ox removes a carboxylic group from GA_{12} to give C-19 precursors, and GA3ox adds a 3B-hydroxyl group on C-3 that provides functional GAs. Bioactive GA_4 and GA_1 are inactivated by GA2-oxidase (GA2ox), which catalyses a 2β-hydroxylation on C-2, creating products that are no longer active (Fig. 3). GA2ox acts on C-19 or C-20 precursors as well as on GA_4 and GA_1, therefore regulating GA content at different points of the biosynthetic pathway (Thomas et al., 1999). The levels of active GAs is maintained constant at the cellular level through a feedback mechanism, in which high GAs content triggers repression of genes encoding GA biosynthetic enzymes, and conversely, it activates expression of genes encoding GA catabolic enzymes.

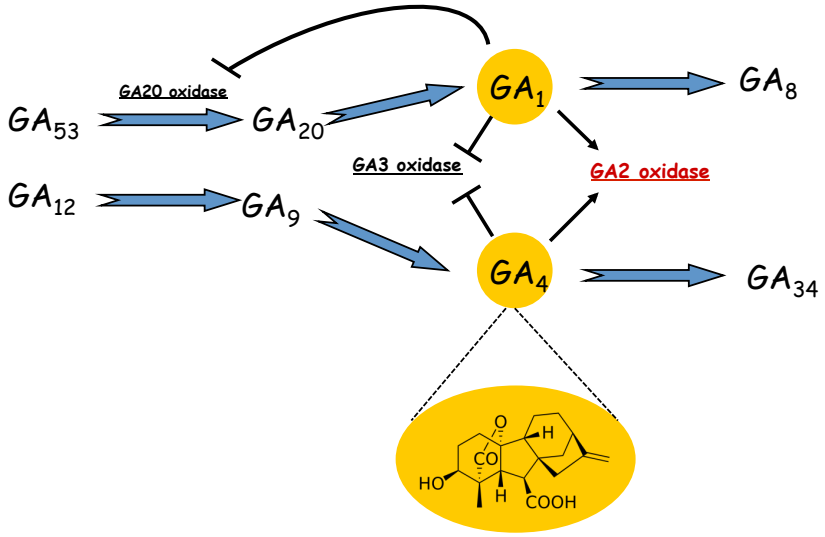

Fig 3. GA biosynthesis occurs through several enzymatic reactions carried out in the final steps by GA20ox and GA3ox.

GA20ox enzymes act on C-20 of GA precursors GA_{53} and GA_{12} to give GA_{20} or GA_9 intermediates. GA_{20} and GA_9 are converted to active forms GA_4 and GA_1 by GA3ox, which adds a hydroxyl group at the C-3. The enzymes GA2ox play important functions in the turnover of GAs by converting GA_4 and GA_1 to inactive products GA_8 and GA_{34}.

GAs control development of growing tissue of *Arabidopsis,* as well as in important crop species. *GA20ox* and *GA3ox* may be active in tissues where cell division and elongation occurs (Itoh et al., 1999; Kaneko et al., 2003). In addition, *GA1* (TPS) promoter sequence fused to a *GUS* reporter gene showed expression mainly in actively growing tissues, indicating that these are the most important sites of GA biosynthesis (Silverstone et al., 1997). In tobacco, *GA3ox* is expressed in elongating and dividing tissues including the rib meristem area, and the root tip (Itoh et al., 1999). In addition, GAs are produced in the embryo of cereal grains, and then transferred to the aleurone tissue where they activate α-amylase, and induce germination (Kaneko et al., 2003). GAs are required to promote several other developmental processes including leaf expansion, seed germination, chlorophyll biosynthesis, hypocotyl elongation and flowering. GA signalling is mediated by DELLA proteins, which belong to the GRAS family and work as transcriptional

repressors. When GAs are present in a modest amount, DELLAs bind and inhibit transcription factors and prevent their binding to DNA (de Lucas et al., 2008; Feng et al., 2008), thus preventing the transcription of GA-regulated genes. However, when GA biosynthesis is stimulated, the hormone binds the GA receptor GIBBERELLIN INSENSITIVE 1 (GID1), which changes its conformation structure, allowing it to bind to DELLA proteins (Shimada et al., 2008). Upon binding with GA-GID1, DELLAs are targets for ubiquitination, and consequently degraded in the 26S proteome pathway (Murase et al., 2008). The degradation of DELLAs releases the GA responsive transcription factors that are required to mediate the GA effect on growth and development. A clear example of the above described mechanism is the PHYTOCHROME INTERACTING FACTORs (PIFs), which work as transcription factors to control plant growth in response to GAs (Feng et al., 2008). In the presence of GAs, PIFs are released from DELLAs, and induce transcription of several genes implicated in the control of hypocotyl growth, chlorophyll synthesis, and cell elongation (de Lucas et al., 2008) (Fig. 4).

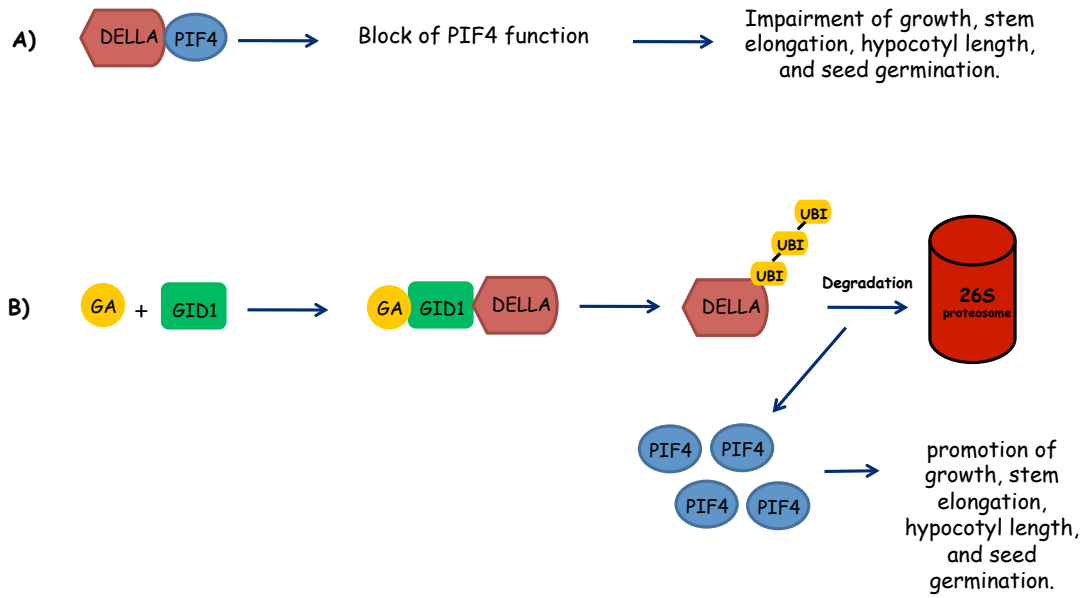

Fig 4. GA dependent function of PIF4.

PHYTOCHROME INTERACTING FACTORs (PIFs) are required to regulate many phenotypic traits, including chlorophyll content, cell elongation, hypocotyl growth, and seed germination. Several PIFs including PIF4, are physically bound by the DELLA transcriptional repressor proteins (A), which inhibit PIF functions. When GAs are synthesised (B), the transcriptional repressor proteins DELLAs are ubiquitinated and degraded, resulting in the release of PIFs.

The relevance of GAs in flowering control has been shown mostly under non-inductive SD conditions, where GAs are essential to promote the switch from the vegetative to the reproductive phase. During transition, the vegetative meristem that produced leaves and other aerial parts of the plant is transformed to an inflorescence meristem, which produces flowers until the senescence phase. Mutants that are affected in GA biosynthesis such as the *ga1-3* mutant, or plants lacking GID receptors such as the *gid a-b-c* triple mutant, did not flower under SDs (Koornneef and Vanderveen, 1980; Griffiths et al., 2006). In wild-type plants, the levels of bioactive GA_4 gradually increased at the SAM until it reached a threshold that triggered flowering (Eriksson et al., 2006). This increase in GA_4 could not be correlated with increased expression levels of genes encoding GA biosynthetic enzymes at the SAM. Therefore, it was proposed that GAs might move from the leaf to the SAM, presumably through the phloem system, similar to FT. This possibility was further supported by experiments in which labelled GA_4 applied in the leaf was detected at the SAM (Eriksson et al., 2006). At the SAM GAs promote the expression of the floral integrator *SOC1* under SDs, (Moon et al., 2003) as well as of the meristem identity gene *LFY*, thus promoting the transition to flowering and floral initiation. The GA effect on *LFY* transcription occurs through GIBBERELLIN RESPONSIVE ELEMENTS (GAREs) located in the *LFY* promoter, whereas it remains to be elucidated how GAs control the expression of *SOC1*.

Induction of flowering by endogenous signals

In the absence of external stimuli the genetic module composed of miR156 and *SPLs* promotes flowering as the plants age. This genetic pathway, called the aging pathway because of its ability to promote flowering during aging, has been extensively studied in *Arabidopsis* (Wang et al., 2009) as well as in the perennial *Arabis alpine* (Bergonzi et al., 2013). In these two plant species *SPLs* are involved in the promotion of flowering and in controlling the switch from the juvenile to adult phase during vegetative growth.

SPL proteins range in size from 131 to 927 amino acids (Cardon et al., 1999; Yu et al., 2010). These protein sequences are characterised by the presence of a conserved motif composed from 79 amino acids, which is required for DNA-interaction through the cis element GTAC (Cardon et al., 1999). Furthermore, this domain contains a nuclear

localisation signal, which in part overlaps with the DNA binding domain (Birkenbihl et al., 2005).

The *SPLs* mRNA sequence contains miRNA responsive elements required for posttranscriptional regulation by miR156 and miR157. At least 11 out of 17 *SPL* genes of *Arabidopsis thaliana* are regulated post-transcriptionally by miR156*,* which binds the last exon or the untranslated region of *SPL* mRNAs (Rhoades et al., 2002; Gandikota et al., 2007).

Previous studies (Wang et al., 2009; Wu et al., 2009) suggested that in *Arabidopsis SPLs* are involved in important developmental processes such as leaf development, fruit formation, transition from juvenile to adult phase and flowering. Several *SPLs* including *SPL4* and *SPL5* regulate trichome formation and distribution, and leaf cell size (Usami et al., 2009). The other members of the group, *SPL9* and *SPL15* control leaf shape (Usami et al., 2009). As the plant ages the transcript levels of miR156 and miR157 decrease in abundance allowing SPL proteins to accumulate and activate directly the transcription of *MIR172* (Wang et al., 2009). Activation of *MIR172* by SPLs leads to the downregulation of miR172 downstream targets *APETALA2 (AP2)-li*ke, *TARGET OF EAT 1 (TOE1)* and *TOE2*, which repress adult traits (Wu et al., 2009) . Therefore, during growth the levels of miR156 decreases, leading to accumulation of SPLs, which activate the transcription of *MIR172,* thus promoting the switch from juvenile to adult stage. At the SAM the abundance of *SPL* mRNAs also increase in response to miR156 downregulation. SPLs activate in the SAM important floral genes such as *AP1*, *SOC1* and *AGL42* (Wang et al., 2009). In addition, SPL3 binds directly to the promoter of *LFY* and of *FRUITFULL (FUL),* a gene with a redundant function to *SOC1* (Wang et al., 2009; Yamaguchi et al., 2009) Recently (Torti et al., 2012) reported that *SPLs* are activated downstream of *SOC1* in the SAM of plants exposed to inductive LDs, thus indicating the presence of a positive feedback loop between *SOC1* and *SPLs* that in turn promote flowering. In agreement with the above results SOC1 was found to bind directly to *SPL3, SPL4* and *SPL5* promoters (Jung et al., 2011). Furthermore overexpression of *SPL3* in a *soc1* mutant background could not suppress the late flowering of *soc1*, suggesting that *SOC1* is required for the *SPL3* mediated early flowering (Jung et al., 2011) . In addition to the role of *SPLs* in the SAM, the activation of *MIR172* by the miR156*/SPLs* module plays also an important role in activating transcription of *FT* in the CC of the leaf (Wang et al., 2009).

These results suggest that *SPLs* have two different spatially distinct functions: in the SAM to promote expression of important floral integrators, and in the leaf to induce *FT*.

AIM OF THE PROJECT

The aim of this research project was to characterize the functions of the plant hormone gibberellin in the contexts of flowering and development using the model plant *Arabidopsis thaliana*.

In the first part of the research (Chapter 2) we focused on the spatial effects that gibberellin plays under long-day conditions that rapidly induce flowering. We generated transgenic plants misexpressing *GA2ox7,* a gene that encodes an enzyme that reduces levels of active gibberellin. The ectopic expression of *GA2ox7* was driven by the tissue specific promoters *SUC2* and *KNAT1* in the companion cells of the leaf and at the shoot meristem, respectively. These genetic approaches allowed us to assess the regulatory roles of the hormone in different plant organs to regulate flowering and other developmental traits. Moreover, it enabled us to place gibberellin in novel genetic hierarchies that were previously not described.

In the second part of the project (Chapter 3) we focused our attention on the genetic mechanisms controlling the biosynthesis of gibberellin during photoperiodic flowering. We employed the *svp-41* mutant, which displayed several GA over accumulation-like phenotypes, to unravel how in *Arabidopsis* gibberellin content increases in response to long-day induction. Our interest was also extended to understand in which plant organs gibberellin biosynthesis occurs during the transition to flowering.

In the third part (Chapter 4) we systematically characterized the phenotypes of *swi3c*, a mutant carrying a mutation in a gene encoding a key subunit of chromatin remodeling complexes. The *swi3c* plants showed phenotypic traits associated with low gibberellin levels. To understand the relation between *SWI3C* and gibberellin pathways several phenotypes of *swi3* mutants were described, and the levels of the hormone were quantified to demonstrate that *SWI3C* is required to regulate gibberellin biosynthesis and perception.

Overall, this Ph.D research project demonstrates how forward and reverse genetic, as well as functional genomics can be used to unravel specific spatial, developmental and molecular actions of a key hormone, whose functions are essential throughout the life cycle of *Arabidopsis*.

Chapter 2: Spatially distinct regulatory roles for gibberellins in the promotion of flowering of *Arabidopsis* under long photoperiods

Aimone Porri, Stefano Torti, Maida Romera-Branchat and George Coupland

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ABSTRACT

The plant growth regulator gibberellin (GA) contributes to many developmental processes, including the transition to flowering. In Arabidopsis GA promotes this transition most strongly under environmental conditions such as short days (SDs) when other regulatory pathways that promote flowering are not active. Under SDs GAs activate transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *LEAFY* (*LFY*) at the shoot meristem, two genes encoding transcription factors involved in flowering. Here the tissues in which GAs act to promote flowering were tested under different environmental conditions. The enzyme GIBBERELLIN 2 OXIDASE 7 (GA2ox7), which catabolizes active GAs, was overexpressed in most tissues from the viral *CaMV 35S* promoter, specifically in the vascular tissue from the *SUCROSE TRANSPORTER 2* (*SUC2*) promoter or in the shoot apical meristem from the *KNAT1* promoter. We find that under inductive LDs GAs are required in the vascular tissue to increase the levels of *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) mRNAs, which encode a systemic signal transported from the leaves to the meristem during floral induction. Similarly, impairing GA signalling in the vascular tissue reduces *FT* and *TSF* mRNA levels and delays flowering. In the meristem under inductive LDs, GAs are not required to activate *SOC1*, as reported under SDs, but for subsequent steps in floral induction, including transcription of genes encoding SQUAMOSA PROMOTER BINDING PROMOTER LIKE (SPL) transcription factors. Thus GA has important roles in promoting transcription of *FT*, *TSF* and *SPL* genes during floral induction in response to LDs, and these functions are spatially separated between the leaves and shoot meristem.

INTRODUCTION

Flowering occurs when the shoot apical meristem (SAM), from which all aerial tissues are derived, undergoes a developmental transition that allows the production of flowers instead of leaves. In *Arabidopis thaliana* this transition is controlled by several pathways that are regulated by endogenous developmental signals or by external environmental cues (Fornara et al., 2010). These pathways include the photoperiodic pathway that promotes flowering in response to long days (LD) characteristic of summer, and the response pathway to the growth regulator gibberellin, which has its strongest effect under short days (SD).

In the photoperiodic pathway, transcription of the *FLOWERING LOCUS T* (*FT*) and *TWIN SISTER OF FT* (*TSF*) genes is activated specifically under LDs (Kobayashi and Weigel, 2007; Turck et al., 2008) These genes encode small proteins that are members of the CEN1, TFL1, FT (CETS) family related to phosphatidyl-ethanolamine binding proteins (Kardailsky et al., 1999; Kobayashi et al., 1999; Pnueli et al., 2001). FT has been demonstrated to move through the phloem system to the SAM (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). FT and TSF interact with the bZIP transcription factor FD, which is expressed at the shoot apical meristem (Abe et al., 2005; Wigge et al., 2005). Genetic analysis demonstrated that FT, TSF and FD all contribute to characteristic changes in gene expression at the SAM during floral transition, including induction of transcription of *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS (SOC1)* and *FRUITFULL* (FUL), which encode related MADS box transcription factors and are among the first genes to be activated after exposure of plants to LDs (Abe et al., 2005; Jang et al., 2009; Samach et al., 2000; Searle et al., 2006; Wang et al., 2009; Wigge et al., 2005). After induction of *SOC1*, expression of many flowering genes is rapidly induced in the meristem. These include members of the family of genes encoding the SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPLs) transcription factors. Three members of this family, *SPL3*, *SPL4* and *SPL5*, are direct targets of SOC1 and FD (Jung et al., 2011), whilst transcriptome profiling and *in situ* hybridizations demonstrated that their expression also requires *FT TSF* and *SOC1 FUL* function (Schmid et al., 2003; Torti et al., 2012). Ectopic expression of *SPL3* accelerates flowering, supporting the idea that they are part of the floral inductive process (Cardon et al., 1997; Wang et al., 2009; Yamaguchi et al.,

2009). Similarly, suppression of the function of many *SPLs* through overexpression of miR156, which targets *SPL* mRNAs, delays floral transition (Schwab et al., 2005; Schwarz et al., 2008; Wu and Poethig, 2006). In turn, the floral meristem identity genes *APETALA 1 (AP1)* and *LEAFY (LFY)* as well as the flowering-time gene *FRUITFULL* (*FUL*) are directly activated by SPL3 (Wang et al., 2009; Yamaguchi et al., 2009), whilst AP1 and LFY confer floral identity on developing primordia (Bowman et al., 1993). Thus a series of direct interactions in the shoot meristem linking SOC1, SPLs and floral meristem identity genes reveals one route from floral induction by LDs to floral development.

Genetic analysis suggests that gibberellins have their most important function in flowering under SD. The *ga1-3* mutant, which is impaired in GA biosynthesis, fails to flower in SD but shows a relatively weak late-flowering phenotype under LD (Wilson et al., 1992). The stronger effect of GA under SDs, is likely due to the photoperiodic pathway masking the effect of loss of GA signaling under LDs (Reeves and Coupland, 2001)**.** A mechanistic basis for the interaction between the photoperiodic and GA pathways is suggested by the convergence of both pathways on the promotion of *SOC1* transcription in the meristem (Achard et al., 2004; Moon et al., 2003; Searle et al., 2006). Furthermore, flowering of *soc1* mutants shows reduced sensitivity to GA treatments (Moon et al., 2003). Previous reports demonstrated that GA activates later events in the meristem during flowering such as the activation of *LFY* transcription (Blazquez et al., 1998), although it is now unclear whether these are an indirect consequence of increased *SOC1* expression. In addition, GA has been reported to affect flowering by other mechanisms, but these are not yet clearly integrated into the flowering network. GA increases expression of miR159 and of its target mRNA encoding the MYB transcription factor MYB33 (Achard et al., 2004), which has been proposed to regulate *LFY* expression (Achard et al., 2004; Gocal et al., 2001; Woodger et al., 2003). Also the *GATA NITRATE INDUCIBLE CARBON-METABOLISM INVOLVED* (*GNC*) and *GNC-LIKE* (*GNL*) genes encode GATA factors that inhibit flowering, and are repressed by GAs (Richter et al., 2010). Finally, *FT* transcript was reduced in the strong GA biosynthetic mutant *ga1-3* after transfer from SD to far-red enriched LD (Hisamatsu and King, 2008). The relevance of this observation to floral induction under standard white light LD conditions has not yet been demonstrated. Overall GA may regulate flowering of Arabidopsis by different mechanisms that are not clearly distinguished.

Bioactive GAs, particularly GA1, GA4 and GA3, are synthesized through a complex pathway (Yamaguchi, 2008). Genes encoding the enzyme GA20 oxidase, which is required to synthesize bioactive GA, are widely expressed in the plant, suggesting that GA is synthesized in most tissues (Rieu et al., 2008b). In addition, GA4 content increases 100 fold in the Arabidopsis shoot apex during the transition to flowering, although this could not be correlated with increased expression of biosynthetic enzymes (Eriksson et al., 2006). The levels of active GAs are also reduced by 2-β hydroxylation catalyzed by GA2 oxidases (GA2oxs) (Rieu et al., 2008a; Schomburg et al., 2003). In Arabidopsis, two classes of GA2oxs have been identified. Class I and II GA2oxs act directly on bioactive GA1 and GA4 to generate inactive hydroxylated forms. In contrast, Class III GA2oxs act earlier in the biosynthetic pathway to reduce the abundance of precursors of bioactive GAs. Overexpression of either class of GA2ox from the viral *CaMV 35S* promoter reduces the levels of bioactive GAs *in vivo* and causes phenotypes associated with GA depletion (Rieu et al., 2008a; Schomburg et al., 2003).

GAs regulate gene expression through a relatively short signal transduction pathway (Harberd et al., 2009). This pathway influences gene expression by promoting the degradation of DELLA proteins (Dill et al., 2004; Fu et al., 2004; McGinnis et al., 2003; Nakajima et al., 2006; Willige et al., 2007). This removal of DELLA proteins releases transcription factors that are otherwise prevented from binding DNA by DELLAs, including PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and PIF5 (de Lucas et al., 2008; Feng et al., 2008).

Here we assess the effect on flowering of overexpressing GA2ox and thereby depleting GA in specific tissues and demonstrate spatially distinct functions in the promotion of flowering under LDs.

RESULTS

Misexpression of *GA2ox7* in different tissues causes GA deficiency phenotypes

Overexpression of *GA2ox7* mRNA from the *CaMV 35S* promoter reduces levels of bioactive GAs (Schomburg et al., 2003). To test the effect of reducing GA levels in specific tissues, *GA2ox7* cDNA was fused to promoters with specific expression patterns that have been used previously to misexpress regulatory proteins (An et al., 2004;

Ranjan et al., 2011). The *KNAT1* promoter, which is active in the shoot apical meristem, and the *SUC2* promoter which is specific to the companion cells of the phloem, were used. The *CaMV 35S* promoter acted as a control to overexpress *GA2ox7* in most tissues. The three gene fusions were introduced into wild-type Columbia plants, and independent transformants were selected (Methods).

Four independent transformants expressing *GA2ox7* transcript at differing levels were identified for each construct. The abundance of *GA2ox7* mRNA was measured by qRT-PCR in seedlings of *35S:GA2ox7* (Fig. 1A), in leaves of *SUC2:GA2ox7* (Fig. 1B) and in apices of *KNAT1:GA2ox7* (Fig. 1C) and was present in each transformant at much higher levels than in wild-type. To determine the spatial expression pattern of *GA2ox7* in transformants carrying each transgene, *in situ* hybridizations were performed (Fig. 1D). In wild-type plants, no signal was detected, consistent with the very low level of expression of *GA2ox7* mRNA detected by qRT-PCR (Fig.1A,B,C). *35S:GA2ox7* plants showed abundant *GA2ox7* mRNA in most tissues, including leaves, vasculature and shoot apical meristem (SAM). By contrast, in *SUC2:GA2ox7* the *GA2ox7* mRNA was detected only in the vasculature, whereas in the *KNAT1:GA2ox7* it was found only in the shoot meristem (Fig. 1D). Thus the heterologous promoters *CaMV 35S*, *KNAT1* and *SUC2* misexpress *GA2ox7* mRNA in the expected patterns.

The transgenic lines were analyzed for height, internode length, leaf radius and chlorophyll content, phenotypes that are strongly impaired in GA-deficient plants (Rieu et al., 2008a). Young transgenic seedlings were darker green and smaller than wild-type plants (Fig. 1E). Misexpression of *GA2ox7* from all three heterologous promoters greatly reduced plant height, as measured by the length of the main shoot before senescence (Fig. 1F,G) or the length of the internode between the last rosette and first cauline leaf (Fig. 1H). *KNAT1:GA2ox7* had the strongest effect on plant height, demonstrating that depleting GA from the SAM impairs stem elongation.

The leaf radius of each of the transgenic plants was significantly shorter than that of wild-type (Tab. 1, and Fig. S1A). The leaves of the transgenic lines also appeared darker green (Fig. S1A), and therefore their chlorophyll levels were measured (Tab. 1). In the leaves of *35S:GA2ox7* and *SUC2:GA2ox7* these were approximately 50% higher than wild-type, whereas no significant differences were observed in the *KNAT1:GA2ox7*. Thus,

GA is required to promote leaf growth in the vasculature and at the SAM, but in the regulation of chlorophyll levels an effect was detected only in the leaf vasculature.

KNAT1:GA2ox7 acts at the SAM to deplete GA, so the reduction of leaf size observed in these plants was unexpected. To test whether low level expression of *KNAT1:GA2ox7* in leaves could contribute to this phenotype, *GA2ox7* mRNA level was measured directly by qRT-PCR. However, *GA2ox7* transcript levels were not significantly different in leaves of *KNAT1:GA2ox7* plants compared to wild-type (Fig S1B). In addition, *GA20ox1* transcript levels were also measured in these samples to assess whether GA levels were likely to be changed in the leaves of *KNAT1:GA2ox7* plants. This gene is under GA negative feedback regulation and its mRNA level is therefore increased in tissues in which GA content is reduced (Phillips et al., 1995; Xu et al., 1995). In *35S:GA2ox7* plants, *GA20ox1* mRNA levels were more abundant compared to wild-type, indicating that as expected these plants contained lower GA (Fig. S1C). In contrast, in leaves of *KNAT1:GA2ox7 GA20ox1* mRNA levels did not differ significantly compared to wild-type (Fig. S1D). In addition, *GA20ox1* expression was tested in apices of *KNAT1:GA2ox7* plants where expression of the transgene is expected to reduce GA levels. In contrast to what was observed in leaves, the level of *GA20ox1* transcript was much higher in apices of *KNAT1:GA2ox7* compared to wild-type plants (Fig. S1E).

Genotype	Rosette radius (mm)	Chlorophyll (micromoles*m^{-2})
Wild-tipe	**30,6** ± 2,1	**241** ± 7,5
35S:GA2ox7 (4)	**16,7** ± 2,28	**376** ± 28
SUC2:GA2ox7 (3)	**17** ± 1,61	**371** ± 12
KNAT1:GA2ox7 (4)	**13,5** ± 1,56	**248** ± 9,6

Table 1. Rosette leaf radius length and chlorophyll concentration of the transgenic lines.

Rosette leaf radius measurements were carried out in 10 individual plants at the end of the vegetative phase prior to bolting. Chlorophyll concentration was estimated in 3 individual plants. The measurements are the means ± SD. Col wild-type was used as control.

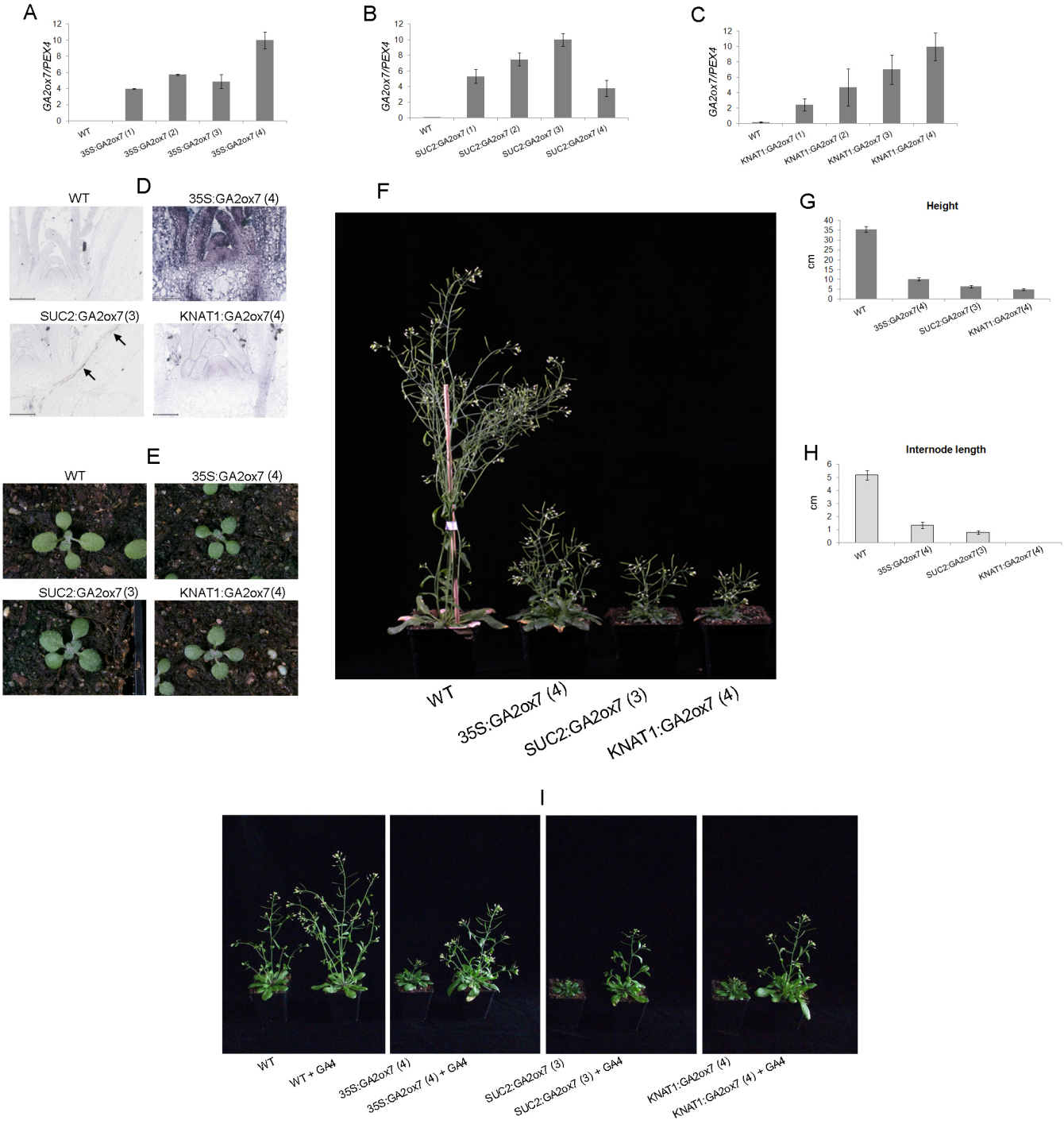

Fig. 1. Phenotypic characterization of *GA2ox7* overexpressor plants.

GA2ox7 transcript levels in seedlings of *35S:GA2ox7* (A), in leaves of *SUC2:GA2ox7* (B) and in apices of *KNAT1:GA2ox7* (C). Samples were harvested from 12-day old plants growing under LD. Data are mean ± s.d. (D) *In situ* hybridization of *GA2ox7* spatial expression pattern in transgenic plants. Apices of 14 day-old plants grown in SDs were harvested. Black arrows indicate detection of *GA2ox7* mRNA. Scale bars: 75 µm (left) 50 µm (right). (E) Phenotypes of young transgenic lines grown in LDs. (F) Phenotypes of adult transgenic lines grown in LDs. (G) Determination of height and internode length (H) of transgenic lines compared to Col wild-type. Data are mean ± s.d. of at least 10 plants. (I) Effect of GA4 treatment (10 µM) on phenotype of the transgenic lines grown in LDs: GA4 was applied on seedlings of *35S:GA2ox7*, in leaves of *SUC2:GA2ox7* and in apices of *KNAT1:GA2ox7*. All tests were performed with four independent transformants for each construct and Col wild-type was used as control.

The above experiment indicated that the leaf phenotypes of *KNAT1:GA2ox7* plants cannot be explained by increased expression of *GA2ox7* nor by reduced levels of GA in mature leaves.

Taken together, the phenotypic characterization data suggest that ectopic expression of *GA2ox7* from tissue specific promoters causes phenotypes associated with GA deficiency. To test this further, the transgenic plants were treated with exogenous GA4 (Methods). The severity of the GA-deficiency phenotypes of the transgenic lines was greatly reduced by the GA applications, supporting the conclusion that reduced levels of bioactive GA are the basis of the phenotypes observed (Fig. 1I).

SUC2:GA2ox7 and _KNAT1:GA2ox7_ plants show different flowering-time behaviours under short days

Mutations impairing GA biosynthesis or signaling delay flowering of *Arabidopsis* most strongly under SDs (Wilson et al., 1992). Therefore the flowering times of all transgenic lines were measured under SDs and compared to wild-type.

35S:GA2ox7 plants flowered much later than wild-type plants under SD (Fig. 2A,D), as previously shown (Schomburg et al., 2003). Under our conditions, the transgenic plants flowered with around 40 rosette leaves more than wild-type plants.

To assess whether reducing GA levels in the phloem and at the shoot apical meristem alters flowering time in non inductive SDs, flowering of *KNAT1:GA2ox7* and *SUC2:GA2ox7* transgenic plants were also scored. *KNAT1:GA2ox7* did not flower during the course of the experiment (Fig. 2B,E), although they had produced around 100 rosette leaves compared with 60 for the wild-type at flowering. Conversely, *SUC2:GA2ox7* plants flowered only slightly later than Columbia (Fig. 2C,F), producing around 70 rosette leaves compared to 60 of wild-type. However, under these conditions, wild-type plants produced several cauline leaves more than *SUC2:GA2ox7* plants, so that the total leaf number at flowering was similar for wild-type and transgenic plants (Fig. 2C).

Taken together, these data suggest that the floral promotive effect of GA under SDs is mainly located at the shoot apical meristem, where depletion of GA largely prevents flowering.

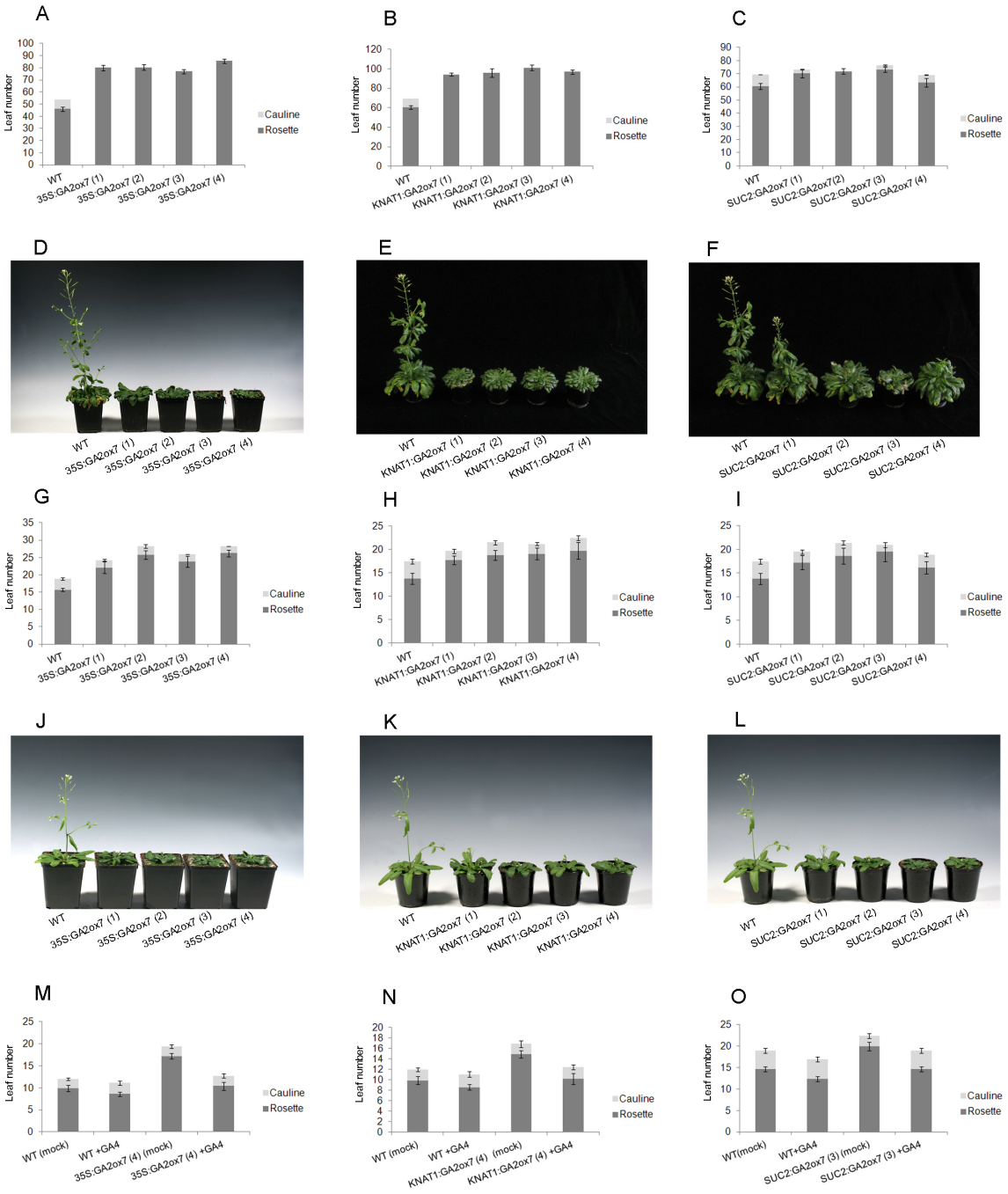

Fig. 2. Flowering time of the transgenic lines under LDs and SDs.

Flowering time of plants overexpressing *GA2ox7* in all tissues from the *CaMV 35S* promoter (A), in the SAM from the *KNAT1* promoter (B) and in the vasculature from the *SUC2* promoter (C) grown in SDs. Data are mean ± s.d of at least 10 plants. Phenotypes of transgenic lines grown under SDs are shown below flowering time graphs (D,E,F). Flowering time of *35S:GA2ox7* (G), *KNAT1:GA2ox7* (H) and *SUC2:GA2ox7* (I) plants under LDs. Data are mean ± s.d. Phenotypes of transgenic lines grown under LDs are shown below flowering-time graphs (J,K,L). GA4 (10 µm) treatment of seedlings of *35S:GA2ox7* (M), of apices of *KNAT1:GA2ox7* (N) and of leaves of *SUC2:GA2ox7* (O). GA treatment was performed throughout the growth of the plant twice a week. Data are mean ± s.d.

_35S:GA2ox7_, _KNAT1:GA2ox7_ and _SUC2:GA2ox7_ show delayed flowering under long days

Although impairment of GA synthesis or signaling most strongly delays flowering under SDs, a weaker effect is also detected under LDs (Wilson et al., 1992). *35S:GA2ox7* also showed delayed flowering under LDs (Fig. 2G,J), as observed previously (Schomburg et al., 2003). Similarly, *KNAT1:GA2ox7* and *SUC2:GA2ox7* were late flowering, forming around 20 leaves compared to 15 for wild-type (Fig. 2H,K,I,L). Thus, ectopic expression of *GA2ox7* in either the vascular tissue or the shoot meristem delays flowering under LDs, but the strongest effect is observed when *GA2ox7* is expressed generally from the *CaMV 35S* promoter.

The severity of the late-flowering phenotype of individual lines was significantly correlated ($P<0.001$) to the level of *GA2ox7* mRNA (Fig. S1F), so that the lines that expressed *GA2ox7* mRNA most strongly were the latest flowering. This observation suggests that the effect of *GA2ox7* on flowering is dosage dependent.

The effect of exogenous GA4 treatment on the late-flowering phenotype of the transgenic plants was also tested. GA4 application accelerated flowering of the transgenic lines under LDs, and at the end of the treatment the transgenic lines flowered with a similar number of leaves to the wild-type mock treated plants (Fig. 2M,N,O).

To test whether the delay of flowering under LDs caused by *KNAT1:GA2ox7* was enhanced by *SUC2:GA2ox7,* the two latest flowering transgenic lines, were crossed and flowering time was scored in the F1 generation (Fig. S1G,H; Methods). The double overexpressor *KNAT1:GA2ox7 SUC2:GA2ox7* flowered later than either progenitor and at a similar stage to *35S:GA2ox7*. Therefore, the effect of overexpressing *GA2ox7* in the leaves and meristem is additive on flowering time under LDs.

Taken together, the flowering-time experiments indicate that under LDs GA acts both in the vasculature and at the SAM to promote flowering. However, the requirement for GA at the meristem is reduced in LDs compared to SDs, whilst in the vascular tissue the effect of GA on flowering appears stronger under LDs than SDs.

_FT_ and _TSF_ mRNA levels are regulated by GA in the phloem under long days

Many of the genes comprising the photoperiodic flowering pathway are expressed in the phloem companion cells, where the *SUC2* promoter is active. Therefore, whether *SUC2:GA2ox7* delays flowering by reducing the transcript levels of the photoperiodic pathway genes *FT*, *TSF*, *CO* and *GI* was tested (Fig. 3A,B,C,D). Several of these genes are regulated by the circadian clock so their RNA levels were measured every 3 hours through a 24 hour cycle under LDs (Methods). In wild-type plants *FT* mRNA level showed the expected diurnal pattern with a strong increase at 12 hours after dawn and a peak at 16 hours. *SUC2:GA2ox7* showed a similar diurnal pattern in *FT* mRNA, but its rise in expression was slightly delayed and its abundance was significantly reduced between 12 and 16 hours after dawn. The *SUC2:GA2ox7* transformants with the highest *GA2ox7* transcript levels (Fig. 1B) showed the strongest reduction in *FT* (Fig: S1I). A similar but less pronounced effect was observed for the mRNA of the *FT* paralogue *TSF* (Figure 3B). In contrast, the mRNAs of *CO* and *GI*, which act earlier in the photoperiodic pathway than *FT* and *TSF*, were not significantly reduced in *SUC2:GA2ox7* compared to wild-type (Fig. 3 C,D).

Several repressors of *FT* transcription have been described, including *SVP* (Lee et al., 2007; Li et al., 2008), *FLC* (Searle et al., 2006), *TEM1* and *TEM2* (Castillejo and Pelaz, 2008). Increased expression of the mRNAs of these repressors in *SUC2:GA2ox7* plants could explain the reduced level of *FT* and *TSF* transcripts, and therefore these mRNAs were quantified in the transgenic plants (Fig. 3E,F,G,H).

No significant difference between *SUC2:GA2ox7* and Col wild-type was observed for *SVP*, *TEM1* and *TEM2* transcript levels, indicating that increased levels of these mRNAs cannot explain the reduced expression of *FT* and *TSF*. *FLC* mRNA levels were slightly increased at the beginning of the light period in the *SUC2:GA2ox7* plants suggesting that the increase in abundance of this mRNA may be the cause of the reduced levels of *FT* and *TSF* mRNAs (Fig. 3F). To test this further, *flc* mutant and wild-type plants were treated with Paclobutrazol (PAC), an inhibitor of GA biosynthesis, and *FT* transcript levels were quantified. Interestingly, *FT* transcript was reduced to similar levels in wild-type and *flc* PAC treated plants (Fig. S1J). This result supports the idea that lowering GA

content reduces *FT* expression and suggests that the effect of GA levels in regulating *FT* is likely independent of *FLC*.

Finally, *GNC* and *GNL* were recently described to act as repressors of flowering downstream of GA (Richter et al., 2010). *GNL* mRNA levels did not differ in *SUC2:GA2ox7* compared to Col (Fig. 3I), showing the same diurnal peak of abundance in both genotypes. *GNC* transcript levels slightly increased 18 hours after dawn in *SUC2:GA2ox7* plants compared to Col (Fig. 3J), but this difference is probably not sufficient to explain the reduced levels of *FT* transcript, which are observed earlier in the diurnal cycle (12 hours after dawn) (Fig. 3A).

An 8.1 Kb fragment was previously described to contain the *FT* promoter and recreates the spatial pattern of expression of *FT* (Adrian et al., 2010; Takada and Goto, 2003). The *SUC2:GA2ox7* transgenic line and Col were crossed to an *8.1KbFTpro:GUS* plant and GUS expression was analyzed in the F1 plants (Fig. 3K). As expected *8.1KbFTpro:GUS*/- seedlings showed GUS signal in the vasculature of the cotyledons and leaves. In contrast, in *8.1KbFTpro:GUS*/- *SUC2:GA2ox7*/- seedlings, which were similarly stained, no GUS signal was detected. Thus, in wild-type plants GA acts to increase *FT* mRNA through the defined 8.1Kb *FT* promoter.

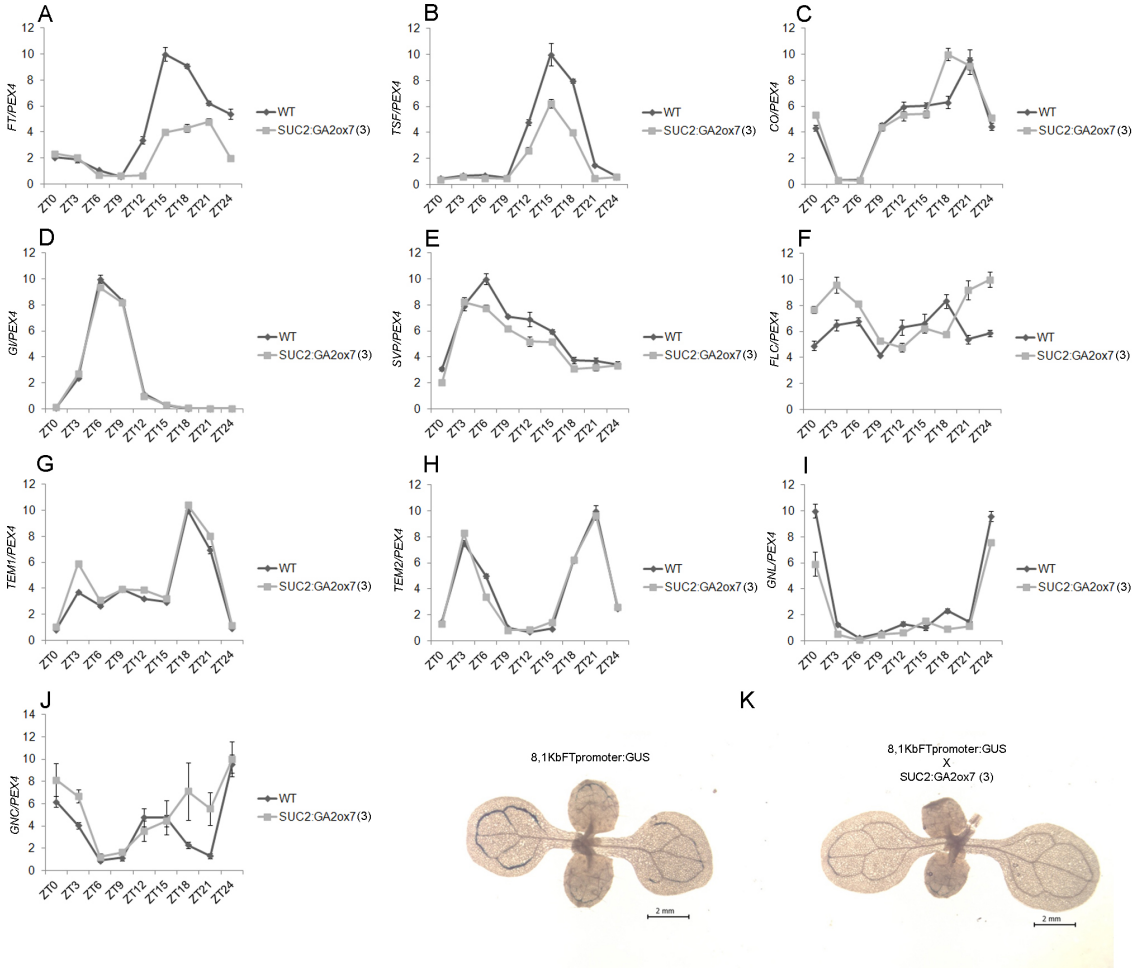

Fig.3. *SUC2:GA2ox7* reduces the expression of photoperiodic genes *FT* and *TSF.*

Temporal expression patterns of *FT* (A), *TSF* (B), *CO* (C), *GI* (D), *SVP* (E), *FLC* (F), *TEM1*(G), *TEM2* (H) and of GA downstream acting genes *GNL* (I) and *GNC* (J) in *SUC2:GA2ox7* plants compared to Col wild-type. mRNA levels were measured by q-RT-PCR in leaves of 12 day-old seedling harvested throughout a long day. All q-PCR analyses were performed with at least 3 independent RNA samples. Time is expressed as hours from dawn (ZT, *zeitgeber*). Data are mean ± s.d. Histochemical localization of GUS activity in 10 day-old seedlings of 8,1Kb *FT* promoter:GUS and 8,1KbFTpromoter:GUS X *SUC2:GA2ox7* (K) grown in LDs. Scale bars: 2 mm.

Ectopic expression of *FT* suppresses the late flowering caused by *SUC2:GA2ox7*

To assess whether the reduced level of *FT* and *TSF* mRNA was the cause of delayed flowering of *SUC2:GA2ox7* plants, a transgene expressing *FT* from a heterologous promoter was introduced into *SUC2:GA2ox7* plants (Methods). Ectopic expression of *FT* can overcome the effect of loss of function of *FT* and *TSF* (Jang et al., 2009; Michaels et al., 2005; Yamaguchi et al., 2005). The *GAS1:FT* construct overexpresses *FT* mRNA only in

minor veins and to a lesser extent than other phloem specific promoters (Corbesier et al., 2007; Haritatos et al., 2000). The *SUC2:GA2ox7* X *GAS1:FT* plants flowered much earlier than those carrying only *SUC2:GA2ox7* and after producing a similar number of leaves to *GAS1:FT* plants (Fig. 4A,B), supporting the idea that the late flowering of *SUC2:GA2ox7* is caused by reduced *FT* mRNA levels.

In addition, the effects of impairing GA signaling in the companion cells on *FT* expression and flowering time were tested by expressing from the *SUC2* promoter the dominant mutant form of GAI that represses GA signaling (Peng et al., 1997). *SUC2:gai-D* plants were late-flowering and showed reduced *FT* mRNA levels, similar to the effects observed in the *SUC2:GA2ox7* plants (Fig. S2A,B).

The above experiments suggested that GA and GA signaling act in the vascular tissue to increase *FT* and *TSF* mRNA levels and thereby promote flowering. Therefore whether *FT* and *TSF* are required in the leaf for GA treatments of leaves to promote flowering was tested. Leaves of *ft-10 tsf-1* double mutants and WT plants grown under SD were treated with GA4 (Fig. 4C,D). WT plants showed significant acceleration of flowering upon GA-treatment, producing 20 leaves fewer than the mock-treated plants. By contrast, GA application to leaves of *ft-10 tsf-1* mutants caused flowering to occur after production of only 10 leaves fewer than the mock treated plants. Therefore, *ft-10 tsf-1* double mutants still respond to GA leaf treatments, but their response is strongly attenuated compared to wild-type plants. This result is consistent with GA leaf treatments acting partly through *FT* and *TSF* to promote flowering. In addition, leaves of *SUC2:GA2ox7* and Col wild-type were also treated with GA and after 24h the *FT* transcript level was quantified (Fig. 4E). Wild-type plants did not show any significant change in *FT* expression after GA application, which is probably due to the saturating level of GA at this stage. In contrast, *SUC2:GA2ox7* showed an approximately 3 fold increase of *FT* transcript in the GA-treated compared with the mock-treated plants. Therefore, depletion of GA in the leaves of *SUC2:GA2ox7* caused *FT* downregulation, which could be restored by applying active GA.

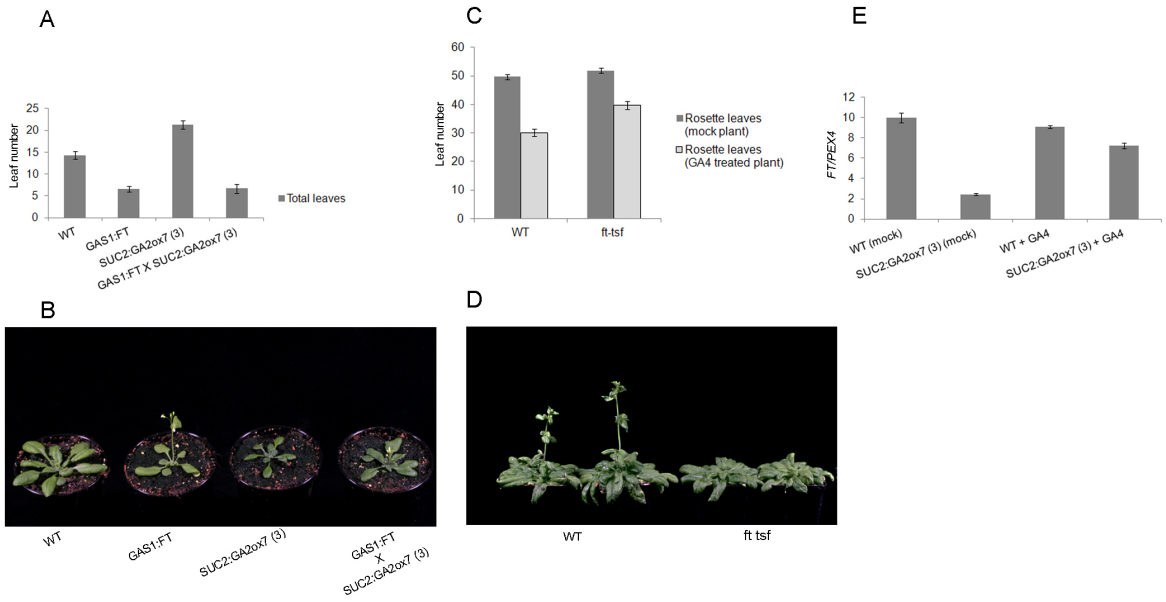

Fig. 4. The *ft tsf* double mutant shows less sensitivity to leaf applications of GA in the acceleration of flowering.

Effect of ectopic expression of *FT* in *SUC2:GA2ox7* plants grown in LDs (A,B). Col wild-type, *SUC2:GA2ox7* and *GAS1:FT* plants were used as controls. Effect of GA4 on flowering time of *ft tsf* and Col wild-type plants under SDs (C,D). GA4 (10 µM) was applied to leaves twice weekly. Effect of GA4 on *FT* expression in *SUC2:GA2ox7* and Col wild-type plants in LDs (E). GA treatment was carried out in leaves of 10 day-old plants and tissues were collected 24 hours after. Data are mean ± s.d.

Induction of *SPL*s but not *SOC1* transcription is delayed in the meristem of *KNAT1:GA2ox7* plants under long days

The level of *FT* mRNA was similar in *KNAT1:GA2ox7* and Col plants under LD (Fig. S2C), confirming that the delay in flowering of this plant occurred by a different mechanism than for *SUC2:GA2ox7* plants.

During the transition to flowering, expression of many genes is induced at the shoot apex, and this can be synchronized by transferring plants from SDs to LDs. To determine how these gene expression patterns are affected by *KNAT1:GA2ox7*, the transgenic plants and Col were grown for 3 weeks in SD and then transferred to LDs. Apices were harvested for *in situ* hybridizations before transfer and then after 3, 5 and 9 days in LDs.

In Col shoot meristems *SOC1* mRNA was not detected after 3 weeks in SDs, but increased in the meristem after 3, 5 and 9 LDs (Fig. 5A). Similarly, in the *KNAT1:GA2ox7*

plants *SOC1* mRNA was detected in the meristem after exposure to 3, 5 and 9 LDs. However, unlike Col plants, flower development was not initiated throughout this period. Consistent with this result, an increase in *SOC1* transcript in apices of Col and *KNAT1:GA2ox7* plants was detected after transfer to LD (Fig. 5B). Thus, the meristem of *KNAT1:GA2ox7* plants responds normally to the LD signal in terms of *SOC1* mRNA induction, demonstrating that GA is required to promote later steps in floral induction.

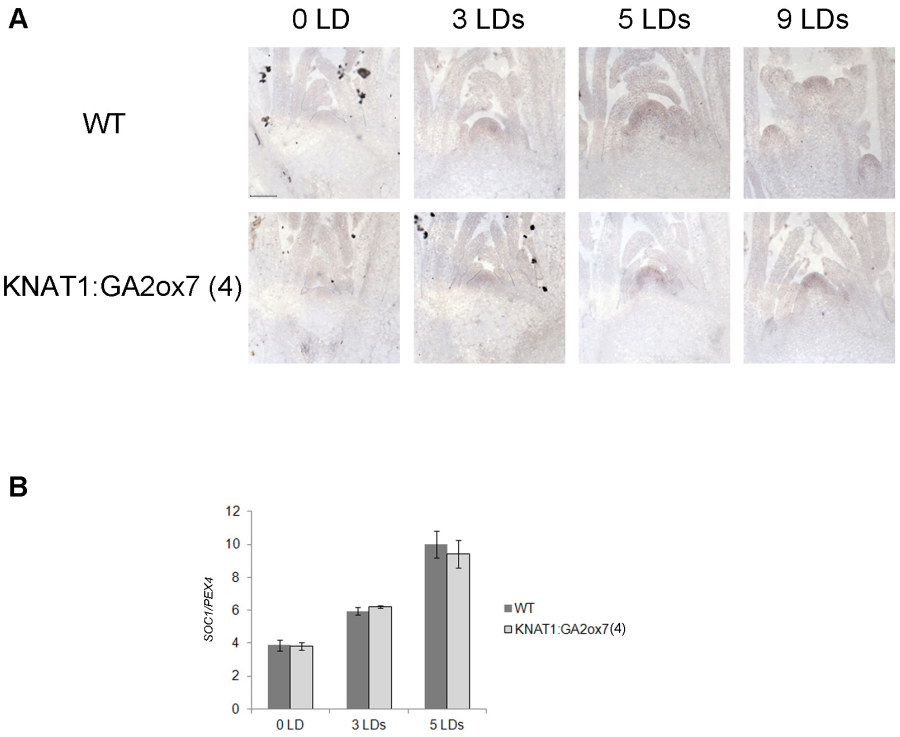

Fig. 5. Temporal and spatial expression pattern of *SOC1* in the transgenic lines.

Time courses of *in situ* hybridization on Col wild-type and *KNAT1:GA2ox7* plants (A). Plants were grown for three weeks in SDs (0 LD) and then transferred to LDs (3LD, 5LD, 9LD). *SOC1* expression levels in apices of *KNAT1:GA2ox7* and Col wild-type (B). Plants were grown for 3 weeks in SDs (0LD) and then transferred to LDs (3LD, 5 LD). Data are mean ± s.d. Scale bar: 75 µm.

The *SPL* genes are expressed in the shoot apical meristem downstream of *SOC1* (Jung et al., 2011; Torti et al., 2012) and play important roles in the activation of floral meristem identity genes *FUL* and *AP1* (Wang et al., 2009; Yamaguchi et al., 2009). Therefore, the expression patterns of *SPL* mRNAs were also studied. In Col plants transferred to LDs the mRNAs of *SPL4* and *SPL5* were strongly detected in the rib meristem region after

exposure to 3-5 LDs (Fig. 6B,C). Similarly, *SPL9* mRNA was detected on the flanks of the meristems of Col plants exposed to 3-5 LDs (Fig. 6D).

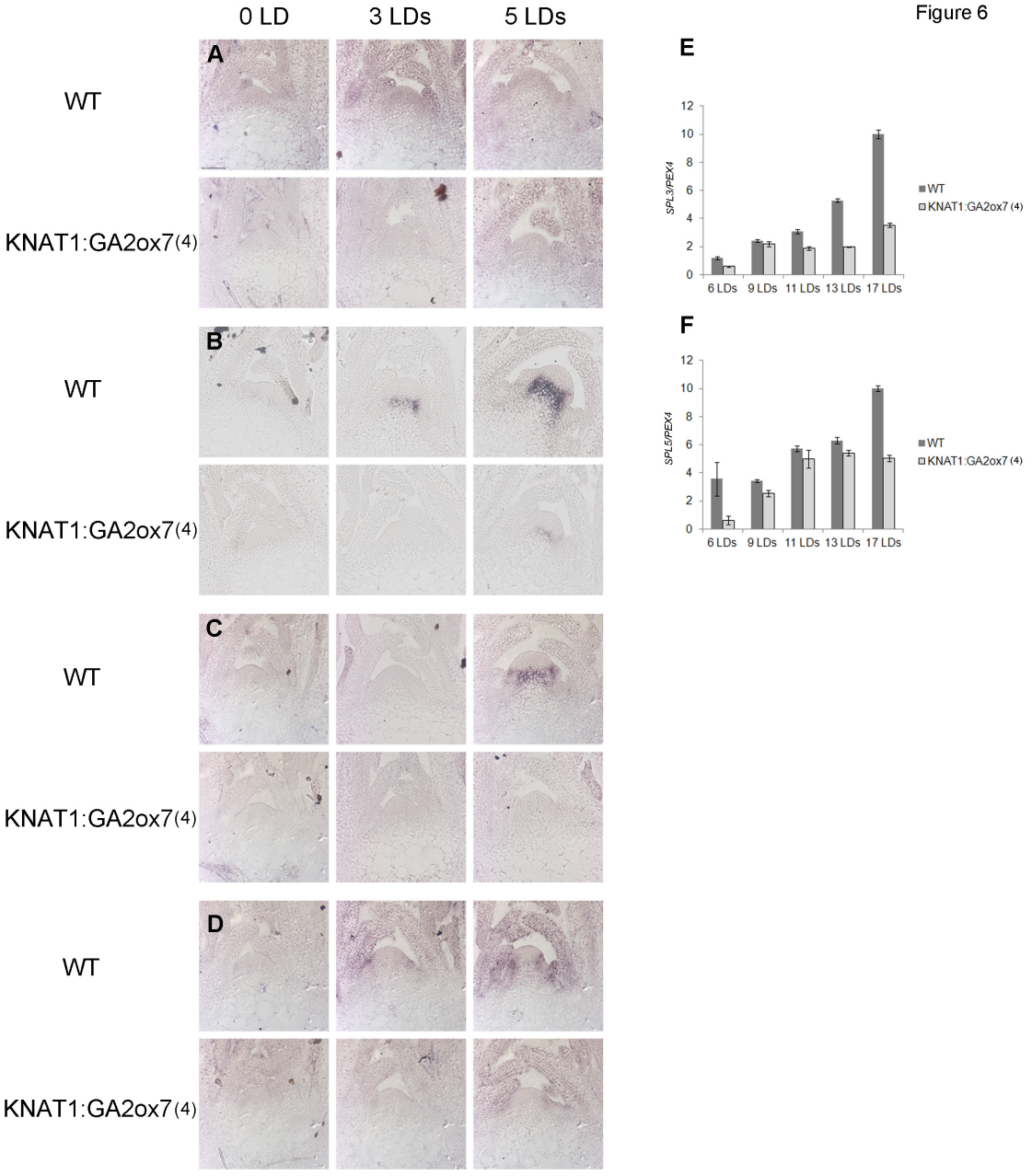

Fig. 6. Temporal and spatial expression patterns of *SPL* genes in the transgenic lines.

Time courses of *in situ* hybridization on Col wild-type and *KNAT1:GA2ox7* plants grown for three weeks in SDs (0 LD) and then transferred to LDs (3LD, 5LD). Specific probes were used to detect mRNAs of *SPL3* (A), *SPL4* (B), *SPL5* (C), *SPL9* (D). Temporal expression pattern of *SPL3* (E) and *SPL5* (F) in apexes of Col wild-type and *KNAT1:GA2ox7* plants grown in continuous LDs. Sample were harvested at 6 LD, 9LD, 11LD, 13 LD and 17LD. Data are mean ± s.d. Scale bar: 50 µm

In contrast, in *KNAT1:GA2ox7*, expression of *SPL4* and *SPL9* mRNAs was strongly reduced so that their mRNAs only appeared weakly after exposure to 5 LDs. *SPL5* mRNA level was even more strongly affected and was undetectable in the shoot meristem 5 LDs after transfer. *SPL3* mRNA was detected throughout the meristem and in leaf primordia in Col plants and increased in abundance during LD induction (Fig. 6A). Conversely, in *KNAT1:GA2ox7 SPL3* expression was strongly delayed and transcript was only weakly detectable after 5 LDs in leaf primordia.

These experiments indicate that although *KNAT1:GA2ox7* does not prevent the early induction of *SOC1* expression in the shoot meristem in response to LDs, it does prevent the subsequent activation of later acting genes such as *SPL3*, *SPL4*, *SPL5* and *SPL9*. The effect of *KNAT1:GA2ox7* on *SPL* gene expression could be exerted at the level of FD, which binds directly to *SPL3, SPL4* and *SPL5* to promote their expression (Jung et al., 2011). Therefore *fd* mutants were treated with active GA and the levels of *SPL3* and *SPL4* mRNAs were quantified in apices (Fig. S2DE). *SPL3* and *SPL4* mRNA levels increased in *fd* mutants treated with GA compared to the mock-treated plants, indicating that GA can activate these *SPL* genes independently of *FD*. However, the level of *SPL* expression is lower than in GA-treated WT plants, so a role for *FD* in this process cannot be excluded (Supplementary Fig. 2D,E).

Expression of *SPL*s is negatively regulated by miR156 at the post transcriptional level (Gandikota et al., 2007; Schwab et al., 2005). Therefore, whether down regulation of *SPLs* in *KNAT1:GA2ox*7 was caused by increased levels of miR156 was tested in apices of wild-type and *KNAT1:GA2ox7* (Fig. S2F).

Apices were harvested after growing plants in LDs for 6, 9, 11, 13 and 17 days. In Col wild-type the levels of miR156 progressively decreased along the time course, as previously described (Wang et al., 2009; Wu and Poethig, 2006), reaching the lowest level at 17 LDs (Fig. S2F). Similarly, in *KNAT1:GA2ox7* the expression pattern of miR156 followed the same kinetics as in wild-type and no significant differences in abundance of miR156 were detected. In contrast, the transcript levels of *SPL3* increased in apices of wild-type plants but not in *KNAT1:GA2ox7* (Fig. 6E). *SPL5* mRNA slightly increased along the time course in *KNAT1:GA2ox7* plants but the transcript levels were significantly reduced compared to wild-type (Fig. 6F).

Taken together, the *in situ* hybridization and the qRT-PCR data suggest that in the shoot apical meristem GA increases *SPL* mRNA levels by acting after *SOC1* mRNA accumulation and not by decreasing miR156 levels.

DISCUSSION

The plant growth regulator GA was previously shown to promote the transition to flowering of Arabidopsis mainly under non-inductive SDs. Here we demonstrated that GA has defined tissue-specific roles during floral induction in response to inductive LDs.

Effects of tissue specific expression of *GA2ox7* on leaf size and height

Gibberellins regulate many phases of development, including height, leaf size and chlorophyll content of Arabidopsis. The strongest effect on plant height was observed in KNAT1:*GA2ox7* plants, suggesting that the major impact of GA in shoot elongation occurs in the meristem. This effect might be caused by ectopic expression of *GA2ox7* in cells in which it is not normally expressed or due to increased activity of *GA2ox7* in cells in which it is expressed in wild-type plants. The expression patterns of the Class III GA2ox encoding genes, *GA2ox7* and *GA2ox8*, are unknown, but expression of Classes I and II GA2 oxidases have been detected in the shoot apical meristem of Arabidopsis, rice and maize (Bolduc and Hake, 2009; Jasinski et al., 2005; Sakamoto et al., 2001).

The severe short internode phenotype of *KNAT1:GA2ox7* plants is similar to that of loss of function GA biosynthetic mutants, consistent with the overexpression of *GA2ox7* depleting GA from the meristem. Also bioactive GA is present within the apex of flowering plants when internodes strongly extend. GA promotes cell division and expansion, suggesting that both contribute to internode elongation in the rib meristem region (Achard et al., 2009; Cowling and Harberd, 1999; Daykin et al., 1997). Although depletion of GA in the meristem showed the greatest effect on stem length and these plants were unable to appreciably extend stem internodes, a significant effect was also observed in the *SUC2:GA2ox7* plants, where GA is depleted in the phloem companion cells.

SUC2:GA2ox7 plants also showed a dark green phenotype associated with increased chlorophyll levels. GA regulates chlorophyll biosynthesis through the transcriptional repressors DELLAs and the downstream acting proteins GNL and GNC (Richter et al.,

2010). Indeed, GA causes downregulation of *GNL* and *GNC* mRNAs leading to reduced levels of protochlorophyllide oxidoreductases (PORs), thus modulating chlorophyll biosynthesis. In agreement with those findings, we showed that overexpression of *GA2ox7* causing depletion of GA in the companion cells led to increased chlorophyll levels in the leaves. However, no difference in abundance of *GNL* and *GNC* transcripts could be detected in total leaf mRNA. Perhaps if *GNC* and *GNL* are expressed throughout the leaf, reduction in expression in companion cells is undetectable in total leaf RNA, alternatively other genes might be implicated in the regulation of GA-mediated chlorophyll biosynthesis. No effect could be observed on chlorophyll content by lowering GA in the SAM, suggesting that GA levels in the meristem do not affect chlorophyll biosynthesis.

The length of the leaf radius was consistently reduced when GA was depleted in companion cells and in the SAM. This phenotype was similar to that reported for *ga20ox1 ga20ox2* double mutants, which show reduced levels of GA4 and GA1 (Rieu et al., 2008a). Our data suggest that GA levels in the companion cells and shoot meristem contribute to this phenotype.

Effect on floral transition of misexpression of *GA2ox7* in phloem companion cells

The effects of the *SUC2:GA2ox7* and *KNAT1:GA2ox7* fusions on flowering time were separable at the physiological and molecular levels. Expression in phloem companion cells from the *SUC2* promoter caused a relatively stronger delay of flowering under LDs than SDs, although the increase in absolute number of leaves was similar under both conditions. In contrast the *KNAT1* fusion caused the strongest effect under SDs, where it prevented flowering. The delay in flowering of *SUC2:GA2ox7* under LDs correlated with reduced levels of *FT* and *TSF* mRNAs, which were not observed in *KNAT1:GA2ox7* plants. A similar correlation between GA levels and *FT* mRNA abundance was previously observed in the *ga1-3* mutant exposed to long days enriched in far-red light (Hisamatsu and King, 2008). However, in those plants GA levels are strongly reduced in all tissues, and GA depletion in other cell types might affect *FT* mRNA levels in the companion cells, as was observed for PHYB (Endo et al., 2005). However, our experiments together with those of Hisamatsu and King (2008) strongly suggest that GA is required in the companion cells for normal levels of *FT* and *TSF* mRNAs under LDs. We also provide

genetic evidence that the reduced levels of *FT* and *TSF* mRNAs are causally related to the late flowering of the *SUC2:GA2ox7* plants. Introduction of a transgene expressing *FT* from a heterologous phloem specific promoter, *GAS1*, suppressed the late flowering of *SUC2:GAox2* plants. Furthermore, GA applications to leaves increased *FT* and *TSF* mRNA levels in *SUC2:GA2ox7* plants, as previously shown for *ga1-3* plants (Hisamatsu and King, 2008), and restored early flowering. That increasing *FT* and *TSF* mRNA levels is required for the full effect of GA applications to the leaves on flowering time, was supported by showing that *ft-10 tsf-1* double mutants were less sensitive to GA leaf applications, although they did still respond to the treatment. Previously, Hisamatsu and King (2008) discussed an *FT* independent role of GA applications, and this is probably explained by a spatially separated function for GA in the shoot meristem, as mentioned in the following section. The mechanism by which GA increases *FT* and *TSF* mRNA levels is presumably via DELLA protein accumulation. Indeed we demonstrated that expression of gai-D, a dominant mutant form of the GAI DELLA protein (Peng et al., 1997), in companion cells reduced *FT* and *TSF* mRNA levels. Therefore, when DELLA proteins accumulate in the companion cells they likely inhibit proteins required for transcriptional activation of *FT*. No effect on mRNAs of previously identified regulators of *FT* was observed, demonstrating that depletion of GA does not affect the transcription of known repressors or activators of *FT*, although we cannot exclude that these proteins are regulated at the post-translational level.

Effect on floral transition of misexpression of *GA2ox7* in the shoot meristem

The role of GA at the apex in the promotion of flowering has mainly been studied under SDs. Under these conditions, GA levels increase at the apex prior to the floral transition, and this correlates with increased expression of the floral meristem identity gene *LFY* (Eriksson et al., 2006). GA also promotes expression of genes acting earlier in floral induction, particularly increasing transcription of *SOC1* (Achard et al., 2004; Moon et al., 2003). Applications of exogenous GA to wild-type plants caused increased abundance of *SOC1* mRNA, whilst in *ga1-3* and *gai* mutants *SOC1* mRNA level was reduced. However, all published analyses of *SOC1* expression in response to GA were carried out by RT-PCR, and as *SOC1* is also expressed in leaves (Michaels et al., 2005), the increase in expression detected in apical samples may not be in the shoot meristem. Also, the effect of GA on

SOC1 mRNA was mainly analyzed at single time points, making it difficult to assess its effect on the dynamics of *SOC1* expression during floral induction. By performing *in situ* hybridizations to follow *SOC1* mRNA in the meristem through a time course of several days after inducing flowering by exposure to LDs, our work identifies a role for GA in the meristem after induction of *SOC1*.

Transfer of wild-type plants from SDs to LDs causes a rapid induction of *SOC1* mRNA in the meristem within 1-3 days (Borner et al., 2000; Samach et al., 2000). The *SPL* genes are induced slightly later, with *SPL4*, *SPL5* and *SPL9* mRNAs rising in the meristem 3-5 days after transfer (Torti et al., 2012; Wang et al., 2009). The dynamics of *SOC1* mRNA induction was not changed in *KNAT1:GA2ox7* plants, indicating that reducing GA in the meristem does not affect *SOC1* induction in the meristem, in contrast to what was observed under SDs (Achard et al., 2004; Moon et al., 2003). However, expression of *SPL3*, *SPL4*, *SPL5* and *SPL9* all occurred markedly later, indicating that GA has a role in floral induction under LDs between activation of *SOC1* transcription and the activation of *SPL* gene expression (Figure 7). In contrast no effects on *SPL9* mRNA or miR156 were detected by RT-PCR in 2 week old plants treated with GA or in *ga1-3* mutants compared to wild-type (Wang et al., 2009), but this single time point would not have been sufficient to detect the effect of GA on the dynamics of *SPL* activation. GA-dependent activation of *SPL* gene expression may contribute to the induction of floral meristem identity genes by GA, because SPLs have been shown to bind directly to floral meristem identity genes such as *LFY* (Wang et al., 2009; Yamaguchi et al., 2009). As transcription of *SPL* genes is induced in the SAM both by the photoperiodic (Torti et al., 2012; Wang et al., 2009) and GA pathways they might both activate *LFY* transcription via SPL proteins. However, the GA and photoperiod pathways are likely to also have additional independent branches leading to *LFY* activation, because they were previously shown to activate *LFY* transcription through independent promoter motifs (Blazquez and Weigel, 2000). The mechanism by which GA regulates *SPL* expression presumably involves post-translational regulation of transcription factors required to increase *SPL* expression. These GA regulated factors might act together with SOC1, which was recently shown to bind directly to *SPL* genes. Taken together our data provide a basis for identifying the molecular mechanisms by which under inductive photoperiods GA signaling facilitates the activation of *FT* transcription in leaves and transcription of the *SPL*s in the meristem.

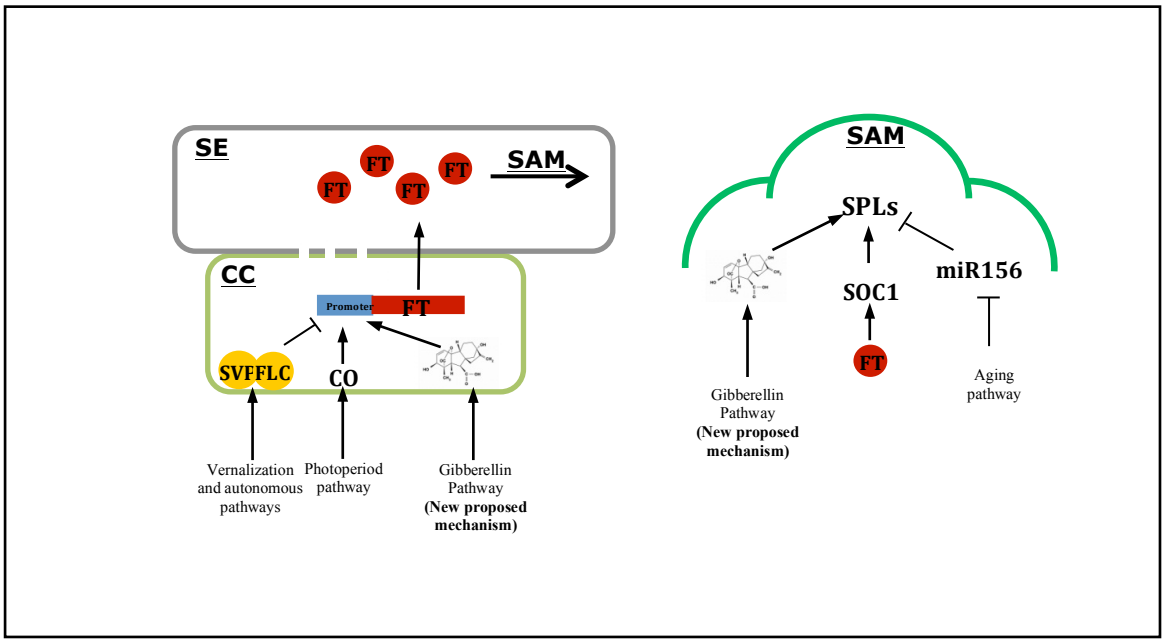

Fig. 7. Spatially separated roles of GA in controlling the floral transition under long days.

GA signaling regulates the floral transition in LDs by increasing *FT* mRNA levels in the leaf vasculature, and of the levels of *SPL* gene mRNAs at the shoot apical meristem. Other pathways also regulate *FT*. FT protein moves to the SAM where it activates expression of the floral integrator *SOC1*. At the SAM GA promotes expression of *SPL3, SPL4, SPL5 and SPL9* and this occurs without transcriptional changes in *SOC1*. CC (companion cell), SE (sieve element) and SAM (shoot apical meristem).

MATERIALS AND METHODS

Growth conditions and plant materials

Plants were grown on soil under controlled conditions of LDs (16 h light/8 h dark) and SDs (8 h light/16 h dark) at 20°C. The level of photosynthetic active radiation was 60 µmol m^{-2} s^{-1} under both conditions. For quantitative PCR, leaves of 12 day-old seedlings were collected every 3 hours in a 24 hour cycle under LDs, and mRNA was extracted. For *in situ* hybridizations, plants were grown for 3 weeks in SD, then shifted to LD, and apices were collected at ZT 8 before transfer, and after 3, 5 and 9 LDs. These analyses were performed in 3 biological replicates.

GAS1:FT SUC2:GA2ox7, *SUC2:GA2ox7 KNAT1:GA2ox7* were obtained by crossing both progenitors. For these crosses *SUC2:GA2ox7* (3) and *KNAT1:GA2ox7* (4) were used.

GA treatment

GA_4 (SIGMA) was stored in ethanol 100% with final concentration of 1mM. Two solutions were then prepared: 1) GA_4 10µM, tween 0,1% ; 2) Pure ethanol 1%, tween 0,1%. GA treatment was carried out by brushing leaves, apices or seedlings of 10 individual plants with solution 1, while solution 2 was applied to the mock plants.

Flowering time determination

Flowering time was determined by counting the number of cauline and rosette leaves of at least 10 individual plants. Data are reported as mean leaf number ± SD and were measured from homozygous lines. Four independent transformants were used for each overexpressor plant

Plasmid construction, plant transformation and transformant selection

The full length *GA2ox7* and *gai* cDNAs were amplified by PCR and used to generate an entry clone via BP reaction (Invitrogen, http://www.invitrogen.com/). The entry clones were used to generate an expression clone via the LR reaction. The plasmids were then introduced into Agrobacterium strain GV3101 (pMP90RK) and transformed into WT Columbia by floral dip.

Determination of chlorophyll concentration

Chlorophyll concentration was estimated by using SPAD-502 leaf chlorophyll meter (Markwell et al., 1995).

***In situ* hybridization and GUS staining**

In situ hybridization was performed according to the method already described (Torti et al., 2012): *SOC1* (Searle et al., 2006), *SPL3* and *SPL9* (Wang et al., 2009; Wu et al., 2009) and *SPL5* (Cardon et al., 1999). Primers to generate *GA2ox7*, *SPL4* probe are in Table S1. GUS staining was performed as previously described (Blazquez et al., 1997).

RNA extraction and Quantitative real-time PCR

Total RNA was isolated from plant tissues by using RNAeasy extraction kit (Qiagen). Transcript levels were quantified by quantitative PCR (Roche) and *PEX4* (At5G25760) was used as a control. Reactions were performed using the primers described in Table S2. Total RNA, including small RNAs, was extracted by using miRNeasy^{TM} Mini Kit (Qiagen). After DNAse treatment (Ambion), the mature form of miRNA156 was then amplified as previously described (Yang et al., 2009)(Peter Huijser, unpublished). All quantitative real-time PCRs were performed with at least 3 independent RNA samples.

Acknowledgments

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Supporting Information

Supporting information can be downloaded from the online version of this manuscript (Porri *et al.,* 2012): http://dev.biologists.org

Fig. S1.

(A) Leaf phenotype of WT, *35S:GA2ox7*, *SUC2:GA2ox7*, *KNAT1:GA2ox7)*. Leaves were dissected at the end of the vegetative phase when plants started flowering.
(B) *GA2ox7* transcript levels in leaves of four independent transformants of *KNAT1:GA2ox7* in LDs. Samples were harvested from 12-day old plants growing under LDs.
(C) *GA20ox1* transcript levels in seedlings of four independent transformants of *35S:GA2ox7* in LDs. Samples were harvested after 12 LDs.
(D) *GA20ox1* transcript levels in leaves of four independent transformants of *KNAT1:GA2ox7* in LDs. Samples were harvested from 12-day old plants growing under LDs.
(E) *GA20ox1* transcript levels in apices of four independent transformants of *KNAT1:GA2ox7* in LDs. Samples were harvested from 12-day old plants growing under LDs.
(F) Scatter plots showing the relationship between flowering time and *GA2ox7* relative expression of the transgenic lines. R^2 = correlational constant. For each comparison, Spearman correlation coefficients were calculated using SAS software. Each of the three correlations coefficients was positive and significant (p value < 0.001).
(G) Flowering time data of *SUC2:GA2ox7 X KNAT1:GA2ox7* in LDs. *SUC2:GA2ox7, KNAT1:GA2ox7* and Col wild-type were used as control. Data are mean ± standard deviation of at least 10 plants. The number of rosette leaves (dark grey bars) and rosette leaves (grey bars) is shown.
(H) Plant phenotypes of *SUC2:GA2ox7 X KNAT1:GA2ox7* in LDs.
(I) *FT* transcript levels in leaves of four independent transformants of *SUC2:GA2ox7* in LDs. Leaves of 12 day-old seedlings were harvested at ZT16. Col wild-type was used as control.
(J) Effect of PAC on *FT* expression in leaves *flc* mutant and Col wild-type plants in LDs. Seedlings were grown on medium containing PAC 0,5µM or DMSO (mock). Samples were harvested at ZT 16 from 12-day old plants growing under LDs.

Fig. S2.

(A) Flowering time of plants overexpressing *gai* in the vasculature from the *SUC2* promoter grown in LDs. Bars are mean ± standard deviation of at least 10 plants.
(B) *FT* transcript levels in leaves in leaves*SUC2:gai* plants compared to Col wild-type. mRNA levels were measured by q-RT-PCR in leaves of 12 day-old seedling harvested 16 hours after dawn.
(C) *FT* transcript levels in leaves of four independent transformants of *KNAT1:GA2ox7* in LDs. Leaves of 12 day-old seedlings were harvested at ZT16. Col wild-type was used as control.
(D) Effect of GA on *SPL3* expression in apices of *fd* mutant and Col wild-type plants in LDs. Seedlings were grown on medium containing GA_4 5µM or ethanol (mock). Samples were harvested from 12-day old plants growing under LDs.
(E) Effect of GA on *SPL4* expression in apices of *fd* mutant and Col wild-type plants in LDs. Seedlings were grown on medium containing GA_4 5µM or ethanol (mock). Samples were harvested from 12-day old plants growing under LDs.
(F) miRNA156 expression levels in apexes of Col wild-type and *KNAT1:GA2ox7* plants grown in continuous LDs. Sample were harvested at 6 LD, 9LD, 11LD, 13 LD and 17LD.

Chapter 3: Photoperiodic flowering signals increase gibberellin biosynthesis in *Arabidopsis* by repressing transcription of SVP

Fernando Andrés*, **Aimone Porri***, Stefano Torti*, Julieta L. Mateos, José Luis García-Martínez, Fabio Fornara, Veronica Gregis, Martin M. Kater and George Coupland

Submitted for publication

*These authors contributed equally to this work and their names are listed in alphabetic order

ABSTRACT

The developmental transition from vegetative growth to flowering is regulated by environmental cues. In *Arabidopsis*, the photoperiodic pathway promotes flowering under long days (LDs) of summer, whereas the growth regulator gibberellin (GA) has its strongest effect under short days (SDs). However, we show that these pathways are unexpectedly linked through regulation of GA biosynthesis. We find that GA20ox2, which catalyzes a rate-limiting step in GA biosynthesis, rises in expression at the shoot apical meristem (SAM) under LDs and that this response requires repression of *SHORT VEGETATIVE PHASE (SVP)*. Mutations in *SVP* increase levels of GA and *GA20ox2* mRNA. Furthermore, *SVP* transcription is repressed by the photoperiodic pathway via *FLOWERING LOCUS T, TWIN SISTER OF FT*, *SUPPRESSOR OF OVEREXPRESSON OF CONSTANS1 and FRUITFULL*. In quadruple mutants for these genes, *SVP* mRNA persists at the SAM, delays flowering and reduces *GA20ox2* expression. We propose that GA biosynthesis is rapidly increased at the SAM under LDs via the repression of *SVP*.

INTRODUCTION

Many plant species initiate flower development in response to particular day lengths. This process induces the transition from vegetative to reproductive development at specific times of year. In *Arabidopsis thaliana* the time of flowering is strongly accelerated in response to long days (LDs). Differences in day length are perceived in the leaves but flowers develop at the shoot apex. In *A. thaliana* FLOWERING LOCUS T (FT) and its paralogue TWIN SISTER OF FT (TSF) contribute to the systemic signal made in the leaves in response to inductive day lengths and thereby promote floral induction at the shoot apical meristem (Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). Closely related proteins promote photoperiodic flowering in distantly related species and also mediate other day-length controlled developmental processes such as tuberisation in potato (Navarro et al., 2011; Tamaki et al., 2007). FT is made in the phloem companion cells and moves through the phloem sieve elements to the shoot meristem where it is proposed to promote the floral transition by interacting with the bZIP transcription factor FD (Abe et al., 2005; Wigge et al., 2005). Based on observations made with rice proteins this interaction is likely to be indirect and occur via a bridging 14-3-3 protein (Taoka et al., 2011). In *A. thaliana* FT and FD are required for transcriptional activation in response to LDs of the floral integrator genes *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* (*SOC1*) and *FRUITFULL* (*FUL*), which encode two MADS box transcription factors that redundantly play a crucial role in promoting the floral transition (Melzer et al., 2008; Torti et al., 2012). The important function of these transcription factors in photoperiodic flowering is supported by genetic data that demonstrate that *soc1-3 ful-2* mutations suppress the early flowering caused by overexpression of *FT* (Melzer et al., 2008). Transcriptional profiling identified several hundred genes that respond at the shoot apex or in the shoot apical meristem to FT signaling (Schmid et al., 2003; Torti et al., 2012), but the regulatory pathways and cellular processes that mediate between FT signaling and floral development remain poorly elucidated.

In addition to the promotion of flowering by the FT pathway, several negative regulators of photoperiodic flowering have been described (Yant et al., 2009). Among these, the role of the MADS box transcription factor SHORT VEGETATIVE PHASE (SVP) is well

characterized at the genetic and molecular levels. Mutations in *SVP* cause extreme early flowering under SDs and slightly earlier flowering under LDs (Hartmann et al., 2000), which correlates with increased levels of the mRNAs of *FT*, *TSF* and *SOC1* (Jang et al., 2009; Lee et al., 2007; Li et al., 2008). Thus *SVP* represses the photoperiod pathway at several positions and in different tissues. In wild-type plants the repressive function of SVP is overcome by exposure to LDs, indicating that SVP increases the amplitude of the photoperiodic response by preventing premature flowering under SDs. SVP plays a similar role in response to winter temperatures (vernalization) where it forms a heterodimer with FLOWERING LOCUS C (FLC) to strongly repress flowering prior to exposure to cold (Fujiwara et al., 2008; Li et al., 2008). Patterns of naturally occurring allelic variation at *SVP* also suggest that it plays a role in adapting flowering time to local conditions (Mendez-Vigo et al., 2013). Thus SVP represents a critical node in the seasonal control of flowering time of *A. thaliana*. Genomic studies proposed several hundred SVP direct targets based on ChIP-chip or ChIP-seq analysis (Gregis et al., 2013; Tao et al., 2012). This global analysis together with specific ChIP-PCR experiments demonstrated that repression of some flowering genes by SVP, including *FT* and *SOC1,* is direct (Lee et al., 2007; Li et al., 2008).

Here we show that an important novel function of SVP is to reduce levels of the growth regulator gibberellin (GA) by repressing *GIBBERELLIN 20-OXIDASE 2*, which encodes a rate-limiting enzyme required for GA biosynthesis (Coles et al., 1999; Huang et al., 1998; Rieu et al., 2008). Previous physiological and genetic analysis demonstrated that GA promotes flowering of *A. thaliana* (Mutasa-Gottgens and Hedden, 2009). Strong mutations of the biosynthetic pathway delay flowering most markedly under SDs (Wilson et al., 1992), but also under LDs (Rieu et al., 2008), and depletion of GA specifically in the shoot apical meristem by overexpression of a catabolic enzyme delays flowering under both conditions (Porri et al., 2012). Similarly, impairment of GA signaling delayed flowering under LDs and SDs (Galvao et al., 2012; Griffiths et al., 2006; Willige et al., 2007). Furthermore, the RAV-family transcription factors TEMPRANILLO 1 (TEM1) and TEM2 repress transcription of both *FT* and *GIBBERELLIN 3-OXIDASE 1* (*GA3OX1*) and *GA3OX2* (Osnato et al., 2012) suggesting a regulatory link between the two pathways. Nevertheless, it remains unclear whether GA biosynthesis is linked to the well-established regulatory network that controls flowering at the shoot apex (Fornara

et al., 2010). Here we show that FT signaling activates *GA20ox2* transcription in the shoot apical meristem under LDs via FUL and SOC1, which directly represses *SVP* transcription at the meristem. We propose that FT signaling by activating *SOC1* transcription biases a repressive loop involving *SOC1* and *SVP* thereby increasing GA accumulation during photoperiodic flowering and stably inducing the floral transition. Our data provide a novel mechanism that underlies the seasonal control of GA biosynthesis and contributes to the floral transition.

RESULTS

Inhibition of floral induction by *SVP* cannot be fully explained by repression of *FT*, *TSF*, *SOC1* and *FUL*

The MADS box transcription factor SVP regulates flowering under SDs and LDs by repressing transcription and reducing steady-state mRNA levels of *FT, TSF* and *SOC1*, which are all required for the photoperiodic flowering response (Turck et al., 2008). By contrast the mRNA abundance of *FUL*, which also acts in the photoperiod pathway and is partially genetically redundant with *SOC1*, is not affected by *SVP* under SDs (Figure S1A, S1B). However, in plants transferred to LDs the levels of *FUL* mRNA are increased in the SAM of *svp-41* mutants compared to Col-0 (Figure S1C, S1D). The relevance of the increase in *FT*, *TSF, SOC1 and FUL* mRNA levels for the early-flowering phenotype of *svp-41* mutants was tested by genetic analysis. The *svp-41 ful-2 soc1-2* and *svp-41 ft-10 tsf-1* triple mutants flowered significantly later than *svp-41* mutants but much earlier than the *ful-2 soc1-2* or *ft-10 tsf-1* double mutants, respectively (Jang et al., 2009; Torti et al., 2012)(Figure 1A). Therefore, *FUL SOC1* and *FT TSF* contribute to the early flowering of *svp-41* mutants but these pairs of genes are not responsible for the full early-flowering phenotype of *svp-41*. To test whether this early flowering can be fully explained by all four genes, the quintuple mutant *svp-41 ft-10 tsf-1 soc1-2 ful-2* was constructed and its flowering time compared to that of the quadruple mutant *ft-10 tsf-1 soc1-2 ful-2*. Under inductive LDs the quadruple mutant flowered after forming around 85 leaves, whereas the quintuple mutant flowered after producing around 50 leaves (Figure 1A, B). Therefore, the *svp-41* mutation causes earlier flowering even in the absence of functional *FT TSF SOC1 FUL* genes.

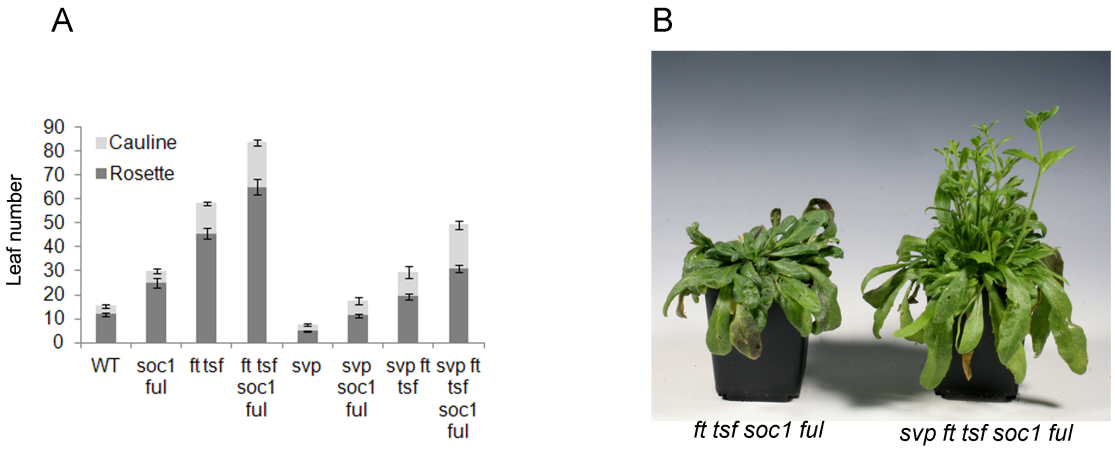

Figure 1. The *svp-41* mutation accelerates flowering in the absence of functional *FT TSF SOC1 FUL* genes.

(A) Leaf number at flowering of plants grown under LDs condition. Data are mean ± standard deviation of at least 10 individual plants. (B) Phenotypes of the quadruple *ft-10 tsf-1 soc1-2 ful-2* and of the quintuple *svp-41 ft-10 tsf-1 soc1-2* mutant plants around 60 days after germination growing under LDs. See also Figure S1.

SVP reduces levels of the GA growth regulator by repressing transcription of the gene encoding the GA-biosynthetic enzyme *GA20-oxidase 2*

Genome-wide transcriptome analysis was used to identify additional genes regulated by *SVP* that could contribute to the early flowering of *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants. Previously, hybridization of Affymetrix tiling arrays was used to identify genes de-regulated in *svp-41* mutants compared to wild-type (Gregis et al., 2013). Among the genes differentially expressed in *svp-41* mutants compared to wild-type were several that contribute to the biosynthesis, catabolism or signaling pathway for the growth regulator GA (Figure 2A), which promotes flowering of *A. thaliana*. Expression of genes involved in GA catabolism and signaling was up-regulated in *svp-41* mutants whereas those contributing to GA biosynthesis were down-regulated. A striking exception to this trend was *GIBBERELLIN 20-OXIDASE 2* (*GA20ox2*),which encodes a GA biosynthetic enzyme and showed an increase in mRNA abundance in *svp-41* compared to wild-type. Therefore SVP directly or indirectly reduces the transcription of *GA20ox2*. SVP acts as a

transcriptional repressor, and therefore whether it binds directly to the *GA20ox2* genomic region *in vivo* was tested. Mutant *svp-41* plants in which the mutation was complemented by a *SVP::SVP:GFP* (Gregis et al., 2009) were used for ChIP-qPCR. No enrichment of the *GA20ox2* locus was detected after ChIP, although positive controls with the known SVP target *SEP3* clearly detected binding of SVP:GFP (Figure S2).

Increased expression of *GA20ox2* mRNA in *svp-41* mutants suggested that these plants might contain higher levels of the growth regulator GA than wild-type plants, and that this could contribute to the early flowering of *svp-41*. Consistent with this idea, comparisons of the *svp-41* and wild-type plants revealed that the mutants exhibit phenotypes that resemble those of plants over-accumulating GA. For example, in addition to early flowering, *svp-41* mutants display a larger rosette radius, lower chlorophyll content and a longer stem (Figure 2B and Table S1). If *svp-41* plants are altered in their GA content, then their responses to exogenously applied GA might differ from those of wild-type plants. Treatment of SD-grown wild-type plants with GA_4 accelerated flowering and reduced chlorophyll content, by contrast no significant changes in these phenotypes were observed after application of GA_4 to *svp-41* mutants (Figures 2C, 2D and S2). The insensitivity of *svp-41* to exogenous application of GA_4 is consistent with *svp-41* mutants containing high endogenous levels of the hormone that saturate downstream responses. By contrast, flowering time and chlorophyll content of *35S::SVP* plants were hypersensitive to GA_4 treatment (Figures 2C and 2D), suggesting that phenotypes associated with high expression of *SVP* are at least partially due to unusually low levels of GA.

Further support for *svp-41* containing increased levels of GA was obtained by direct quantification of GA and by analysis of expression of *GA20ox1 (GA5),* which is regulated by GA via negative-transcriptional feedback control (Phillips et al., 1995; Xu et al., 1995). The microarray data showed that levels of *GA20ox1* mRNA were significantly lower in *svp-41* mutants than in wild-type plants, consistent with the mutant containing elevated levels of GA (Figures 2A).

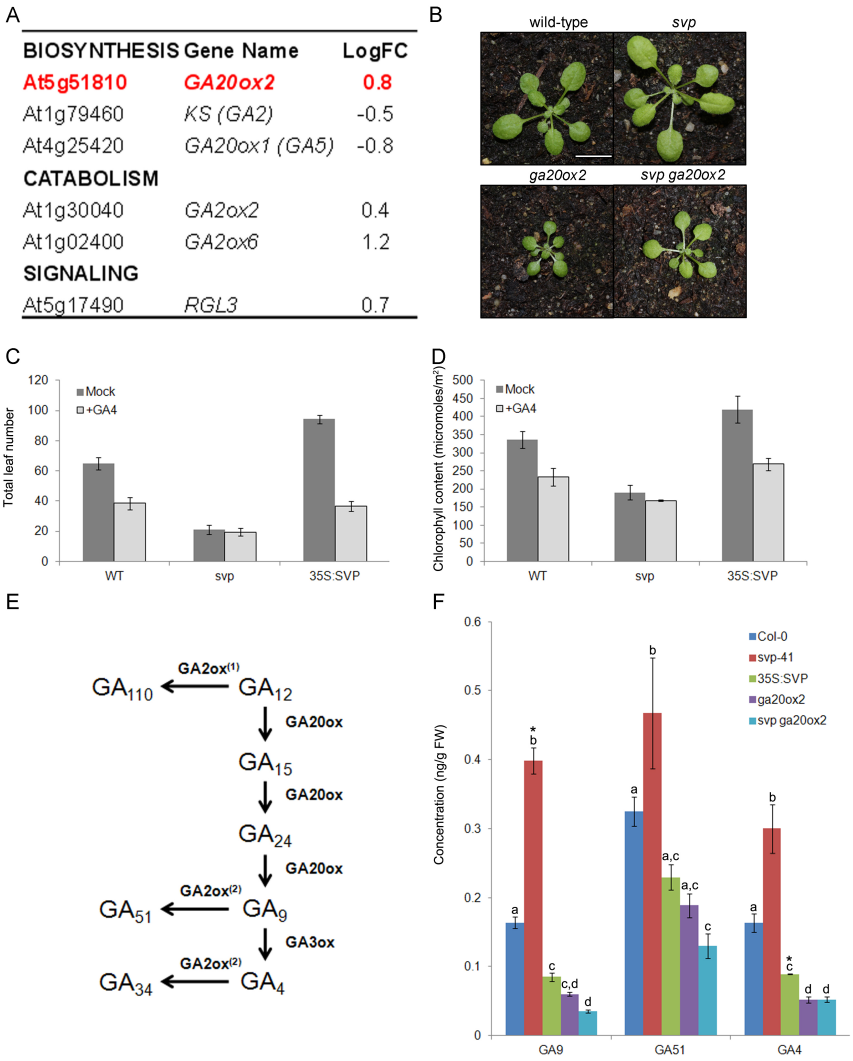

Figure 2. *SVP* reduces GA content through the transcriptional repression of *GA20ox2*.

(A) List of the GA-related genes differentially expressed in *svp-41* mutant compared to wild-type plants according to the microarray experiments performed by Gregis et al. (2013). (B) Phenotype of seedlings of wild-type, *svp-41* mutant (upper panel), *ga20ox2-1* mutant and *svp-41 ga20ox2-1* double mutants (lower panel). Bar = 10 mm. (C) Flowering time and (D) chlorophyll content measurement of wild-type, *svp-41* and *35S::SVP* plants after treatments with GA_4 (light bars) or mock (dark bars). All plants in (A) to (D) were grown under SDs. N = 10-12. (E) Schematic representation of the non-13-hydroxylated GA-biosynthetic pathway in Arabidopsis (adapted from Yamaguchi, 2008). (1) GA2ox7 and -8; (2) GA2ox1, -2, -3, -4 and -6. (F) Concentration of GAs in aerial part of seedlings grown for 2 weeks under SDs. The values are the mean ± SEM of three biological replicates (ng/g FW). Letters shared in common between the genotypes indicate no significant difference in GA concentration (Pairwise Multiple Comparison Procedures, Student-Newman-Keuls Method, P<0.05). (*) Two biological replicates. See also Figure S2 and Table S1.

To explore this idea further, we quantified the concentration of GA forms belonging to the non-13-hydroxylated pathway that mainly contributes to the biosynthesis of GA_4 (Figure 2E) (Yamaguchi, 2008). The levels of the final GA products of this pathway (GA_9, GA_{51} and G_{A4}) were significantly increased in *svp-41* and reduced in *35S::SVP* compared to wild type (Figure 2F).

Whether increased expression of *GA20ox2* contributes to the over-accumulation of GA and the early-flowering phenotype of the *svp-41* mutant was then tested. As shown in Figure 3A, the loss-of-function *ga20ox2-1* mutant flowered slightly later than wild-type (11.1% more leaves) under SDs, however when this mutation was introduced into the *svp-41* mutant it strongly delayed flowering (27.7% more leaves). Moreover, the GA over-accumulation phenotypes observed in *svp-41*, including the leaf radius and chlorophyll content, were suppressed in the *svp-41 ga20ox2-1* double mutant (Figure 2B and Table S1). In addition, GA quantification analyses demonstrated that *GA20ox2* was the main contributor to the GA_9, GA_{51} and GA_4 over-accumulation in the *svp-41* mutant because the levels of these forms were strongly reduced in the *svp-41 ga20ox2-1* double mutant (Figure 2F). Therefore, repression of *GA20ox2* is an important aspect of the role of *SVP* in modulating GA biosynthesis and the phenotypes controlled by this pathway, including flowering time.

The increase in *GA20ox2* mRNA was also detected in the *svp-41 soc1-2 ful-2 ft-10 tsf-1* quintuple mutant compared to the *soc1-2 ful-2 ft-10 tsf-1* quadruple, consistent with it contributing to the earlier flowering phenotype of the quintuple (Figure 3B). Support for the role of GAs in promoting flowering independently of *FT*, *TSF*, *SOC1* and *FUL* was obtained by applying GA4 to the quadruple and quintuple mutants. Strikingly, GA4 treatment accelerated flowering of the quadruple mutant (Figure 3C), but had no effect on flowering time of the quintuple mutant (Figure 3C). Taken together, these results suggest that GAs promote flowering by acting either downstream or in parallel to the photoperiodic pathway containing *FT, TSF, SOC1* and *FUL* and that this process is regulated by the floral repressor *SVP*.

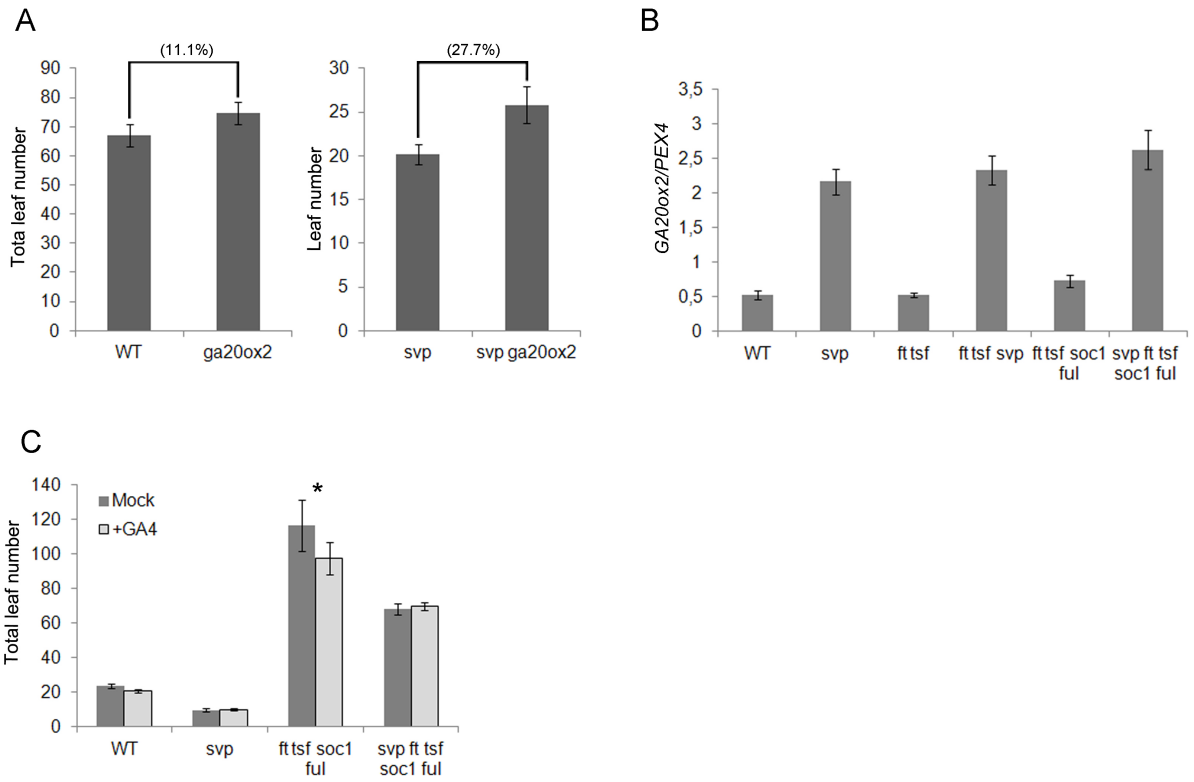

Figure 3. *SVP* regulates flowering time through transcriptional regulation of *GA20ox2*.

(A) Flowering time of wild-type plants compared to *ga20ox2-1* (left graph) and *svp-41* compared to *svp-41 ga20ox2-1* plants (right graph) grown under SDs. The numbers in brackets indicate the differences in flowering time expressed as a percentage. (B) *GA20ox2* mRNA levels in 2 weeks old seedlings of *ft-10 tsf-1* and *soc1-2 ful-2* in the presence or absence of *SVP*. Wild-type and *svp-41* plants were used as controls. Samples were collected 8 h after dawn under SDs. (C) Effect of GA_4 treatment on flowering phenotype of *svp-41*, *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutants growing under LDs. Treatment was carried out with at least 10 individual plants and wild-type was used as control. The asterisk indicates that there is a statistically significant difference between the treated and non-treated *ft-10 tsf-1 soc1-2 ful-2* plants (P = 0.007).

SVP regulates flowering and the expression of *GA20ox2* in the SAM

SVP represses *FT* and *TSF* in the leaves and *SOC1* in the SAM. In the absence of the *FT TSF* photoperiodic signals produced in the leaves, the *svp-41* mutation still accelerates flowering and this is associated with an increase of *GA20ox2* mRNA. Therefore, *SVP* might act downstream of *FT* and *TSF* to repress *GA20ox2* in the SAM. We tested this possibility by quantifying the expression of *GA20ox2* mRNA in different plant organs. As shown in the Figure 4A, *GA20ox2* mRNA is more abundant in apices than leaves of wild-type seedlings, and this difference is enhanced in the *svp-41* mutant.

The effect of misexpression of *SVP* in the SAM on *GA20ox2* expression was also tested. A *pKNAT1::SVP* transgene that drives *SVP* expression in the shoot meristem was introduced into the *svp-41* mutant. The transgenic plants showed a significant delay of flowering under LDs and SDs compared to the *svp-41* mutant, indicating that *SVP* expressed in the SAM is sufficient to repress flowering (Figure 4B). In addition, *GA20ox2* mRNA level was lower in apices of these transgenic plants than in apices of *svp-41* mutants, confirming that SVP represses the transcription of *GA20ox2* in the SAM (Figure 4C) and that this is associated with delayed flowering. Thus, in wild-type plants SVP represses *GA20ox2* expression at the shoot apex.

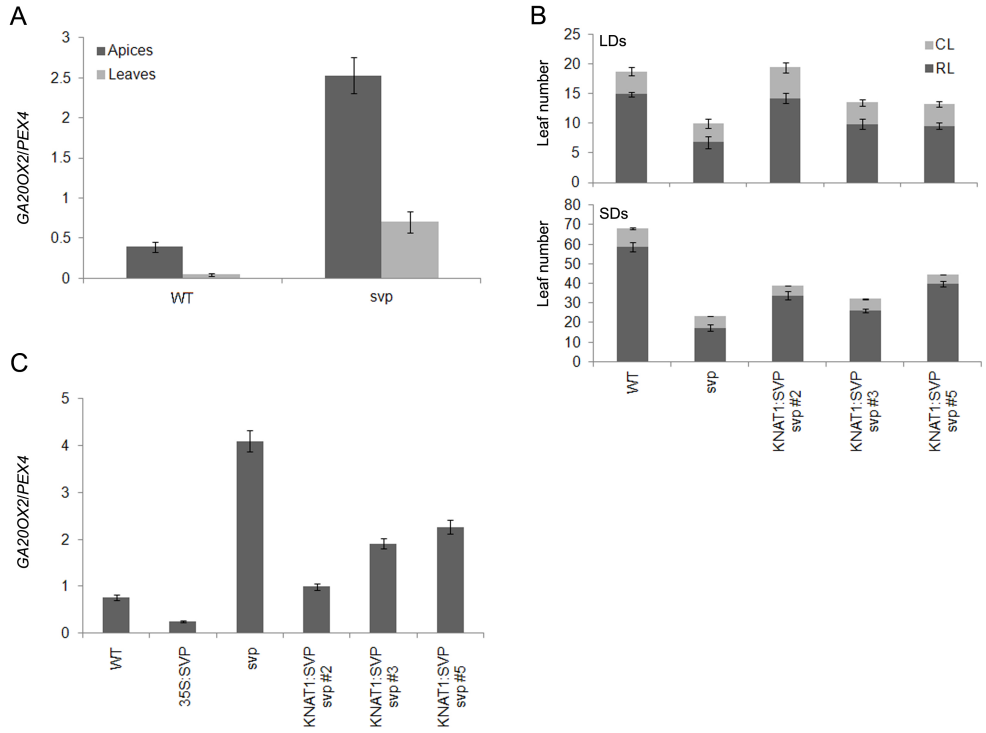

Figure 4. SVP controls floral transition and *GA20ox2* transcription in the SAM.

(A) Levels of *GA20ox2* mRNA in apices and leaves of wild-type and *svp-41* plants. (B) Effect of the misexpression of *SVP* in the SAM on flowering time under LDs (upper panel) and SDs (lower panel). CL: cauline leaves, RL: rosette leaves. (C) Levels of *GA20ox2* mRNA in apices of transgenic plants misexpressing *SVP* compared to WT and *svp-41* mutant grown for 2 weeks under SDs.

During photoperiodic induction of flowering FT-signaling mediates the down regulation of *SVP* and thereby induction of GA biosynthesis

SVP mRNA levels are reduced in the shoot apical meristem during floral induction (Jang et al., 2009), and the above data predict that this is associated with increased *GA20ox2* mRNA abundance and higher GA levels. To test the dynamics of *SVP* down regulation, we studied the temporal and spatial expression patterns of *SVP* mRNA at the SAM of wild-type plants grown in SDs and then transferred to inductive LDs. The *SVP* mRNA was strongly detected at the meristem of wild-type plants under SDs in agreement with the function of *SVP* as a repressor of flowering (Figure 5A). However, after transferring plants to LDs, *SVP* mRNA decreased from the centre of the meristem of plants at 3 LDs and was detectable only in floral primordia at 5 and 7 LDs, representing a later function of *SVP* in floral development (Gregis et al., 2008; Liu et al., 2009). Thus, during photoperiodic induction LD signals repress activity of the floral repressor *SVP* in the shoot apical meristem. To test whether this reduction is associated with changes in the levels of *GA20ox2* mRNA, qRT-PCR was performed with cDNA extracted from apices of wild-type plants transferred from SDs to LDs. The levels of *GA20ox2* mRNA significantly increased at the apex of these plants after exposure to 3, 5 and 7 LDs, consistent with the idea that reduced *SVP* mRNA level is associated with increased expression of *GA20ox2* at the apex (Figure 5B).

To characterize *GA20ox2* spatial expression pattern at the SAM of wild-type plants, GUS staining was performed in *pGA20ox2::GA20ox2:GUS* plants growing under LDs and tissue was harvested prior (8 LDs), during (11 LDs) and after (14 LDs) the transition to flowering (Figure 5C). GUS signal was weakly detected in the centre of the SAM of *pGA20ox2::GA20ox2:GUS* plants 8 LDs after germination, (Figure 5C). However, at 11 LDs, *GA20ox2:GUS* expression was strongly increased (Figure 5C), at the base of the SAM in the rib meristem region. After the floral transition, 14 LDs after germination, GUS expression was maintained mainly in the elongating region of the rib meristem (Figure 5C). Therefore, *GA20ox2* expression occurs in a specific area of the SAM and correlates with the switch from vegetative growth to flowering. Furthermore *SVP* and *GA20ox2* have reverse temporal expression patterns at the SAM during flowering in LDs.

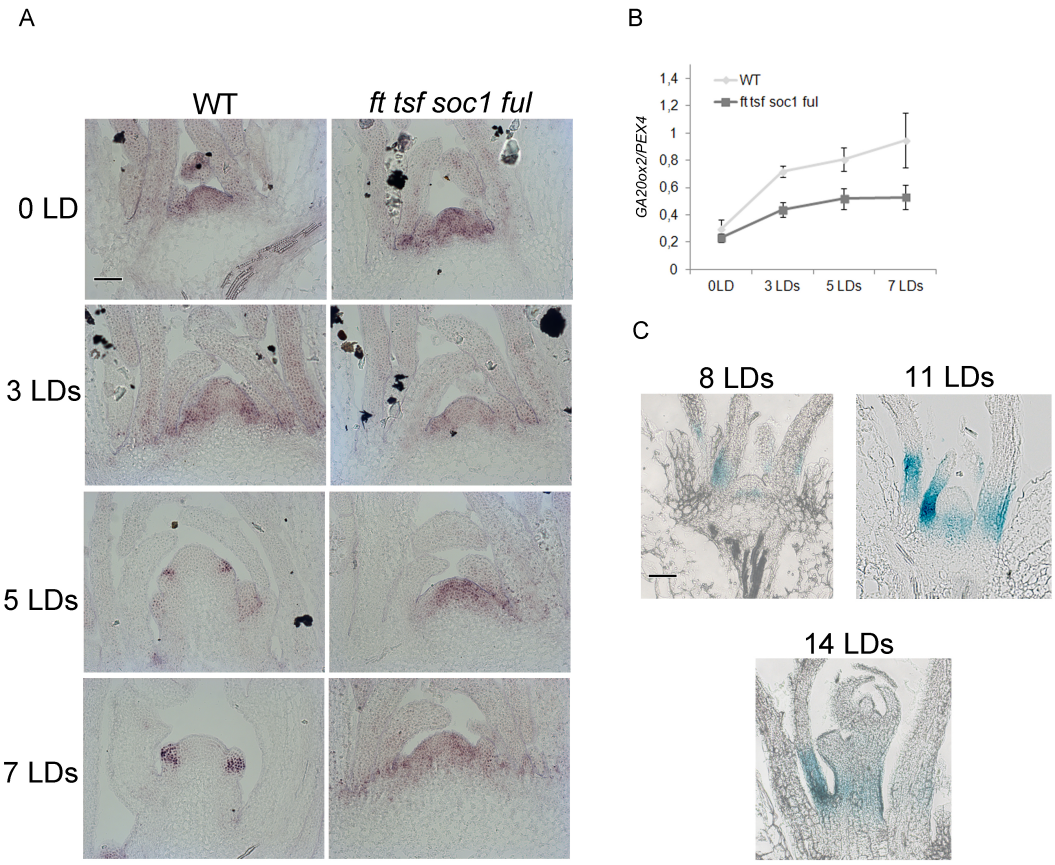

Figure 5. Photoperiodic regulation of GA biosynthesis.

(A) Spatial pattern of SVP mRNA detected by *in situ* hybridization during a time course of *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 3 weeks in SDs (0LD) and then transferred to LDs (3 LDs, 5 LDs, 7 LDs). A specific probe was employed to detect mRNA of *SVP* at the shoot apex. Scale bar: 50 µm. (B) Temporal expression pattern of *GA20ox2* mRNA in apices of wild-type, *ft-10 tsf-1* and *soc1-2 ful-2* mutant plants grown for 3 weeks in SDs (0 LD) and then shifted to LDs (3 LDs, 5 LDs, 7 LDs). All samples were harvested 8 hours after dawn. (C) Histochemical localization of GUS activity at SAM of *pGA20ox2::GA20ox2:GUS* seedlings harvested at the beginning (8 LDs), during (11 LDs) and after (14 LDs) the transition to flowering. Scale bars = 1 mm. See also Figure S3.

In *A. thaliana* the photoperiodic response is mediated by increased expression of *FT* and *TSF* in the leaf followed by upregulation of *SOC1* and *FUL* in the meristem (Turck et al., 2008). During floral induction, SOC1 binds directly to the promoters of several floral integrator genes including *SVP* (Immink et al., 2012). Therefore, whether the module *SVP/GA20ox2* is controlled by the photoperiod pathway was tested by studying the

temporal and spatial expression patterns of *SVP* in meristems of *ft-10 tsf-1 soc1-2 ful-2* mutant plants shifted from SDs to LDs. In contrast to wild-type plants (Figure 5A), *SVP* mRNA was still strongly detectable at the center of the meristem of *ft-10 tsf-1 soc1-2 ful-2* plants even after 7 days exposure to LDs, demonstrating that the *FT TSF SOC1 FUL* pathway is required to repress expression of *SVP* during LD induction. Furthermore, *SVP* transcript persisted at the meristem of the double mutants *soc1-2 ful-2* and *ft-10 tsf-1* for at least 7 days after their transfer from SDs to LDs (Figure S3). In agreement with these results, the levels of *GA20ox2* mRNA were significantly reduced in the apex of these *ft-10 tsf-1 soc1-2 ful-2* plants compared to wild-type (Figure 5B).

_GA20ox2_ is responsible for the SVP-mediated activation of SPL transcription factors during floral induction

Depletion of GA from the shoot apical meristem was previously shown to reduce expression of genes encoding SQUAMOSA PROMOTER BINDING PROTEIN-LIKE (SPL) transcription factors during floral induction under LDs (Porri et al., 2012). In addition, the levels of *SPL3*, *4* and *5* transcripts are regulated by *FT, TSF* and by the downstream acting genes *SOC1* and *FUL* (Torti et al., 2012; Wang et al., 2009). We employed the *svp-41* mutation to distinguish the roles of the *FT, TSF, SOC1, FUL* pathway and GA biosynthesis in the transcriptional activation of *SPL3* and *SPL4*. Therefore, the spatial and temporal expression patterns of *SPL3* and *SPL4* were compared in shoot apical meristems of *svp-41 ft-10 tsf-1 soc1-2 ful-2* and *ft-10 tsf-1 soc1-2 ful-2* plants after transfer from SDs to LDs. No *SPL4* expression was detected in either genotype under SDs, but in *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants *SPL4* mRNA was detected at the base and on the flanks of the shoot apical meristem after exposure to 5 LDs and became strongly detectable after 7 LDs (Figures 6A and S4). By contrast, in the meristem of *ft-10 tsf-1 soc1-2 ful-2* no *SPL4* mRNA was detectable after similar treatments (Figures 6A and S4).

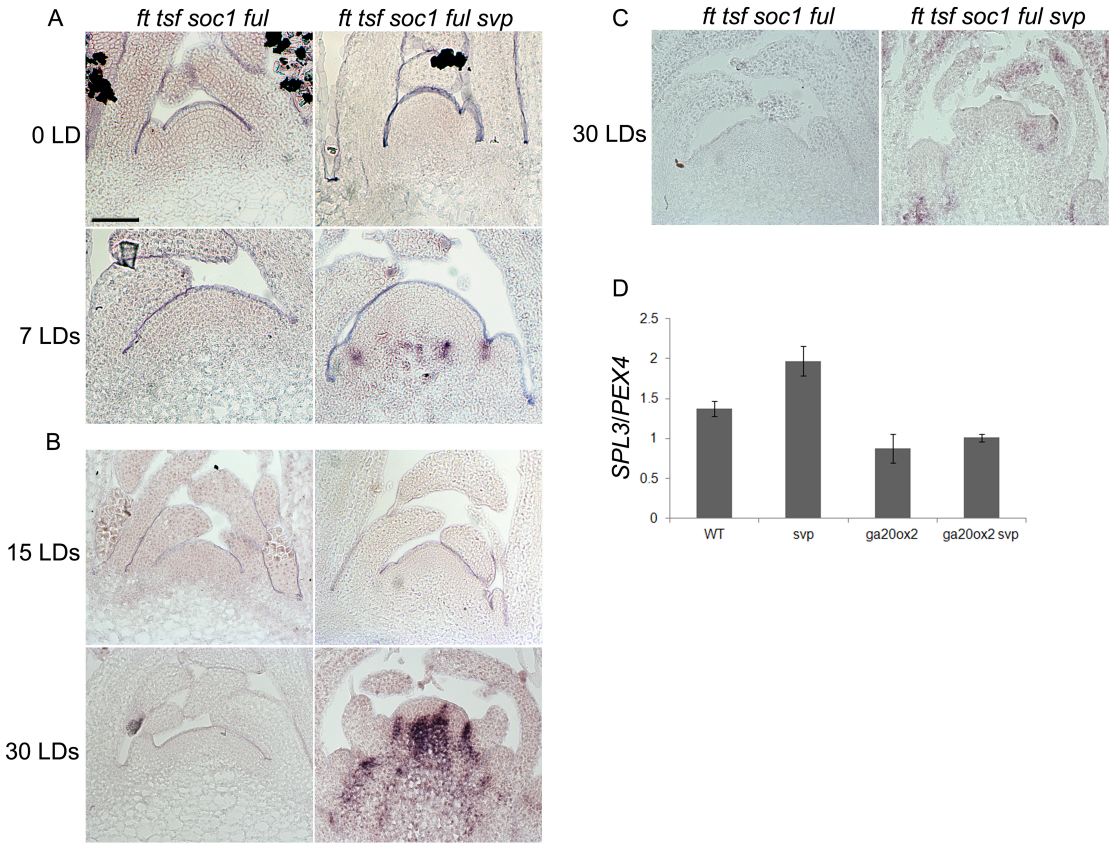

Figure 6. Transcriptional activation of *SPL* gene mRNA is regulated by *SVP* and *GA20ox2*.

(A, B) Pattern of expression of *SPL4*: (A) *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants were grown for 3 weeks in SDs (0LD, upper panel) and then transferred to LDs for 7 additional days (lower panel). (B) *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants were grown for 15 (upper panel) and 30 LDs (lower panel) after germination. (C) Expression levels of *SPL3* in *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 30 LDs. (D) Quantification of the mRNA levels of *SPL3* in wild-type, *svp-41*, *ga20ox2-1* and *svp-41 ga20ox2-1* seedlings grown for 2 weeks under SDs. Scale bars = 50 µm. See also Figure S4.

In addition, *SPL4* mRNA was strongly detected in the meristem of 30 day old *svp-41 ft-10 tsf-1 soc1-2 ful-2* plants grown continuously under LDs that were undergoing the transition to flowering while the meristem *of ft-10 tsf-1 soc1-2 ful-2* showed no *SPL4* mRNA at the same time (Figures 6B and S4). Similarly, expression of *SPL3* was detected in the meristem *of svp-41 ft-10 tsf-1 soc1-2 ful-2* but not in *ft-10 tsf-1 soc1-2 ful-2* at 30 LDs (Figure 6C). Thus, the presence of the *svp-41* mutation accelerates expression of *SPL4* and *SPL3* in the absence of *FT, TSF, SOC1* and *FUL*, which could be due to the increased GA levels present in the *svp-41* mutant. To test this further, the transcript

levels of *SPL3* were quantified in apices of *svp-41 ga20ox2-1* double mutants and compared with *svp-41*, *ga20ox2-1* and wild-type. The transcript levels of *SPL3* were higher in *svp-41* apices compared to wild-type and *ga20ox2-1* (Figures 6D). By contrast, in apices of *svp-41 ga20ox2-1,* abundance of *SPL3* mRNA was reduced compared to *svp-41* and similar to wild-type and *ga20ox2-1*. Therefore, the increased levels of *SPL3* in *svp-41* mutants are dependent on *GA20ox2* activity.

DISCUSSION

In *Arabidopsis thaliana* several genetic pathways determine the timing of floral induction (Andres and Coupland, 2012). These genetically separable pathways mediate responses to seasonal cues such as day length and winter temperatures as well as to endogenous signals including the growth regulator GA. However whether the environmentally regulated pathways controlling floral transition are linked to those regulating GA metabolism is not clear. Here we show that SVP, a MADS box transcription factor with a central role in flowering-time control in response to vernalization and day length, represses GA biosynthesis. Mutations in *SVP* are associated with higher levels of GA_4, the main bioactive GA in Arabidopsis, which was previously shown to promote flowering (Eriksson et al., 2006). SVP expression reduces transcription of *GA20ox2*, which encodes a rate-limiting enzyme in synthesis of GA_4 (Hedden and Phillips, 2000; Rieu et al., 2008). In wild-type plants *GA20ox2* expression rises in the meristem in response to LDs that induce flowering and we show that this is mediated by FT TSF acting through the MADS box transcription factors SOC1 and FUL to repress *SVP*. We propose that an early stage in floral transition in response to LDs involves FT TSF activation of *SOC1* allowing the repression of *SVP* and thereby leading to an increase in GA biosynthesis in the shoot meristem.

Regulation of GA biosynthesis by day length

GA contributes to flowering under inductive LDs and non-inductive SDs. Under SDs flowering is delayed and correlates with a gradual increase in bioactive GA at the shoot apex (Eriksson et al., 2006). Furthermore mutations that impair GA biosynthesis prevent flowering under SDs (Wilson et al., 1992). Such observations led to the idea that GA is essential for flowering under SDs, whilst under LDs the requirement for GA is reduced

because the photoperiodic flowering pathway acting through CONSTANS (CO) and FT TSF accelerates flowering (Reeves and Coupland, 2001; Wilson et al., 1992). Nevertheless, genetic analysis also argues for a role for GA in floral induction under LDs. Mutations that inactivate the GA receptors or strongly reduce GA biosynthesis delay flowering under LDs (Griffiths et al., 2006; Willige et al., 2007). GA biosynthesis is also increased by exposure to LDs in rosette species such as *A. thaliana* or spinach, which is associated with increased expression of GA20ox isoforms and is linked to shoot elongation as well as earlier flowering (Lee and Zeevaart, 2007; Xu et al., 1997). Similarly, the *GA3ox1* and *GA3ox2* genes of *A. thaliana* are co-regulated with *FT* by the TEM transcription factors (Osnato et al., 2012). Here, we provide a mechanism by which increased GA levels at the shoot apex are coordinated with the floral transition under LDs. Our data demonstrate that under LDs the GA and photoperiodic pathways do not simply act in parallel and converge on integrator genes such as *SOC1*, but that GA biosynthesis is regulated by the photoperiodic pathway through SOC1 leading to the downregulation of *SVP* and thus increased expression of GA biosynthetic genes.

We monitored the expression pattern of *pGA20ox2::GA20ox2:GUS* (Plackett et al., 2012) in the meristem and found that under LDs *GA20ox2* expression rises in the rib meristem during floral induction. This region of the meristem promotes stem elongation (bolting) and floral promoter genes change in expression in this region in Arabidopsis after exposure to LDs (Jacqmard et al., 2003; Torti et al., 2012). This indicates that *GA20ox2* expression in this region might have roles in the onset of bolting and floral development and in synchronizing these events during the onset of reproductive development in Arabidopsis (Jacqmard et al., 2003). These results are in agreement with previous observations that *GA20-oxidases* are involved in stem elongation and that mutations in *GA20ox2* delay flowering under LDs (Rieu et al., 2008; Xu et al., 1997). The flowering-time defect of the *ga20ox2-1* mutant under LDs is enhanced by mutations in two other paralogues (Plackett et al., 2012), suggesting that these also contribute to GA biosynthesis under these conditions. Nevertheless, in our experiments only *GA20ox2* was negatively regulated by SVP, suggesting that the boost in GA biosynthesis conferred by the photoperiodic flowering pathway acts predominately through this paralogue. The increase in *GA20ox2* expression observed in the rib meristem under LDs indicates that GA biosynthesis increases specifically in the meristem after down regulation of SVP. This

result contrasts with the gradual increase in GA levels under SDs, which could not be correlated with elevated expression in GA biosynthetic genes suggesting that under these conditions GA is synthesized in other tissues and transported to the meristem (Eriksson et al., 2006). The GA synthesized via *GA20ox2* expression in the rib meristem might move locally into other regions of the shoot meristem, because GA influences the expression of genes such as *LEAFY* and *SPL9* in more apical regions of the meristem (Blazquez and Weigel, 2000; Porri et al., 2012). However it cannot be excluded that non-cell autonomous factors acting downstream of GA move from the rib meristem into more apical regions.

SVP mediates between the photoperiodic pathway and GA regulation

A progressive decrease in *SVP* mRNA in wild-type plants shifted from SDs to LDs is accompanied by a complementary increase in *GA20ox2* mRNA. The reduction of *SVP* mRNA requires the activity of the *FT TSF SOC1* and *FUL* genes because *SVP* mRNA strongly accumulates at the meristem of the quadruple mutant *ft-10 tsf-1 soc1-2 ful-2* even after several days under LDs. This effect probably occurs mainly at the meristem, since mutations of either *FT* or *CO* genes did not result in a significant decrease of *SVP* mRNA level in entire seedlings at early stages of development, as previously shown (Li et al., 2008). Therefore, under LDs *FT TSF* and their downstream target genes *SOC1* and *FUL* act to repress *SVP,* which leads to an increases in *GA20ox2* mRNA and GA levels at the SAM. SOC1 directly represses *SVP* by binding directly to its promoter (Immink et al., 2012) highlighting the effect of the photoperiod pathway. On the other hand, *SOC1* expression is upregulated in *svp-41* mutants (Jang et al., 2009), and SVP binds directly to the *SOC1* promoter (Gregis et al., 2013; Li et al., 2008), indicating that SVP directly represses *SOC1*. These data demonstrate reciprocal repression of SVP/SOC1, so that SVP represses expression of *SOC1* and *vice versa*. Consistent with this model *SVP* and *SOC1* show mutually exclusive temporal expression patterns at the shoot apical meristem with *SVP* being expressed during the vegetative phase while *SOC1* is activated during the transition to flowering (Jang et al., 2009). Thus, one possibility is that in the vegetative shoot apex *SVP* is activated early during development and acts to repress *SOC1*, whereas during flowering the strong induction of *SOC1* by FT TSF overcomes *SVP* repression and allows SOC1 to repress *SVP* (Immink et al., 2012). In SD, GAs gradually induce *SOC1*

expression, which in turn represses *SVP* transcription, and this could explain the repressive effect of the gibberellin pathway upstream of *SVP* observed under these conditions (Li et al., 2008).

Influence of GA on shoot apical meristem activity

The influence of GA on meristem activity was demonstrated by the finding that homeobox transcription factors involved in meristem identity and maintenance control GA levels. In the shoot meristem GA levels are reduced by these factors preventing differentiation and maintaining meristem activity, whereas on the flanks of the meristem where these transcription factors are not expressed, GA levels rise and contribute to organ differentiation (Bolduc and Hake, 2009; Hay et al., 2002). In maize KNOTTED is expressed in the vegetative meristem and binds directly to a gene encoding GA2ox, an enzyme that reduces bioactive GA levels, to activate its expression (Bolduc and Hake, 2009). Similarly in *A. thaliana* the SHOOTMERISTEMLESS homeobox transcription factor reduces expression of *GA20ox1* in the shoot meristem (Hay et al., 2002). This led to models in which homeobox transcription factors repress GA levels in the shoot meristem preventing differentiation and maintaining meristem activity, while on the flanks of the meristem where these transcription factors are not expressed, GA levels rise and contribute to organ differentiation (Bolduc and Hake, 2009; Hay et al., 2002). Our data demonstrate that the MADS domain transcription factor SVP also participates in the control of GA by repressing *GA20ox2* mRNA levels in the vegetative meristem. It remains to be tested whether the action of the homeobox transcription factors and SVP are related or whether they independently repress GA biosynthesis, perhaps by repressing different *GA20ox* paralogues.

During floral induction GA levels rise in the meristem, and our data indicate that this is in part due to repression of *SVP* transcription. It has been shown that the transcription of genes with defined roles in floral transition responds to increasing GA levels (Blazquez et al., 1998; Moon et al., 2003). Several genes encoding SPL transcription factors, including *SPL3*, *SPL4*, *SPL5*, and *SPL9* are activated in response to GA (Galvao et al., 2012; Porri et al., 2012). In agreement with these data, the expression of *SPL4* and *SPL5* is increased in *svp-41* mutants (Torti et al., 2012) even in the absence of *FT TSF* or *SOC1 FUL*, supporting the idea that *SVP* acts downstream of the photoperiod pathway to regulate GA levels

and therefore *SPL* gene transcription. The primary mechanism by which GA acts to regulate transcription is likely to be by promoting DELLA protein degradation and thereby releasing transcription factors to regulate transcription of their target genes (de Lucas et al., 2008; Feng et al., 2008). SPL transcription factors are also targets of GA regulation at this post-translational level (Yu et al., 2012). Thus SPL transcription factors may be targets for activation by GA at different levels of regulation and these in turn are direct activators of *FUL* and *LFY* (Wang et al., 2009; Yamaguchi et al., 2009), perhaps providing one mechanism by which *LFY,* a floral meristem identity gene, is activated by GA (Blazquez et al., 1998).

Perspectives

We have demonstrated that the SVP transcription factor has a central function at the shoot apex in co-ordinating GA biosynthesis with the floral transition. SVP represses expression of GA biosynthetic enzymes during vegetative growth, but is downregulated by the photoperiodic flowering pathway allowing GA levels to rise (Figure 7). Our genetic analysis and previous description of SVP targets demonstrates that this transcription factor blocks flowering by repressing expression of *FT*, *TSF*, *SOC1, FUL* and *GA20ox2*. We now show that all of these genes can be placed within a temporal pathway that responds to photoperiod, suggesting that SVP has evolved to block this pathway at several locations and ensure that flowering does not occur prematurely before exposure to appropriate day lengths or to winter cold.

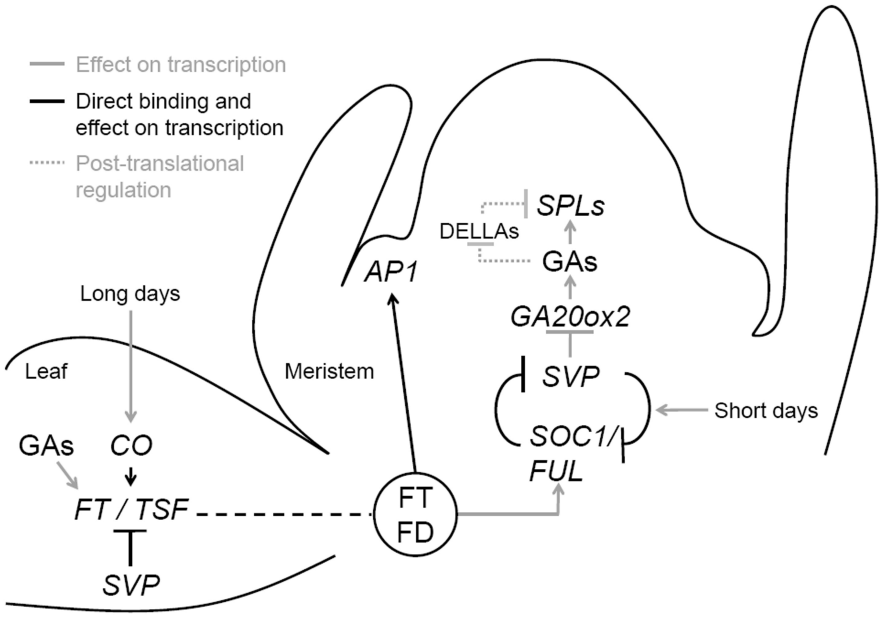

Figure 7. Proposed mechanism for the activation of GA biosynthesis in the shoot apical meristem during photoperiodic flowering.

In plants exposed to LDs the transcription of *FT* and *TSF* is induced in the leaves. The FT protein moves to the SAM (black dashed line) and interacts with FD. The FT-FD module is proposed to activate the transcription of downstream floral promoter genes, such as *AP1*, *SOC1* and *FUL*. SOC1 (and probably also FUL) represses *SVP* expression by direct binding to its promoter and enables the upregulation of *GA20ox2*. The induction of *GA20ox2* transcription in the SAM leads to an increase of GA content required for high transcriptional activation of the *SPL* genes and for release of SPL proteins from DELLA repression during photoperiodic flowering.

MATERIALS AND METHODS

Growth conditions and plant materials

For all studies *Arabidopsis thaliana* (L.) ecotype Columbia (Col-0) was used as wild-type. Plants were grown on soil under controlled conditions of LDs (16 h light/8 h dark) and SDs (8 h light/16 h dark) at 20°C. The level of photosynthetic active radiation was 150 µmol m^{-2} s^{-1} under both conditions. The *svp-41* mutant and the *35S::SVP* transgenic plants were previously described (Hartmann et al., 2000), the double *ft-10 tsf-1* and triple *ft-10 tsf-1 svp-41* mutants were described (Jang et al., 2009) as was the double mutant *soc1-2 ful-2* (Torti et al., 2012). These plants were crossed to generate the quadruple *ft-10 tsf-1 soc1-2 ful-2* and the quintuple *ft-10 tsf-1 soc1-2 ful-2 svp-41* mutants. The GA biosynthetic mutants *ga20ox2-1* and *ga20ox1-3* were reported before (Rieu et al., 2008) as well as the *GA20OX2::GA20OX2:GUS* lines (Plackett et al., 2012).The *SVP::SVP:GFP svp-41* transgenic line used for ChIP experiments (SEP1) has been previously described (Gregis et al., 2009).

GA treatment

The GA_4 stock (SIGMA) was prepared in 100% ethanol with final concentration of 1mM. GA treatments were performed by spraying 10-12 plants with either a GA solution (GA_4 10 µM, Silwet 77 0,02%) or a mock solution (ethanol 1%, Silwet 77 0,02%).

Quantification of gibberellins

About 100-200 mg (fresh weight) of frozen material were used to extract and purify the GAs, as described in Seo et al. (2011). Separated GAs were analyzed by electrospray ionization and targeted-SIM using a Q-Exactive spectrometer (Orbitrap detector; ThermoFisher Scientific). [17,17-^{2}H]GAs were added to the extracts as internal standards for quantification and the concentrations of GAs determined using embedded calibration curves and the Xcalibur program 2.2 SP1 build 48. The full description of these methods can be found as Supplemental Experimental Procedures SEP2.

Flowering-time analysis

Flowering time was determined by counting the number of cauline and rosette leaves of at least 10 individual plants.

***In situ* hybridization and GUS staining**

In situ hybridization was performed according to the method already described in Bradley et al.(1993) and Porri et al. (2012). Probes employed: *SPL3* (Wang et al., 2009; Wu et al., 2009), *SVP* (Torti et al., 2012) and *SPL4* (Porri et al., 2012). GUS staining was performed as described (Adrian et al., 2010).

Plasmid construction, plant transformation and transformant selection

Full length *SVP* cDNAs were amplified by PCR and used to generate an entry clone via BP reaction (Invitrogen). The entry clones were sub-cloned via the LR reaction into the binary vector *pKNAT1::GW* (An et al., 2004) to generate *pKNAT1::SVP*. The plasmids were then introduced into *Agrobacterium* strain GV3101 (pMP90RK) to transform *svp-41* mutant plants by floral dip (Clough and Bent, 1998).

Determination of chlorophyll concentration, leaf radius and stem length

Chlorophyll concentration was estimated by using SPAD-502 leaf chlorophyll meter (Markwell et al., 1995). Leaf radius and stem length were performed manually using a ruler.

RNA extraction and quantitative real-time PCR

Total RNA was isolated from plant tissues by using RNAeasy extraction kit (Qiagen) and treated with DNA-free DNase (Ambion) to remove residual genomic DNA. 1µg of total RNA was used for reverse transcription (Superscript III, Invitrogen). Transcript levels were quantified by quantitative PCR in a LightCycler 480 instrument (Roche) using the *PEX4* gene (At5G25760) as a standard. The sequences of the primers to quantify de expression of *SVP, SOC1, FUL* and *SVP* were described in Torti el al (2012) and the ones for *SPL3, SPL4* and *GA20OX1* were described in Porri et al (2012).

Statistical analysis

All the statistical analyses were performed by using SigmaStat 3.5 software.

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Supporting Information

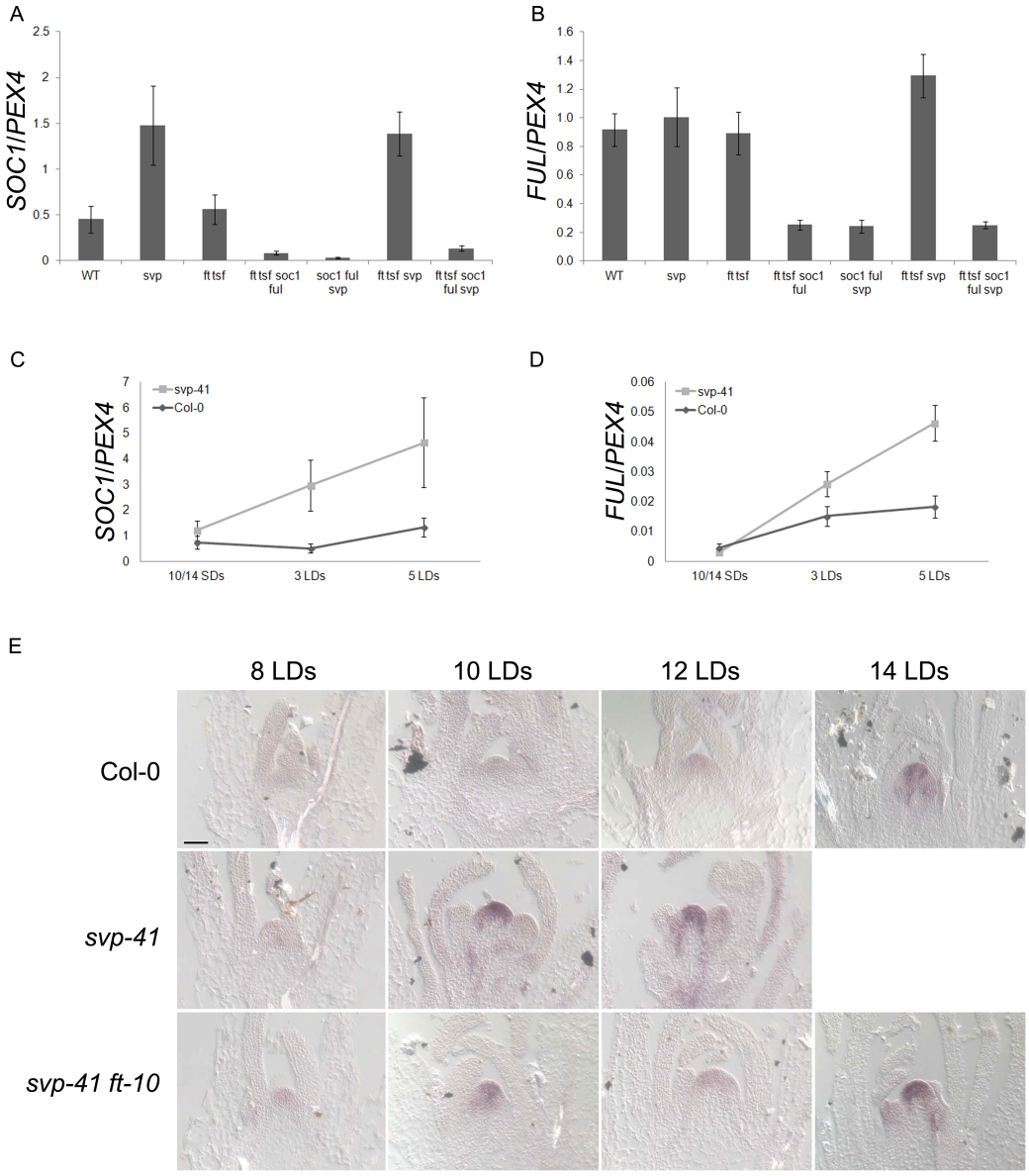

Figure S1. Transcriptional control of SVP downstream targets.

Expression levels of *SOC1* (A, C) and *FUL* (B, D) in different genetic background (A, B) and in a shift experiment (C, D). In (A) and (B) the plants were grown for 2 weeks under SDs and the seedlings were harvested at ZT8. In (C) and (D) the wild type and *svp-41* plants were grown for 14 and 10 SDs while they were still at vegetative stage, respectively and then transfer to LDs for 3 and 5 additional days. The apices of these plants were harvested at ZT8. The panel (E) shows the spatial pattern of *FUL* mRNA during a time course under LDs in wild type, *svp-41* and the *ft-10 svp-41* plants grown under for 8, 10, 12 and 14 LDs.

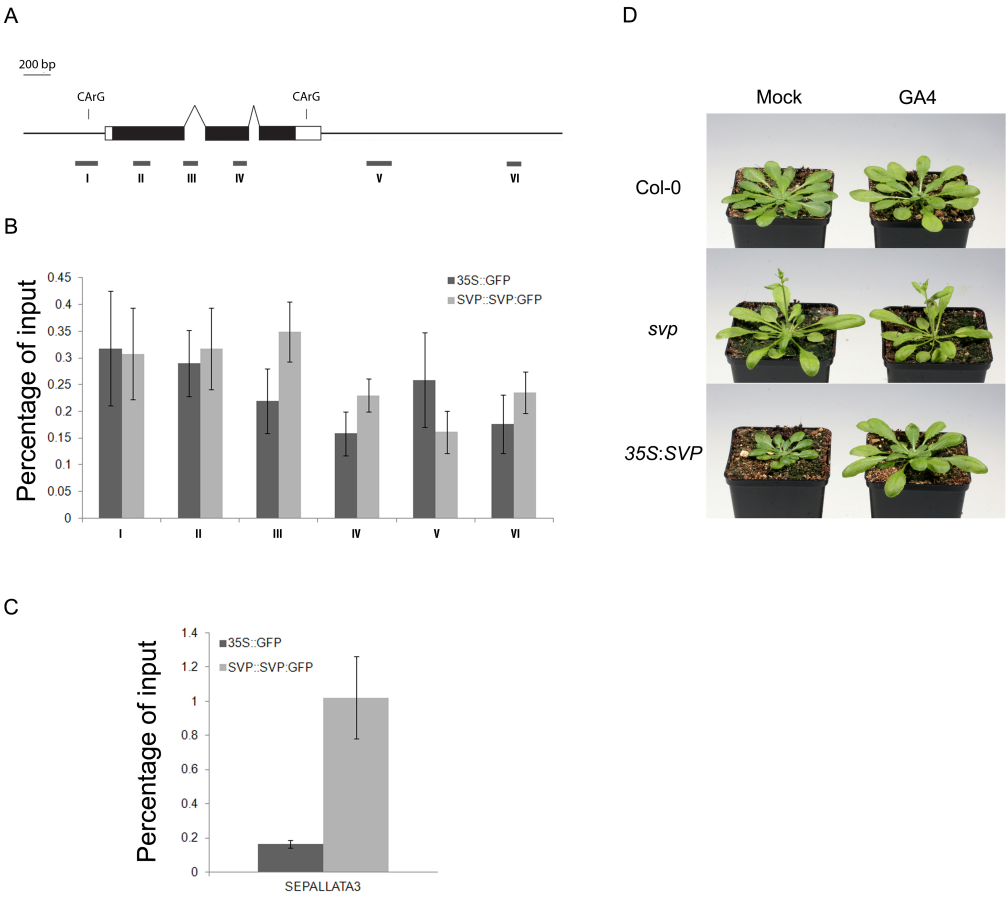

Figure S2. ChIP analysis of SVP:GFP at the *GA20ox2* locus and response to GA treatments in SVP mutants and overexpressors.

SVP direct binding analysis to GA20ox2 by ChIP-PCR. (A) Schematic diagram show the *GA20ox2* genomic region. Exons are represented by black boxes, introns by black line and 3´and 5´UTR regions are represented white boxes. Consensus binding sequence (CArG box) of MADS domain proteins are depicted. Gray boxes denote fragments spanning the locus examined by ChIP enrichment test. (B) ChIP analysis of SVP-GFP binding to different regulatory regions of *GA20ox2* described in (A). (C) A *SEP3* fragment of the promotor was amplified as a positive control for ChIP experiments. Results are represent as percentage of input. Error bars represent SD. (D) Phenotype of wild type (top panel), *svp-41* (middle panel) and *35S::SVP* (lower panel) plants after GA4 treatment under SDs condition. GA4 was applied two times per week at ZT8.

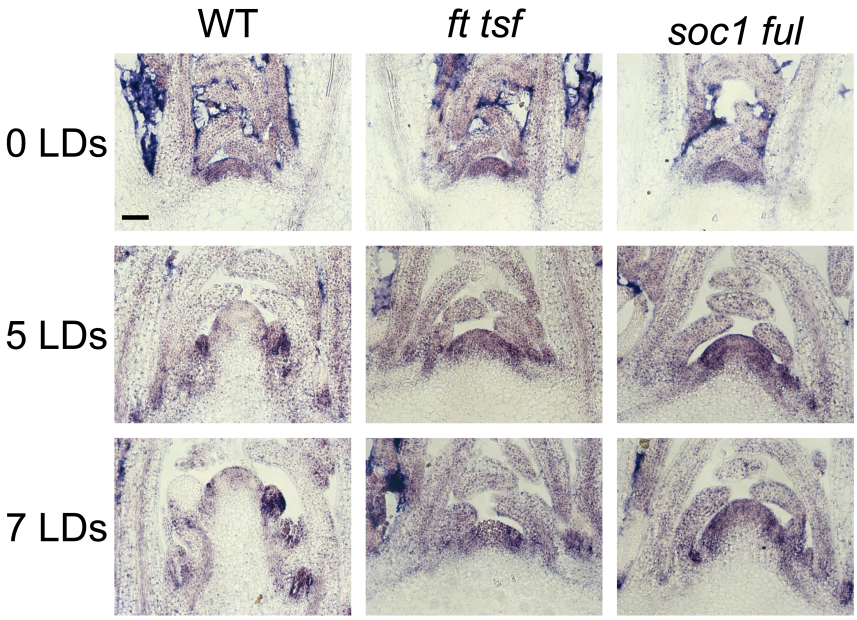

Figure S3. Photoperiodic control of *SVP* expression involves *FT TSF* and *SOC1 FUL*.

Temporal and spatial expression patterns of *SVP* at the meristem of *ft-10 tsf-1* and *soc1-2 ful-2* double mutants plants grown for 3 weeks in SDs (0 LD) and then transferred to LDs (7 LDs). Scale bar: 50 µm.

Genotype	Chlorophyll content (µmoles/m^2)	Height (cm)	Radius (cm)
WT	210.0 ± 11.9[a,b]	45.0 ± 3.4	2.6 ± 0.3[a]
svp	183.6 ± 16.4	51.9 ± 5.0	3.2 ± 0.6
ga20ox2	218.5 ± 15.7[a]	33.8 ± 6.4	1.7 ± 0.2
svp ga20ox2	200.9 ± 11.9[b]	39.7 ± 3.4	2.3 ± 0.3[a]

Table S1. Phenotypic characterization of *svp* and *svp ga20ox2* double mutants.

Mean values among the treatment groups show statistical differences (P = <0.001). Mean values among the treatment groups indicated with the same letter do not show statistical significant difference. Leaf radius and chlorophyll content were estimated in 14 old-day plants grown in SDs, the stem elongation measurement was carried out just before senescence started. N = 10

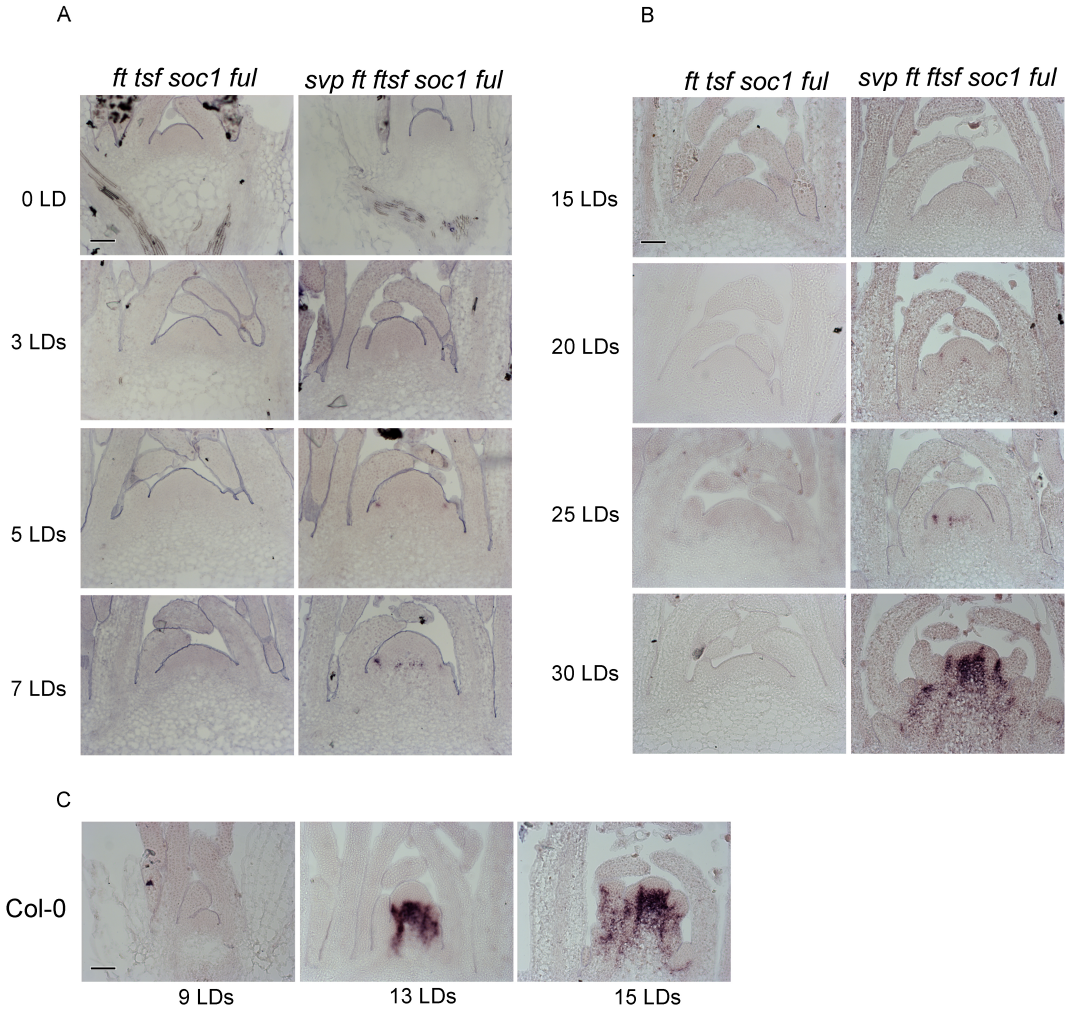

Figure S4. *SVP* regulates *SPL4* expression downstream of the photoperiod pathway.

Temporal and spatial expression patterns of *SPL4* at the meristem of *ft-10 tsf-1 soc1-2 ful-2* and *svp-41 ft-10 tsf-1 soc1-2 ful-2* mutant plants grown for 3 weeks in SDs (0 LD) and then transferred to LDs (3 LDs, 5 LDs, 7 LDs) (A) and for 15, 20, 25 and 30 LDs (B). (C) Pattern of *SPL4* mRNA expression at the meristem of wild type control under LDs (9, 13 and 15 LDs). Scale bar: 50 µm.

Chapter 4: DELLA-interacting SWI3C core subunit of SWI/SNF chromatin remodeling complex modulates gibberellin responses and hormonal crosstalk in *Arabidopsis*

Elzbieta A. Sarnowska, Anna T. Rolicka, Ernest Bucior, Pawel Cwiek, Takayuki Tohge, Alisdair R. Fernie, Yusuke Jikumaru, Yuji Kamiya, Rainer Franzen, Elmon Schmelzer, **Aimone Porri**, Sebastian Sacharowski, Dominika M. Gratkowska, Dorota L. Zugaj, Aleksandra Taff, Anna Zalewska, Rafal Archacki, Seth Davis, George Coupland, Csaba Koncz, Andrzej Jerzmanowski, Tomasz J. Sarnowski

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ABSTRACT

SWI/SNF-type chromatin-remodeling complexes (CRCs) are involved in regulation of transcription, DNA replication and repair, and cell cycle. Mutations of conserved subunits of plant CRCs severely impair growth and development, however the underlying causes of these phenotypes are largely unknown. Here we show that inactivation of SWI3C, the core component of Arabidopsis SWI/SNF CRCs, interferes with normal functioning of several plant hormone pathways and alters transcriptional regulation of key genes of gibberellin (GA) biosynthesis. The resulting reduction of GA_4 causes severe inhibition of hypocotyl and root elongation, which can be rescued by exogenous GA-treatment. In addition, the *swi3c* mutation inhibits DELLA-dependent transcriptional activation of *GID1* GA-receptor genes. Down-regulation of *GID1a* in parallel with the DELLA repressor gene *RGA* in *swi3c* indicates that lack of SWI3C also leads to defects in GA-signalling. Together with recent demonstration of function of SWI/SNF ATPase BRAHMA in the gibberellin pathway, these results reveal a critical role of SWI/SNF CRC in the regulation of GA biosynthesis and signalling. Moreover, we demonstrate that SWI3C is capable of *in vitro* binding to, and shows *in vivo* BiFC interaction in cell nuclei with the DELLA proteins RGL2 and RGL3, which affect

transcriptional activation of *GID1* and *GA3ox* genes controlling GA perception and biosynthesis, respectively. Furthermore, we show that SWI3C also interacts with the O-GlcNAc transferase SPINDLY (SPY) required for proper functioning of DELLAs, and acts hypostatically to SPY in the GA-response pathway. These findings suggest that DELLA-mediated effects in GA-signaling as well as their role as a hub in hormonal cross-talk may be, at least in part, dependent on their direct physical interaction with complexes responsible for modulation of chromatin structure.

INTRODUCTION

The SWI/SNF-type chromatin remodeling complexes (CRCs) are evolutionary conserved in eukaryotes. They carry a central Snf2-type ATPase in association with several core subunits that correspond to orthologs of SNF5, SWI3 and SWP73 proteins of the yeast prototype of SWI/SNF CRCs. In mammals, the core non-catalytic subunits of SWI/SNF-type complexes, such as SWI3, directly interact with nuclear hormone receptors and co-activators (DiRenzo et al., 2000; Zraly et al., 2006; John et al., 2008). All known core subunits of SWI/SNF complexes are conserved in plants. The Arabidopsis genome encodes four SNF2 ATPases and four SWI3-type proteins, which build various SWI/SNF complexes with different subunit composition (Sarnowski et al., 2005). Mutations affecting the Arabidopsis SWI/SNF subunits cause characteristic alterations in plant development and responses to environmental factors. As yet, detailed characterization of knockouts of *BRM* and *SYD* ATPase and four *SWI3* genes (*SWI3A*, *SWI3B*, *SWI3C* and *SWI3D*) has been reported (Sarnowski et al., 2002, 2005; Farrona et al., 2004; Bezhani et al., 2007; Archacki et al., 2009). However, the exact molecular mechanisms by which these mutations cause complex developmental and physiological defects are so far largely unknown.

Our earlier studies revealed that in Arabidopsis the BRM ATPase and SWI3C CRC subunits fulfill most of their functions by acting in a common complex. However, we also found that BRM has additional and specific functions that are independent of SWI3C (Archacki et al., 2009). Transcriptome analysis of *brm* and *syd* mutant lines indicated that these mutations modify the expression of genes in several signaling pathways, including the gibberellin (GA) and ABA hormone pathways (Bezhani et al., 2007; Saez et al., 2008). Gibberellin is responsible for regulation of growth and other basic processes,

including germination, shoot and root elongation, flower development, flowering time, seed development and maturation, and aging (Fleet and Sun 2005). The best-studied downstream elements in the gibberellin pathway are the DELLA proteins that act as general repressors of GA-stimulated processes (Peng et al., 1997, Silverstone et al., 1998). Upon accumulation, GA is perceived by the GID1 nuclear receptors (GIBBERELLIN INSENSITIVE DWARF 1; GID1a, GID1b and GID1c in Arabidopsis) (Ueguchi-Tanaka et al., 2005; Nakajima et al., 2006), and the GA-GID1 complex binds to DELLAs (Griffith et al., 2006; Willige et al., 2007; Ueguchi-Tanaka et al., 2007). This enables interactions with the F-box protein SLEEPY (SLY1)/GIBBERELLIN INSENSITIVE DWARF2 (GID2) that mediates polyubiquitinylation and subsequent proteasomal degradation of the DELLA repressors (Sasaki et al., 2003; Dill et al., 2004). The activity of DELLAs is likely also regulated by other pathways. The enzyme O-GlcNAc transferase encoded by the *SPINDLY* (*SPY*) gene was shown to enhance the repressor activity of DELLAs (Silverstone et al., 2007; Shimada et al., 2006). We have recently demonstrated that BRM affects the expression of a significant number of GA-responsive genes, including *GA3ox1*, and that the level of active GA is markedly decreased in the *brm* null mutant (Archacki et al., 2013).

Here, we show that proper regulation of plant responses to several hormones requires the function of core SWI3C subunit of SWI/SNF CRCs and provide novel clues regarding a possible mechanism underlying SWI/SNF-mediated regulation of the GA hormone response pathway. We show that inactivation of SWI3C results in developmental abnormalities that are characteristic for Arabidopsis mutants impaired in GA biosynthesis and signaling. The *swi3c* mutation, similarly to the *brm* mutation, markedly decreases the levels of bioactive GA derivatives by causing pathway-wide alteration in the transcription of genes involved in the biosynthesis and inactivation of gibberellins. Furthermore, the *swi3c* mutation also down-regulates the expression of *GID1* GA-receptor genes, which may affect the GA perception in leaves. Moreover, SWI3C physically interacts in the nucleus with several DELLA proteins, and with SPY, which appears to act upstream of SWI3C in the GA-response pathway. Physical interactions of SWI3C with DELLAs and SPY suggest that the function of SWI3C-containing SWI/SNF CRCs may be required for some of the DELLA-mediated effects, like activation of *GID1* and *GA3ox* genes involved in GA perception and biosynthesis, respectively.

RESULTS

The *swi3c* mutant shows altered ethylene, ABA, brassinosteroid, gravitropic and gibberellin responses and confers GA-related growth and developmental defects

During initial characterization of the *swi3c* T-DNA insertion mutants (Sarnowski et al., 2005), we observed that seedlings carrying either the *swi3c-1* or *swi3c-2* mutant alleles showed similarly altered phenotypic traits compared to wild type when germinated on media containing different phytohormones. Subsequently, we used the *swi3c-1* mutant to examine in more detail several hormone responses in germination and seedling growth assays. When germinated in the presence of increasing concentrations of ABA, the *swi3c* mutant displayed reduced germination rate compared to wild type indicating that similarly to BRM (Han et al., 2012), inactivation of the SWI3C SWI/SNF subunit results in enhanced sensitivity to ABA (Figure S1A). Dark grown *swi3c* seedlings developed short hypocotyls and roots when germinated in the presence of ethephon, which is hydrolyzed to ethylene in the medium, and the ethylene precursor aminocyclopropane-1-carboxylic acid (ACC), suggesting an increased ethylene sensitivity (Figure S1B). In response to brassinosteroid (BR) treatment, light-grown *swi3c-1* plants responded with enhanced hypocotyl elongation compared to wild-type (Figure S1C). Finally, the *swi3c-1* mutation showed a defect of root gravitropic response compared to wild type indicating that, in addition to inhibition of root elongation, the *swi3c-1* mutation also prevented auxin-dependent gravitropic root bending (Figure S1D). These preliminary germination and growth assays thus highlighted an alteration of multiple hormonal responses underlying the severe developmental defects observed in the *swi3c* mutant (Sarnowski et al., 2005).

In comparison to other hormones, the *swi3c* mutant showed markedly enhanced growth and flowering responses to external feeding with bioactive gibberellins GA_{4+7}. Compared to wild-type, GA_{4+7}-treatment resulted in acceleration of flowering of *swi3c* (counted in number of leaves to flowering), which also flowered earlier than wild-type without GA-treatment under short-day condition (Fig.1A; Sarnowski et al., 2005). However, unlike wild-type, *swi3c* plants did not display an increase of leaf blade size upon GA treatment (Fig. 1B). By contrast, treatment with 1µM GA_{4+7} completely suppressed the defects of hypocotyl and root elongation of *swi3c* mutant seedlings, which developed like wild-

type in the presence of GA (Fig.1C and D and Fig. S2). At higher (10µM) concentration, GA_{4+7} marginally inhibited root but not hypocotyl elongation of both wild-type and *swi3c* seedlings (Fig. S2).

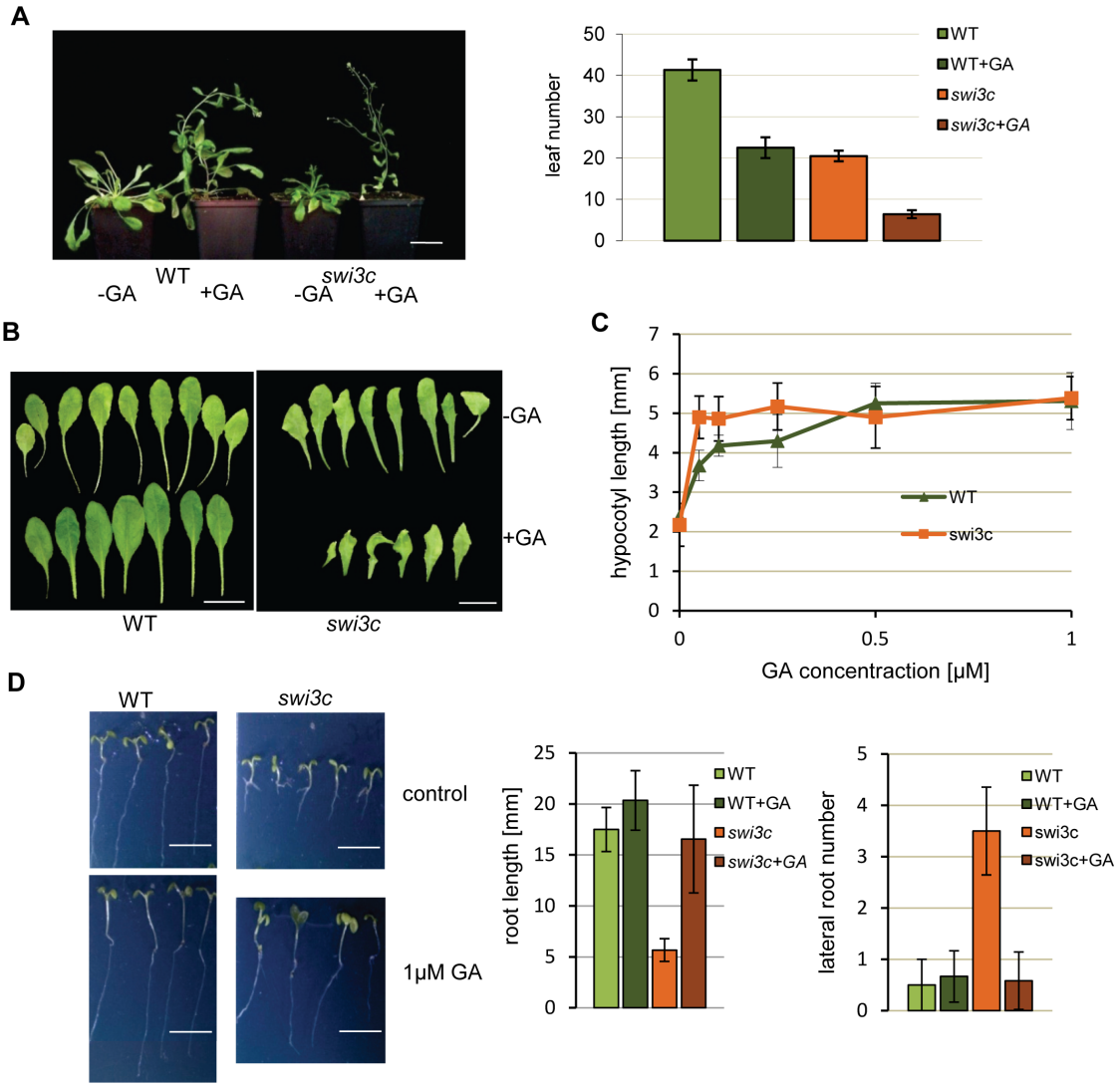

Figure 1. **The *swi3c* mutation confers GA-related growth defects.**

A, Compared to wild-type, GA_{4+7}-treatment resulted in acceleration of flowering time in the *swi3c* mutant that also flowers earlier than wild-type without GA-treatment under short-day condition. Six weeks old *swi3c* and wild-type plants grown in SD conditions untreated or treated with 100µM GA_{4+7}. Scale bar 5 cm. B, The leaves of *swi3c* mutant did not show blade expansion after GA treatment indicating an organ specific defect in GA response. Scale bar 1cm. C, Treatment with 1µM GA_{4+7} completely suppressed the defect of hypocotyl elongation of *swi3c* mutant seedlings. D, Treatment with 1µM GA_{4+7} suppressed the defects of hypocotyl and root elongation of *swi3c* mutant seedlings.

In addition, GA-treatment abolished characteristic branching of *swi3c* mutant roots on 0.5% sucrose-containing MS-medium (Fig. 1D). Compared to wild type, the *swi3c* seedlings proved to be insensitive to the GA biosynthesis inhibitor paclobutrazol (PAC). Even at very low concentration (2.5 nM), PAC-treatment reduced the hypocotyl length of wild-type seedlings. By contrast, PAC-treatment of *swi3c* seedlings stimulated hypocotyl shortening only when PAC concentration was increased to 1µM (Fig. S2). In summary, several developmental defects observed in the *swi3c* mutant proved to be similar to those of GA-deficient mutants. Furthermore, suppression of root and hypocotyl elongation defects by GA_{4+7} indicated that, similarly to mutations of the BRM ATPase (Archacki et al., 2013), the hypocotyl and root elongation defects caused by inactivation of the SWI3C SWI/SNF CRC core subunit were due to deficiency of GA biosynthesis.

We reported previously that the Arabidopsis *swi3c* mutation results in complex pleiotropic developmental defects (Sarnowski et al., 2005). Some of these pleiotropic deficiencies, such as enhanced leaf-curling and alterations in the development of flower organs, were also identified in the *brm* mutant, and are thus typical for plants deficient in the function of SWI/SNF CRCs (Archacki et al., 2009). By contrast, other phenotypic traits of the *swi3c* and *brm* mutants, in particular their dark-green leaf color and semi-dwarf stature resemble those of GA-deficient mutants that show reduced GA-biosynthesis and accumulation of DELLA proteins (Koornneef and van der Veen, 1980). The Arabidopsis mutants *gid1a, gid1b* and *ga1-3* deficient in GA perception and biosynthesis, respectively, display reduced germination, abnormal seed shape and irregular cell division patterns in the seed coat (Iuchi et al., 2007). Using scanning electron microscopic (SEM) studies, we found that the epidermal cell layer of irregularly shaped *swi3c* mutant seeds is similarly characterized by highly abnormal patterns of cells, which differ in both size and shape from seed-coat epidermal cells of wild-type (Fig.2A). Next, we examined the structure of mature wild-type and *swi3c* embryos using cross-sections of seeds embedded into wax after 24h of imbibition and fixation with paraformaldehyde. Cross-sections of matured *swi3c* embryos revealed aberrant development characterized by larger embryo size, increased cell number, and improperly developed cotyledons compared to wild-type. This indicated that *swi3c* mutation altered normal regulation of cell division not only in seed epidermis but also

during embryogenesis (Fig.2B). Compared to wild-type, the *swi3c* mutant had higher density of cells per unit surface area of the leaf epidermis (Fig. 2C). In addition to organ specific changes in cell number and size, the transcription of genes encoding the cell cycle inhibitors KRP2, SIM and SMR1 showed a marked reduction in the *swi3c* mutant (Fig. 2D). Together, these results were consistent with our observations indicating that SWI3C-containing SWI/SNF CRCs are involved in the regulation of multiple hormonal pathways and suggested that, at least part of complex *swi3c* mutant phenotype resulted from aberrant GA biosynthesis and/or signaling.

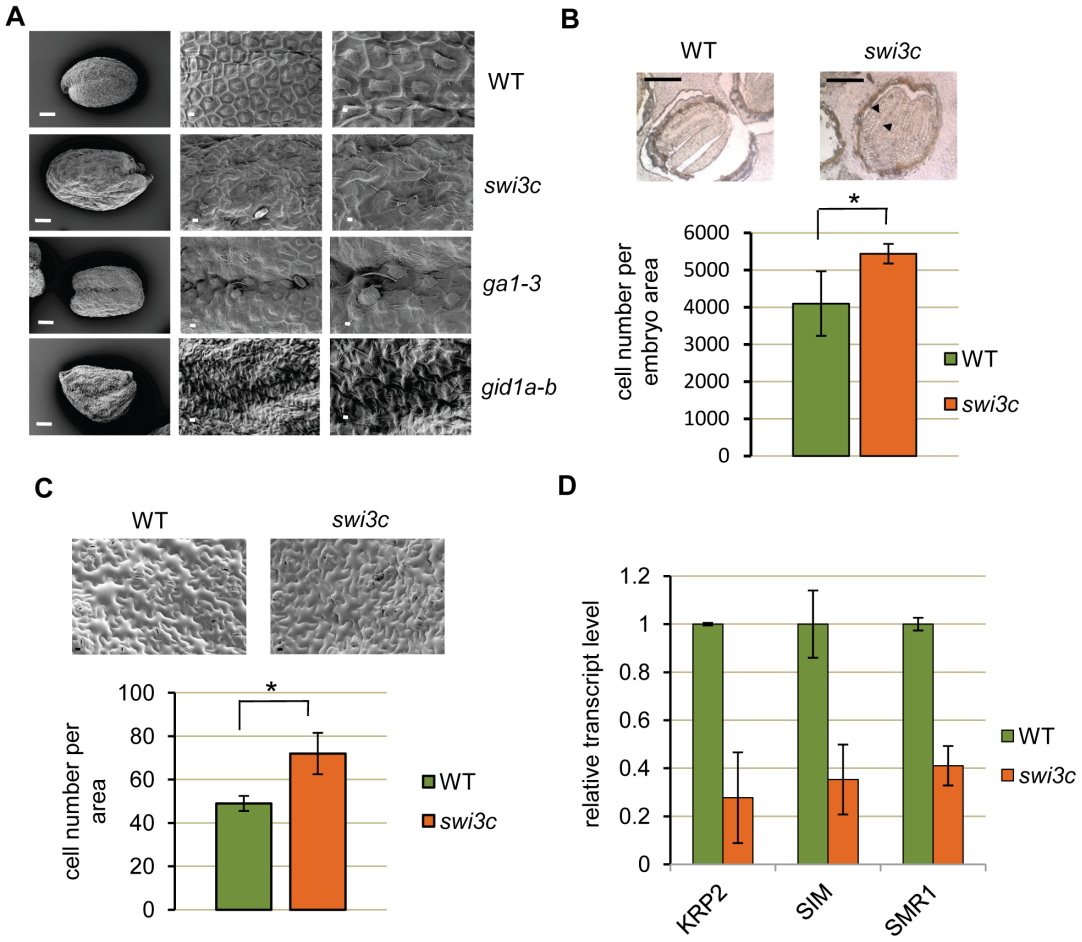

Figure 2. The *swi3c* mutation confers GA-related developmental defects.

A, The SEM analysis of seed coat structure of *swi3c* mutant indicate similar changes to those observed in GA pathway mutants *ga1-3* and *gid1a-b*. Scale bars: 100µm, left column, and 10 µm middle and right columns. B, Cross-sections of mature *swi3c* embryos. Arrowheads indicate improperly developed cotyledons. Scale bar 500 µm. * p value <0.05. C, The cell number of 4 weeks old LD grown *swi3c* mutant leaves is increased. Scale bar 10 µm. * p value <0.05. D, The expression levels of cell cycle inhibitors are markedly reduced in the *swi3c* mutant.

The *swi3c* mutant has decreased level of bioactive gibberellin GA_4

To verify the latter conclusion inferred from physiological assays, we compared the levels of GA biosynthesis intermediates, bioactive GAs and inactive GA metabolites in *swi3c* and *ga1-3* mutant and wild-type plants, which were collected at the end of day and at the end of night during a diurnal growth period. Quantitative measurements of GAs revealed that, similarly to *ga1-3*, the *swi3c* mutant contained reduced levels of bioactive GA_4, as well as GA_{34}, the inactive metabolite of GA_4 (Fig. 3A and S4B, Supplemental Table 1A and B). We did not observe an accumulation of GA_9 but found that the *swi3* mutant accumulated higher levels of GA_{15}, GA_{19} and GA_{51} compared to wild-type, indicating a shift of GA-biosynthesis towards the inactive GA_{51} derivative rather than active GA_4. Consequently, similarly to *brm* (Archacki et al. 2013), the swi3c mutant appeared to be deficient in the biosynthesis of active gibberellins.

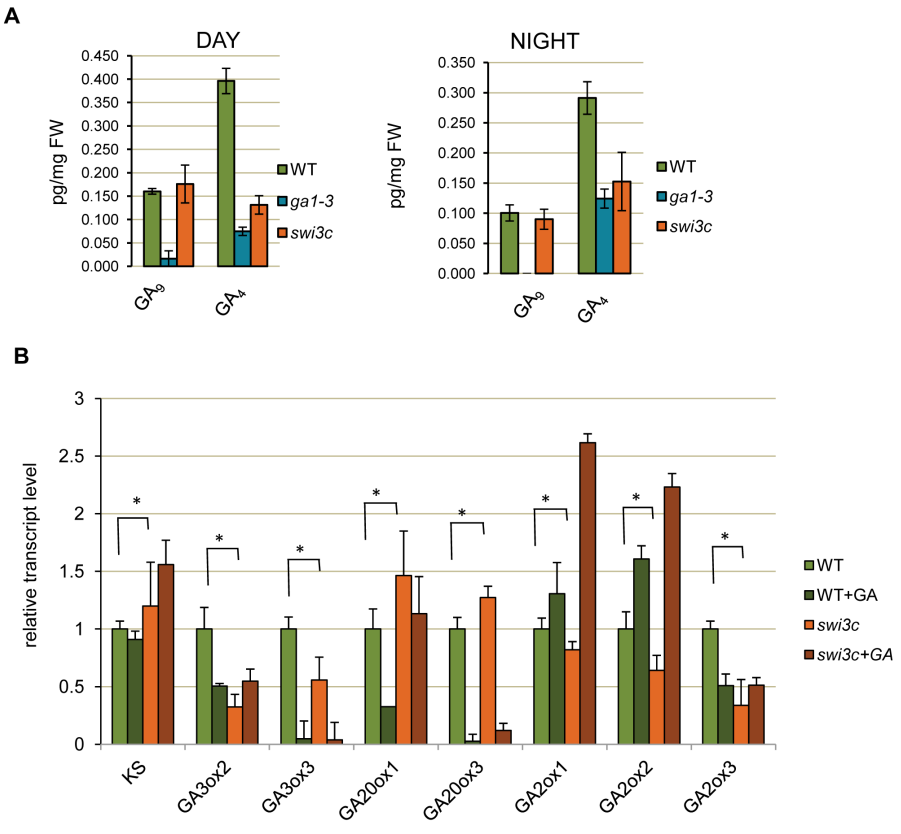

Figure 3. *swi3c* mutant has decreased GA_4 content and shows altered transcriptional regulation of GA pathway genes.

A, Four weeks old LD grown wild-type, *swi3c* and *ga1-3* plants were collected at the end of night and end of day and subjected to GA analysis. Both *swi3c* and *ga1-3* have decreased level of bioactive GA_4. B, Transcription of genes acting in GA biosynthesis and metabolism shows coordinate changes in *swi3c* mutant. Reduction of bioactive GA in *swi3c* mutant correlates with decreased expression of *GA3ox2 and GA3ox3* genes, as well as overexpression of *GA2ox1* and *GA2ox2*. * p value <0.05.

Transcription of genes acting in GA biosynthesis and metabolism show coordinate changes in the *swi3c* mutant

Next, we examined the abundance of transcripts encoding key enzymes of GA metabolism, including *KS* (ent-kaurene synthase B)*, KAO1* and *KAO2* (ent-kaurenoic acid hydroxylase)*, KO* (ent-kaurene oxidase)*, CPS* (ent-copalyl diphosphate synthase)*, GA3ox1-3* (gibberellin 3-beta-dioxygenase)*, GA20ox1* and *GA20ox3* (gibberellin 20-oxidase) and *GA2ox1-3* (gibberellin 2-beta-dioxygenase) in soil-grown *swi3c* seedlings. Compared to wild-type, the *KS* (*GA2*) transcript level was slightly elevated in *swi3c* mutant in both absence and presence of GA_{4+7}-treament, whereas the *KAO1* transcript was marginally elevated only when *swi3c* was treated with $GA_{4+7.}$ The KS ent-kaurene synthase B catalyzes a second step in cyclization of GGPP to ent-kaurene, whereas the KAO1 ent-kaurenoic acid hydroxylase controls the further three steps in GA biosynthetic pathway from ent-kaurenoic acid to GA_{12} (Hedden and Phillips, 2000). More importantly, compared to wild-type in the absence of GA-treatment, the *swi3c* mutant showed a two-fold reduction, of transcript level of *GA3ox2* encoding GA 3-beta-dioxygenase 2, which catalyzes the hydroxylation of GA_9 and GA_{20} to bioactive GA_4 and GA_1, respectively (Curaba et al., 2004). There was also no compensation of *GA3ox2* by *GA3ox3* gene, which acts in GA-dependent regulation of flower organ development, as the expression of the latter gene was marginally reduced in *swi3c*. The expression of *GA3ox2* was however restored to the wild-type level in GA-treated *swi3c* plants (Figure 3B). In parallel, the transcript levels of *GA2ox1*, *GA2ox2* and *GA2ox3* genes, which code for gibberellin 2-oxidases that inactivate the GA_{19}-derived GAs, including GA_9 and GA_{20} precursors of bioactive GA_4 and GA_1, were reduced 0.8 to 2.8-fold in *swi3c* mutant. In comparison, transcript levels of *GA20ox1* and *GA20ox3* genes, involved in the synthesis of precursors of bioactive GAs, were slightly higher in *swi3c* compared to wild type. The treatment with GA_{4+7} increased the abundance of *GA2ox1* and *GA2ox2* transcripts over two-fold to a level 60-80% higher than in wild-type, revealing that inactivation of bioactive GAs was enhanced in *swi3c* mutant when plants were treated with exogenous GA. Together, these observations are consistent with extensive deregulation of the GA-mediated feed-back control of GA biosynthesis pathway (Griffiths et al., 2006) in *swi3c* mutant.

The *swi3c* mutation alters the regulation of *GID1* GA-receptor genes and numerous known DELLA target genes

Although direct measurements of GA levels clearly indicated that the *swi3c* mutation caused GA-deficiency, some of the developmental defects of *swi3c* plants (e.g. formation of curling leaves and expansion of leaf-blades) were not restored to wild-type by GA-treatment (Fig. 1B). To check whether this was due to alteration of tissue specific GA perception in *swi3c*, we compared the abundance of *GID1* GA-receptor transcripts in leaves of wild-type and *swi3c* plants. Transcription of *GID1a*, encoding the most abundant form of GA-receptor expressed at the highest level in all plant organs except roots (Griffiths et al., 2006), showed over two-fold reduction in *swi3c* mutant. The transcript level of *GID1a* in *swi3c*, both without and with GA treatment, was comparable to that in wild-type plants upon GA-mediated feedback inhibition of *GID1a* (Figure 4A). *GID1b*, which is expressed at higher level than *GID1a* in roots but similarly inhibited by GA (Griffiths et al., 2006), showed slightly higher transcript levels but no GA-inhibition in *swi3c*. Finally, *GID1c* that is expressed at very low level compared to *GID1a* and *GID1b* in most plant organs, showed GA-stimulated, rather than inhibited, transcription in *swi3c*. In comparison, transcription of *RGA*, encoding one of the five DELLA repressors, was reduced two-fold in *swi3c* leaves, but restored to wild type levels by GA-treatment. Additionally, the analysis of expression of genes encoding other DELLA proteins indicated that the *RGL1*, *RGL3* and *GAI* transcription levels were reduced 1.5 to 2 fold whereas the RGL2 transcript level was two-fold elevated in *swi3c* leaves (Fig S3). Altered transcription of *GID1* genes in the absence of GA and a lack of their GA-mediated feedback inhibition in *swi3c* leaves thus suggested that SWI3C-contaning SWI/SNF chromatin remodeling complexes are required for proper transcriptional regulation of the GA receptors. As the *GID1a* and *GID1b* genes are considered to be direct targets of the DELLA repressors, we also tested several known DELLA target genes encoding SCL3, a member of the GRAS family of putative transcriptional regulators, the MYB nuclear transcription factor, XERICO E3 ubiquitin ligase, IQD22 protein of the IQD (IQ domain) family of calmodulin (CaM) binding proteins, WRKY27 transcription factor, bHLH137 and bHLH154 basic helix-loop-helix (bHLH) DNA-binding superfamily proteins, and Exp-PT1, a protein predicted to be localized in the nucleus. (Zentella et al., 2007). The transcript

levels of these known DELLA target genes showed 1.5 to 4-fold reduction in the *swi3c* mutant (Fig. 4B). Taken together, de-regulation of *GID1* genes, alteration of expression of all DELLA genes, and altered transcriptional regulation of several known DELLA target genes observed in the *swi3c* mutant suggested that SWI/SNF CRC complexes play a role in the regulation of DELLA repressors, and thereby DELLA-dependent activation and GA-mediated feedback inhibition of transcription of GID1 GA-receptor (Griffiths et al., 2006) and other DELLA target genes (Zentella et al., 2007).

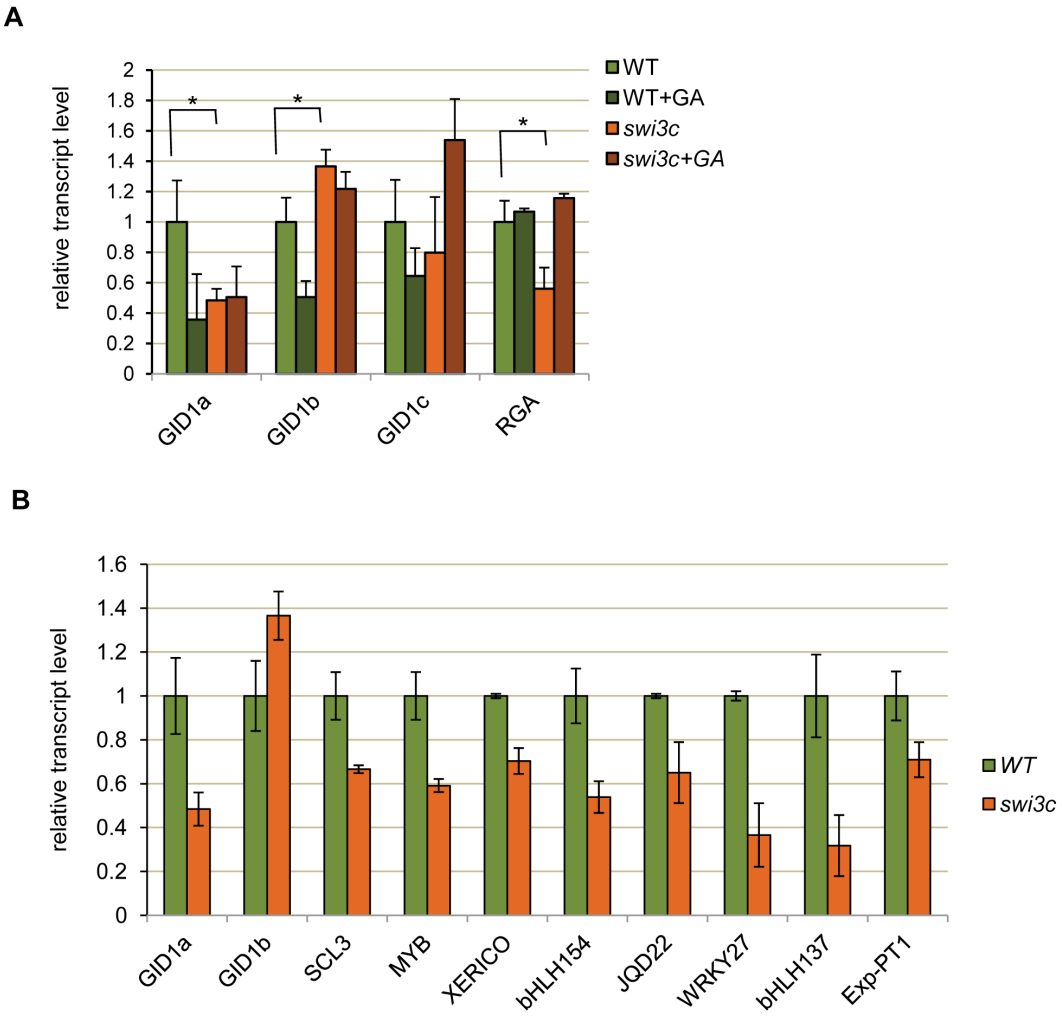

Figure 4. Altered transcriptional regulation of *GID1*, *RGA* and DELLA target genes in the *swi3c* mutant.

A, The *swi3c* mutation causes altered regulation of *GID* genes of GA-receptors, and the *RGA* gene coding for a DELLA protein. * p value <0.05. B, The direct target genes for DELLA repressor proteins show altered transcription in the *swi3c* mutant. * p value <0.05.

SWI3C physically interacts with DELLAs and the O-GlcNAc transferase SPINDLY in the nucleus

DELLA repressors of GA-responses do not bind directly to DNA, and are thus thought to regulate the expression of their target genes through interactions with transcription factors (as demonstrated for the PIF bHLH transcription factors promoting hypocotyl elongation (de Lucas et al., 2008, Feng et al., 2008) and/or chromatin modification complexes (Zentella et al., 2007; Sun 2010). The failure of proper DELLA-dependent activation and GA-mediated repression of *GID1* and *GA3ox* genes in the *swi3c* mutant raised the possibility that SWI3C-containing SWI/SNF complexes may somehow mediate the effects of DELLA repressor on these target genes, perhaps analogously to involvement of animal CRCs with nuclear receptors (Zraly et al., 2006). Since both DELLA and SWI3C (Sarnowski et al., 2005 and our unpublished results) proteins self-activate the reporter gene when fused to the GAL4 binding domain in yeast, we were unable to test the interaction using two-hybrid assay (YTH). Therefore, we used bimolecular fluorescence complementation (BiFC) assays (Hu et al., 2002), in which SWI3C fused to the N-terminal domain of split YFP (YFN-SWI3C) was transiently co-expressed with DELLA repressors in fusion with the C-terminal domain (YFC) of split YFP in epidermal cells of *Nicotiana benthamiana*. Similarly, we used an YFC-fusion of SPY to determine whether this O-GlcNAc transferase required for activation of DELLAs was recruited by SWI3C-containing SWI/SNF CRCs. Using high-resolution confocal microscopy, we detected reconstitution of YFP activity in epidermal cell nuclei revealing *in vivo* interaction of YFN-SWI3C with the YFC-fused DELLA repressors RGL2 and RGL3, and SPY (Fig. 5A). Subsequently, we performed control BiFC assays, in which YFC-fusions of RGL2, RGL3 and SPY were individually co-expressed with YFN-fusions of the red fluorescent proteins (YFN-RFP), whereas an YFC-RFP fusion was expressed simultaneously with YFN-SWI3C. The lack of YFP reconstitution in each case and detection of control RFP signal in both cytoplasm and nuclei confirmed the specificity of observed BiFC interactions of SWI3C with RGL2, RGL3 and SPY (Fig. S5A). The interaction of SWI3C with SPY was next confirmed by YTH (Fig. S5B). To test the robustness of observed protein interactions, we performed additional stringent *in vitro* protein-binding assays. SWI3C was fused to an N-terminal maltose-binding protein-6xHis tag (MBP-6xHis). Subsequently, equal amounts

of purified MBP-6xHis-SWI3C and control MBP-6xHis proteins were immobilized on Ni-NTA resin and used for pull-down assays with total protein extracts from plants expressing one of the 9MYC-tagged DELLA proteins RGA, RGL1 and RGL2, respectively. None of the 9MYC-tagged DELLAs, which were loaded at equal amounts onto the different matrices, were retained on the control MBP-6xHis protein (Fig. 5B and C) and Ni-NTA resins (Fig. 5D). By contrast, anti-c-Myc immunoblotting of proteins eluted from the MBP-6xHis-SWI3C matrix detected *in vitro* binding of all three DELLA proteins confirming specific interaction of RGA, RGL1 and RGL2 with SWI3C (Fig. 5D). Together with the *in vivo* BiFC assays, the results of these *in vitro* pull-down assays indicated that SPY and at least three DELLA proteins interact with core SWI3C subunit of SWI/SNF CRCs. While the observed protein-protein interactions did not resolve whether SPY and DELLAs bind to SWI/SNF together or separately (Fig. 5E), they provided a first mechanistic clue for the observed role of SWI3C in regulation of GA responses.

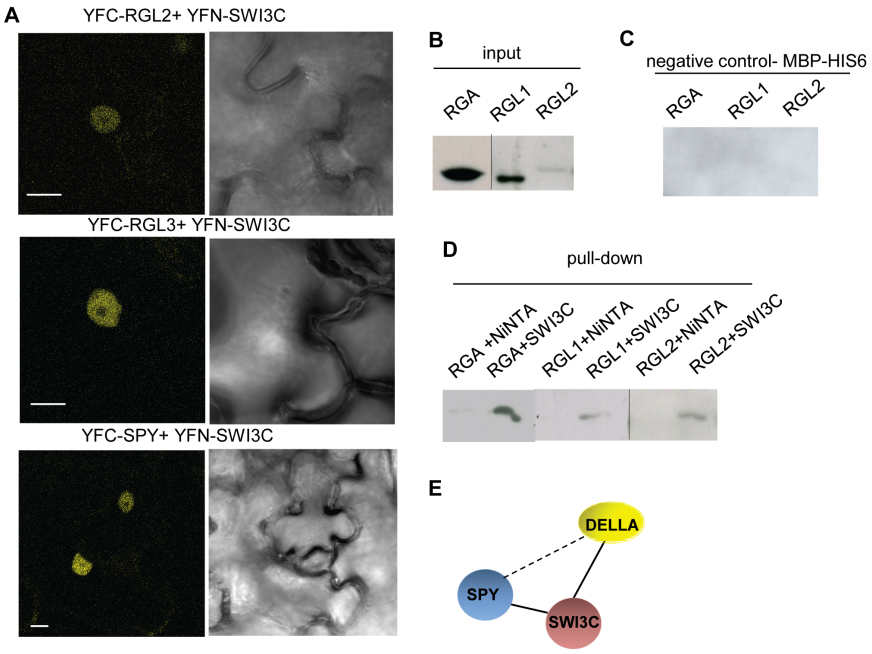

Figure 5. SWI3C interacts with the DELLA and SPY proteins.

A, BiFC analysis of *in vivo* interactions between SWI3C and RGL2, RGL3 and SPY. DIC – differential interference contrast image. Scale bar 10 µm. B, 9MYC-tagged RGA, RGL1 or RGL2 protein levels in the input plant total protein extracts used in pull-down assays. C, Control pull-down assays with NiNTA-bound MBP-His6 protein used as negative control. D, Pull-down assay with recombinant SWI3C protein with MBP-His6 tag and total protein extracts from plants overexpressing the 9Myc-tagged RGA, RGL1 and RGL2 DELLA proteins. NiNTA, protein fraction isolated from bacteria without induction of SWI3C-MBP-His6 construct was combined with DELLA protein extracts as additional negative control. E, Schematic visualization of DELLA, SPY and SWI3C interactions. Full lines indicate direct interactions, dashed line indicate functional relation between SPY and DELLA.

Genetic interactions between the *swi3c* and *spy-1* mutations

Inactivation of the SPY O-GlcNAc transferase in Arabidopsis dramatically reduces fertility. The *spy-1* mutant develops short siliques that produce very low amount of seed at normal temperature. However, seed production is restored to nearly normal when *spy-1* is grown at 18˚C (Jacobsen and Olszewski, 1993). Given that the *swi3c* mutant produces very few seeds when grown at normal 22°C day and 18°C night temperature, we tested whether analogously to *spy-1*, this defect could be due to temperature sensitivity of the *swi3c* mutant. Indeed, when grown at 14 or 16°C under (16h/8h day/night) diurnal cycle, the *swi3c* mutant produced approximately two-fold longer siliques containing a higher number of viable seeds (Fig. 6A, Fig. S4A). Surprisingly, this suggested that low temperature partially lifted the need for chromatin remodeling for some growth processes connected to seed production in *swi3c*. Since we found that SWI3C interacts with SPY, it was interesting to determine whether *spy-1* mutation alters the phenotypic traits of the *swi3c* mutant. In fact, *swi3c* and *spy-1* single mutants showed close phenotypic similarity, except that *swi3c* seedlings developed curling rosette and cauline leaves, and had frequent defects of carpel and stamen development in their flowers. Introduction of the *spy-1* mutation into *swi3c* background resulted in the development of *spy-1*-like non-curling leaves, but the *swi3c spy-1* double mutant displayed similar developmental defects of stamens and carpels, and even more retarded vegetative growth as the *swi3c* single mutant (Fig. 6B to E). In regard to the latter phenotypic traits, the effect of the *swi3c* mutation on leaf development appeared thus hypostatic to those of *spy-1*. However, compared to *swi3c*, the *spy-1* single mutant flowered earlier, with about half the number of leaves, whereas the *swi3c spy-1* double mutant flowered in comparison even earlier (Fig. 6F). Furthermore, the *spy-1* mutation synergistically shortened the lengths of flower organs and resulted in complete sterility in combination with *swi3c*. Thus, SPY turned out necessary for proper execution of flower and seed developmental programs, which were impaired in a temperature-dependent manner by the *swi3c* mutation.

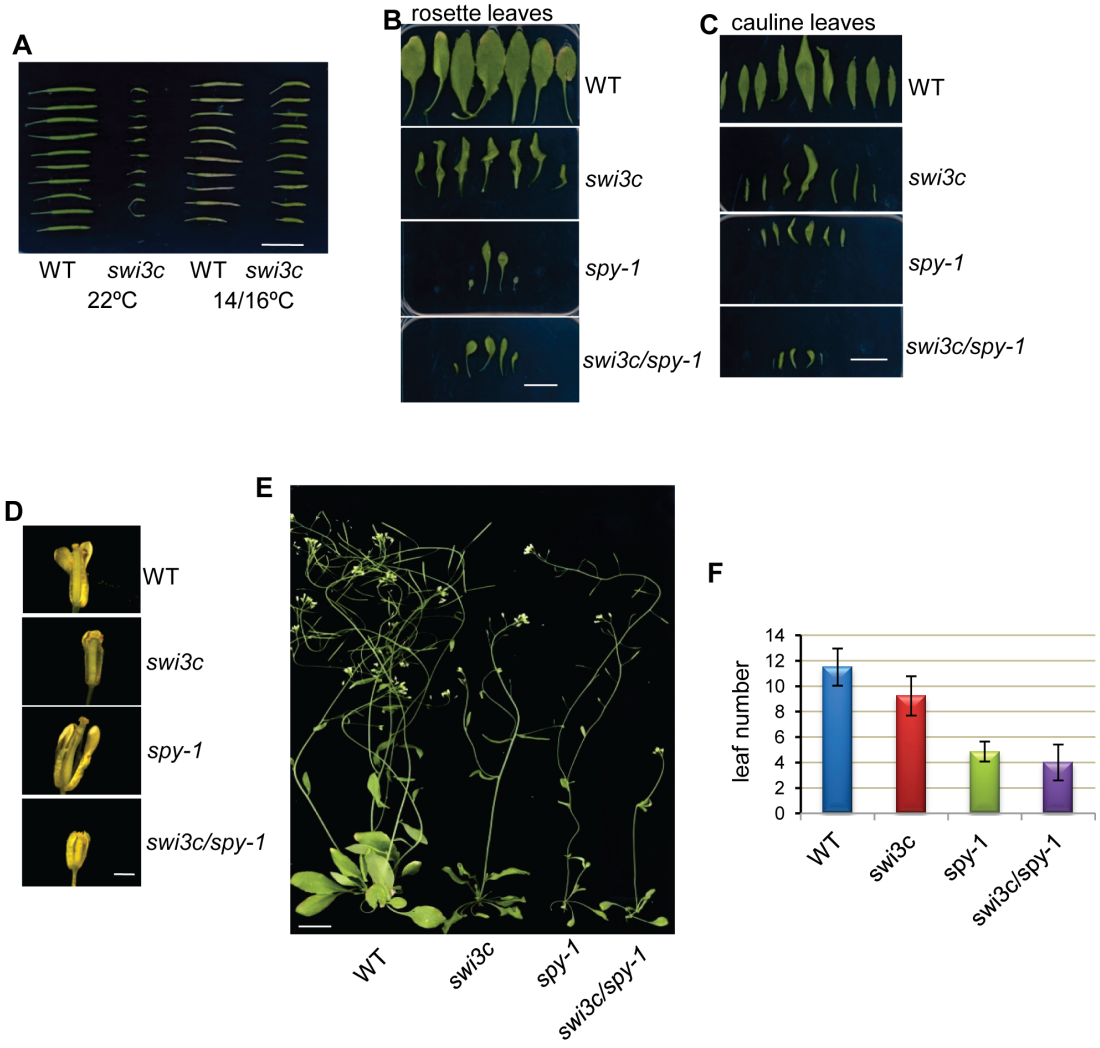

Figure 6. Genetic interaction between *swi3c* and *spy-1* mutations.

A, *swi3c* mutant has greatly reduced fertility when grown under optimal conditions, while the growth at lower temperature (16°C day/14°C night) stimulates the elongation of *swi3c* siliques and increases fertility, resembling the behavior of *spy-1* mutant. B, *swi3c spy-1* double mutant exhibits rosette leaf phenotype similar to *spy-1* with no twisting and curling characteristic for *swi3c*. C, Similarity of cauline leaf phenotype of *swi3c spy-1* double mutant and *spy-1*. D, The flowers of *swi3c spy-1* double mutant have similar developmental changes of carpels and stamens as *swi3c* single mutant, but the double mutant is sterile. E, Twenty eight day old *swi3c spy-1* plants have *spy-1*-like phenotype but show even more retarded vegetative growth and sterility. F, *swi3c spy-1* mutant flowers slightly earlier than *spy-1*. Numbers of leaves were compared at the time of flowering.

DISCUSSION

Studies in yeast and animals document that a major function of SWI/SNF complexes is the control of nucleosome dynamics at gene promoters and enhancers (Euskirchen et al., 2011). A particularly well studied role of SWI/SNF CRCs in animals is their interaction with nuclear receptors. The binding of steroid hormones by nuclear receptor enables their interactions with co-activators, one of which is the SWI/SNF complex. Consistently, genes regulated by steroid hormones are *in vivo* targets for regulation by SWI/SNF CRCs (Zraly et al., 2006; Belandia and Parker, 2003). As in mammals, SWI/SNF chromatin remodeling complexes in Arabidopsis are involved in transcriptional regulation of genes controlling important developmental and hormonal pathways. Our recent study revealed that BRM, a major SWI/SNF ATPase in Arabidopsis, is involved in regulation of GA signaling (Archacki et al., 2013). The *brm* mutation was also found to result in de-repressed expression of the *ABI5* gene that encodes a bZIP transcription factor regulating ABA sensitivity of germinating seeds (Han et al., 2012). Present characterization of phenotypic defects caused by inactivation of the core SWI3C subunit of SWI/SNF CRCs indicate complex alteration of several hormone regulatory pathways. Among these, the *swi3c* mutation simultaneously affects the ABA, ethylene, brassinosteroid and gibberellin signaling pathways by differentially modulating plant responses to these hormones (Fig.7). We reported previously that many, but not all, phenotypic traits of the *swi3c* mutant overlap with those of *brm* impaired in the function of the BRAHMA SNF2 ATPase subunit (Sarnowski et al., 2005; Archacki et al., 2009). Therefore, it is not surprising that both *brm* and *swi3c* mutations result in similar enhancement of ABA hormone sensitivity, which further supports our notion that these subunits act in the same SWI/SNF CRC. Collectively, the above data implicate the SWI/SNF complexes in crosstalk and integration of different hormonal pathways in Arabidopsis. To reveal possible molecular basis of such a role, we decided to concentrate on the characterization of SWI/SNF subunit mutants to gibberellins.

The *swi3c* mutant is characterized by a semi-dwarf growth habit and other traits, such as altered cell division patterns in embryos, seed coat epidermis and leaves, which resemble those of GA-deficient mutants. In the present study we demonstrate that many of the developmental defects observed in the *swi3c* mutant, in particular defective

elongation of hypocotyls and roots during seedling development, can be suppressed and restored to wild type by exogenous GA_{4+7}-treatment. Quantitative analysis of precursors, active forms and inactive derivatives of gibberellins revealed that, similarly as in *brm* mutant, the amount of bioactive GA_4 hormone is largely reduced in *swi3c* seedlings. Systematic qRT-PCR analysis of transcription of genes involved in GA biosynthesis and inactivation showed that the main reason for reduced biosynthesis of GA_4 is the down-regulation of transcription of *GA3ox2* and *3* (gibberellin 3-beta-dioxygenase 2 and 3) genes that control the production of bioactive gibberellins GA_4 and GA_1 in different organs (Curaba et al., 2004). In addition, exogenous GA-treatment enhanced the activation of *GA2ox1* and *GA2ox2* gibberellin 2-oxidase genes leading to a conversion of accumulating GA_{19} precursor towards inactive GAs, such as GA_{51} in the *swi3c* mutant.

The biosynthetic *GA3ox* and *GID1a* GA-receptor genes were reported to show DELLA-dependent activation and GA-dependent feedback inhibition (Griffiths et al., 2006). Because of GA-feedback regulation of *GID1* transcription, the defect of GA biosynthesis has also consequences for GA signaling *via* the receptors. In accordance, we found that *GID1a*, encoding the most abundant GA-receptor, displays reduced transcription and lack of apparent GA-inhibition in leaves of the *swi3c* mutant. Down-regulation of the *GID1a* in mutant leaves may be one of the reasons that *swi3c* leaves failed to respond to a similar extent as wild type to externally provided GA by typical leaf blade expansion (Fig. 1B).

The activity and stability of DELLA repressors is negatively controlled by GA-binding and activation of GID1 receptors, and subsequent formation of stable GID1-DELLA and GID1-DELLA-SLY protein complexes required for DELLA's inactivation and destruction, respectively (Murase et al., 2008; Hartweck, 2008). Although we observed that the *DELLA* genes, except *RGL2*, are also down-regulated in the *swi3c* mutant, altered transcriptional activation of the *GID1a*, *GA3ox* and several known DELLA target genes suggested that the SWI3C subunit might also be implicated in the control of DELLA's activity at the protein level. This prompted us to examine whether SWI3C could directly interact with and thus play a role in the binding of DELLAs to the SWI/SNF chromatin-remodeling complex. We also included in these studies the *SPY* gene encoding one of the two Arabidopsis O-GlcNAc transferases shown to act as potent negative regulator of

GA signaling (Jacobsen and Olszewski, 1993). While it has been suggested that at the molecular level SPY may act by N-acetyl-glucosamination of DELLAs leading to their activation or stabilization, this has not been demonstrated experimentally and the real targets of SPY in GA signaling are still largely unknown (Silverstone et al., 2007, see for review Schwechheimer and Willige, 2009). Since N-acetyl-glucosamination of serine and threonine residues is now recognized as a highly dynamic post-translational modification of numerous nuclear and cytoplasmic proteins acting in key signal transduction pathways (Slawson et al., 2006), we decided to examine potential interaction of SPY with SWI3C. Both *in vivo* BiFC and *in vitro* protein-protein interaction studies, as well as supplementing yeast two-hybrid tests in the case of SPY, indicated that SWI3C is indeed capable of *in vitro* and *in vivo* interactions and therefore may be responsible for forming complexes between SWI/SNF, the DELLA RGA, RGL1 and RGL2 proteins and SPY. While it is by no means clear whether DELLA, SPY and SWI/SNF occur in the same complex, the existence of these interactions suggest a potential role of chromatin structural modifications in functioning of both DELLA proteins and SPY.

The stabilization of DELLAs in *ga* mutants impaired in gibberellin biosynthesis (i.e., as their degradation is inhibited in the absence of GA-mediated activation of GID1 receptors) results in severe retardation of growth. In the *spy-1* mutant, the growth inhibitory activities of DELLAs are greatly decreased despite their remarkable stabilization, and therefore the *spy-1* mutation alleviates most inhibitory effects of DELLAs also in the GA biosynthesis mutants. Interestingly, the rice homolog of SPY has been shown to function in GA signaling not *via* changes in the amount or stability of rice DELLA protein SLR1, but probably through control of suppressive function of SLR1 (Shimada et al., 2006). The resemblance of *spy-1* and *swi3c* responses to low temperature may therefore suggest that the function of SPY, including possibly the control of DELLAs growth-suppressive function, can be linked to its role in active chromatin remodeling. It remains an intriguing question whether the association of DELLA proteins and SPY with SWI3C and their possible consequences for chromatin remodeling by the SWI/SNF complexes, could underlay SPY effect on DELLA activity. It will be therefore important to further explore whether some of the developmental defects observed in the *spy-1* mutant at normal temperature are due to inability to remodel nucleosomes at SWI/SNF-bound nuclear target loci.

The reduced activity of DELLAs could explain decreased transcription of the *GID1a* GA-receptor, other DELLA target genes and *GA3ox2/3* genes (i.e., implying altered regulation of their activators and repressors; see e.g. Richter et al., 2010), as well as defects in the perception and biosynthesis of active gibberellins in the *swi3c* mutant. Given that both the availability of bioactive GAs and transcriptional activation of the major GID1a receptor are simultaneously impaired, DELLAs could confer a pronounced growth inhibition in the *swi3c* mutant. External GA-treatment, decreasing DELLA levels by their GID1-mediated inactivation, alleviates the inhibition of hypocotyl and root elongation by the *swi3c* mutation. It remains to be determined whether the release of PIF basic helix-loop-helix (bHLH) transcription factors from their inactive DELLA-complexes plays a role in this process, and whether SWI/SNF CRCs also play a role in DELLA-dependent or independent recruitment, or phytochrome B-aided destabilization of PIFs (de Lucas et al., 2008). In contrast to GA-mediated suppression of hypocotyl and root elongation defects, the expansion of leaf blades is not restored and leaf curling is not abolished efficiently by GA-treatment of the *swi3c* plants. This may indicate that low GID1a availability in leaves is not sufficient for GA-induced complete inactivation of DELLAs in this organ, which might reflect a requirement for a functional SWI/SNF complex mediating interaction of GID1s with DELLAs in the chromatin context. It is therefore remarkable that the *spy-1* mutation diminishing the activation of DELLAs restores the curling leaf phenotype in the *swi3c* mutant background. This indicates either a SPY-dependent and DELLA-independent effect, or that independently of their simultaneous recruitment by SWI3C, SPY can still control the activation of DELLAs, possibly by interacting with one of the other three SWI3-type SWI/SNF subunits. This might also explain why *swi3c* mutation shows only partial hypostatic behavior in respect to *spy-1* mutation, and why the phenotype of the *swi3c* mutants is milder compared to those in mutants in other *swi3* subunits.

The results of this investigation are summarized and placed in the context of current knowledge about hormone cross-talk in Fig. 7. DELLA growth repressors are known to be under the influence of multiple signals including auxins, ABA, brassinosteroids and ethylene that arrive and modify at different levels the main GA pathway (Sun, 2010; Fu and Harberd, 2003; Han et al., 2012; Achard et al., 2009; Marocco et al., 2010). DELLAs negatively affect also the ABA, ethylene and brassinosteroids pathways, which

collectively defines them as important hubs in the integration of environmental and developmental signals. All the above mentioned hormonal pathways were shown here to require functional SWI/SNF for normal activity. The key new element of this work is the discovery that in addition to controlling the transcription of DELLA genes, the SWI3C subunit of SWI/SNF complex also directly interacts with DELLAs, which could explain possible involvement of SWI3C in controlling DELLA's activity at the protein level. The SWI/SNF chromatin remodelers appear to be uniquely positioned regarding the control of central regulatory hub of DELLAs and therefore emerge as likely important integrators of cross-talk between several hormone signalling networks.

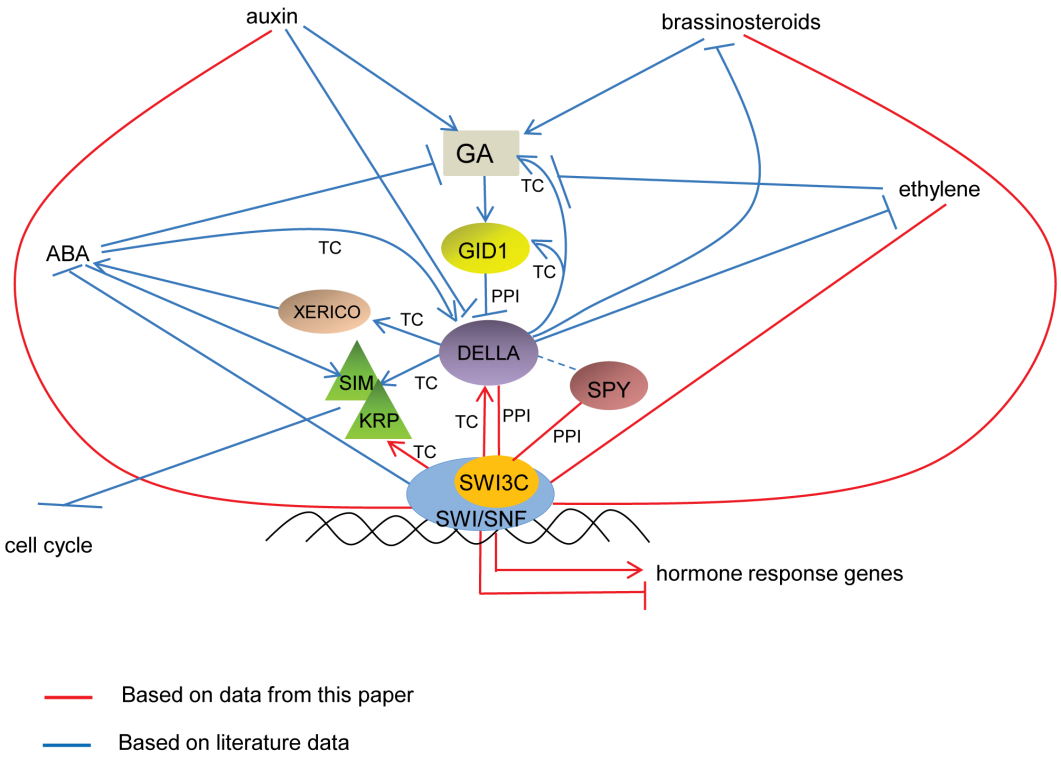

Figure 7. A hypothetical model of regulatory network centered at SWI/SNF and DELLAs, on the basis of published data and the results of this paper.

The SWI/SNF complex influences various hormonal pathways either by controlling a response to a hormonal treatment or by direct interaction with elements of hormonal pathways or their target genes modulating hormonal crosstalk in Arabidopsis. TC- transcriptional control; PPI- protein- protein interaction. Red lines correspond to data presented in this paper. Blue lines represent published data (Sun, 2010; Fu and Harberd, 2003; Han et al., 2012; Achard et al., 2009; Marocco et al., 2010).

MATERIALS AND METHODS

Plant lines and growth conditions

The *swi3c-1* mutant (referred further as *swi3c*) was characterized previously (Sarnowski et al., 2005). Lines overexpressing 9MYC-tagged RGA, GAI, RGL1, RGL2, RGL3 proteins respectively were kindly provided by Dr. Xing Wang Deng (Feng et al., 2008). A *swi3c spy-1* double mutant, was isolated by crossing homozygous *swi3c* plants with a *spy-1* line followed by PCR and phenotype-based screening of mutant alleles in the segregating F2 population. Primers used for genotyping are listed in Supplemental Table S2. Plants were grown under long-day (LD), short-day (SD) or darkness conditions (16h light/8h dark or 8h light/16h dark, respectively) at 18–23°C or using 8h/16h night/day conditions at 14-16°C, 70% humidity and 200 µM m^{-2} s^{-1} light intensity. Seedlings were cultivated in medium containing ½ Murashige and Skoog (MS) salts (Sigma-Aldrich), 0.5% (w/v) sucrose and 0.8% (w/v) agar (pH 5.8) supplemented with various concentrations of GA_{4+7}, paclobutrazol (PAC), ACC, ethephon, brassinolide or abscisic acid (ABA). In the case of ABA treatment, medium without sucrose was used. Wild-type and *swi3c* seeds were sown on ½ MS plates containing different concentrations of PAC, ABA or brassinolide and cultivated for 7 days in SD conditions. To test their ethylene response wild-type and *swi3c* plants were grown in the darkness for 7 days. After this period, seedlings were harvested and subjected for subsequent analyses. To analyze GA responses, plants were grown in soil and treated with 100 µM GA4+7 by spraying twice a week, or were grown on ½ MS medium supplemented with 0.05-10 µM GA4+7. The gravitropism assays were performed on vertically placed square Petri plates. Plants were grown for 6 days and plates were turned 90° CCW and grown for further 4 days.

Quantitative real-time PCR (qRT-PCR) analyses

Fourteen days old LD-grown wild-type and *swi3c* seedlings were sprayed with 100 µM GA4+7 or with water, as control. RNA was extracted from seedlings using the RNeasy plant mini kit (Qiagen), and DNA was removed by DNase-treatment using a TURBO DNA-free kit (Ambion). A first-strand cDNA synthesis kit (Roche) was used to prepare cDNA from 2.5 µg of RNA. Aliquots (3 µl) of 5-fold diluted cDNA samples were used as templates in 20 µl reactions containing SYBR Green Master mix (BioRad) and specific

primers for PCR amplification. The final primer concentrations were 0.5 µM, the annealing temperature was set at 56°C and extension was performed in 72°C. The RT-qPCR data recorded and were analyzed using iQ-PCR (BioRad) or FAST7500 (Applied Biosystems) equipment and software as recommended by the manufacturers. Transcripts of the *PP2A* and *UBQ5* genes were used as normalization controls. Each experiment was performed using at least two independent biological replicates, and the specificity of real-time PCR products was confirmed by melting curve analysis. Specific primers used in qPCR reactions are listed in Supplemental Table S2.

Construction of vectors used in bimolecular fluorescence complementation (BiFC)

To obtain YFN-SWI3C and YFC-DELLA (RGA, GAI, RGL1, 2 and 3) or SPY fusions for BiFC (Hu et al., 2002) analysis, the open reading frames of cDNAs encoding SWI3C, SPY and DELLA proteins were PCR amplified and cloned into the binary vectors pYFN43 or pYFC43 (Belda- Palazon et al., 2012), respectively, using the Gateway (Invitrogen) recombination approach. *In vivo* interactions between proteins were detected by BiFC using Leica TCS SP2 AOBS a laser-scanning confocal microscope (Leica Microsystems, Mannheim, Germany). Excitation of YFP was with the Argon laser line at 514 nm, of RFP with a 563 nm diode laser, detection of YFP fluorescence was at 518-555 nm and of RFP at 568 – 630 nm. The specificity of observed signals was confirmed by measuring the fluorescence emission wavelength (lambdascan). Tobacco epidermal cells were infiltrated Agrobacterium GV3101 (pMP90) strains carrying plasmids encoding SWI3C, DELLA or SPY fusions, and the p19 helper-vector (Voinnet et al., 2003), and analyzed by confocal microscopy 3 days later. YFN-RFP and YFC-RFP fusions were used to detect transformed cells in the BiFC assays. At least five nuclei were analyzed in each of three separate experiments.

Overexpression of ATSWI3C and pull-down of DELLA proteins

The coding region of *SWI3C* gene was cloned into the pDEST-MBP 6xHIS vector (Invitrogen) to express the fusion protein in bacteria. Native SWI3CMBP6xHis protein was purified according to protocol 14 (Qiaexpressionist, Qiagen). Nuclear extracts were prepared from 4-weeks old *Arabidopsis* plants overexpressing the 9MYC-tagged DELLA proteins RGA, RGL1, RGL2, RGL3 and GAI (Feng et al., 2008). 0.5 g plant tissue was

ground in liquid nitrogen and resuspended in IP-1 buffer (20 mM Hepes-KOH; pH 8.0, 0.15M KCl, 0.2% Triton, 10% glycerol, 0.1 mM PMSF, 5 mM β-mercaptoethanol, Complete EDTA - free), incubated for 15 minutes at 4°C, centrifuged for 15 minutes at 15000 x g to yield a supernatant used in further analyses. For pull-down assays, the SWI3C protein was bound to Ni-NTA agarose beads and incubated with total protein extracts of 9MYC-DELLA expressing plants in IP-1 buffer for 2 hours at 4°C. Beads were washed eight times with IP-1 buffer and boiled followed by SDS-PAGE (12%) separation and immunoblotting of proteins using a c-Myc primary (dilution 1: 1500, Covance) and an anti-mouse HRP secondary antibody (dilution 1:10000, Sigma).

Seeds embedding and tissue sectioning

Seeds were fixed with paraformaldehyde as described (Torti et al., 2012; Porri et al., 2012). To allow penetration of the fixative, the seeds were vacuum infiltrated, and the samples incubated on ice overnight. The following day, the fixative was replaced with a graded ethanol: water series at 4°C (85% ethanol, 4 h; 95% ethanol, 4 h; 100% ethanol, overnight; 100% ethanol, fresh). The samples were stored at 4°C in 100% ethanol until embedding. Paraffin embedding in Paraplast Plus (McCormick) was performed using an automated Leica ASP300 tissue processor (Leica Microsystems, Wetzlar, Germany). Wax blocks were stored at 4°C until sectioning with a rotary microtome (Leica, Wetzlar, Germany). The Paraplast was removed from the semithin sections with pure Histoclear before images were taken with a light microscope (Leica DMRB, Leica Microsystems, Wetzlar, Germany) and cell measurements and counting were carried out by using ImageJ software. For scanning electromicroscopic (SEM) analysis of the seed surface, seeds were mounted on stubs using double sided adhesive and conductive tabs, and sputter coated with platin before imaging with a Zeiss Supra 40VP SEM (Carl Zeiss NTS, Oberkochen, Germany).

Yeast two-hybrid protein interaction studies

Yeast two-hybrid assays performed with the plasmids pGBT9 and pGAD424, containing a full-length cDNA of the Arabidopsis SWI3C gene as described previously (Sarnowski et al., 2002). To obtain other pGBT9 and pGAD424 constructs, full-length cDNA of SPY was PCR amplified using primers with suitable restriction sites (Supplemental Table S2) and

cloned in the pCR-TOPO-TA vector (Invitrogen). After sequencing, the cDNAs were excised by restriction endonucleases and cloned into the vectors pGBT9 and pGAD424. Yeast strain Y190 was transformed with the pGBT9 and pGAD424 constructs encoding the protein pairs to be tested, and each construct in combination with either empty pGBT9 or pGAD424, as controls. The level of reporter β-galactosidase activity of each yeast strain was monitored using the replica filter lift method described in the Clontech Yeast Protocols handbook.

Measurement of endogenous phytohormones

For the analysis of the endogenous hormone level, the aerial parts of four weeks old wt, *swi3c*, and *ga1-3* plants were collected, flash frozen in liquid nitrogen and subjected to further analysis. Phytohormones were quantified using a 6410 Triple Quad LCMS (Agilent Technologies, Santa Clara, CA, USA) with an Agilent 1200 series rapid resolution liquid chromatography system fitted with a ZORBAX Eclipse XDB-C18 column (1.8 µm, 2.1 x 50 mm) as described (Plackett et al., 2012). Isotope labeled internal standard were obtained from Olchemin (Olomouc, Czech), Icon Isotopes (Summit, NJ, USA) and Sigma Aldrich (OAKVILLE, on, Canada) and Tokyo Kasei (Tokyo, Japan)(Seo et al., 2011).
Upon request, all novel materials described in this publication will be made available in a timely manner for non-commercial research purposes, subject to the requisite permission from any third-party owners of all or parts of the material. Obtaining any permissions will be the responsibility of the requestor.

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Supporting Information

Supporting information can be downloaded from the online version of this manuscript (Sarnowska *et al.,* 2013): http://www.plantphysiol.org

Supplemental Table 1. The level of GA-intermediates in the *swi3c* and *ga1-3* mutants. A, Samples collected at the end of the day. B, Samples collected at the end of the night.

Supplemental Table 2. Primers used in this work.

Figure S1. The *swi3c* mutant shows altered responses to exogenously applied hormones: ethylene, ABA and brassinosteroids and an altered gravitropic response. A, the *swi3c* mutant plants are hypersensitive to ABA treatment. B, The *swi3c* mutant exhibits enhanced response to both exogenous and endogenous ethylene demonstrated by shortened and thickened hypocotyl, and exaggerated apical hook. C, *swi3c* plants are hyposensitive to exogenous brassinosteroids, (D) *swi3c* plants demonstrate defective gravitropic response. Arrows indicate gravitropic vector (bar 1 cm).

Figure S2. The *swi3c* mutation confers GA-related growth and developmental defects suppressed by the GA_{4+7}-treatment and insensitivity to the GA biosynthesis inhibitor paclobutrazol (PAC).

Figure S3. The *swi3c* mutant exhibits altered expression of genes encoding DELLA represors.

Figure S4. Low temperature similarly affects fertility of *swi3c* and *spy-1*. GA biosynthesis is altered in *swi3c*. A, Silique length and number of seeds produced by wild-type and *swi3c* plants grown in normal conditions and at decreased temperature. B, The level of GA-intermediates in the *swi3c* and *ga1-3* mutants.

Figure S5. SWI3C interacts with the SPY and DELLA proteins. A, Negative controls for BiFC analysis of SWI3C-SPY and SWI3C-DELLA interactions. Scale bar 10 µm. B, Yeast two-hybrid assay indicating SWI3C-SPY interaction. Left: negative controls, right: replica lift assay YTH test.

Chapter 5: GENERAL DISCUSSION

Tissue specific effects of GA in plant development

The plant growth regulator GA controls several developmental processes including leaf expansion, stem elongation, germination as well as plant size and flowering. The phenotypic characterization of plants showing reduced GA levels or impaired GA signalling highlighted the importance of this hormone throughout the plant life cycle (Yamaguchi, 2008). However, little is known about the tissue specificity of GA action, and the particular cell types in which GA acts to control plant development are still unclear. Here we assess the effect of GA in regulating crucial aspects of plant development by overexpressing *GA2ox7* from the *KNAT1* and *SUC2* promoters, which drive expression of the transgene specifically in the SAM and in the CC, respectively. *GA2ox7* gene is a member of the *GA2ox* family, which includes another 7 genes in *Arabidopsis*, all encoding enzymes that reduce active GA or GA precursor levels (Schomburg et al., 2003; Rieu et al., 2008a). GA2ox7 enzymes act on C19-GA precursors, and in *Arabidopsis* the activity of several GA2oxs has been shown to be crucial for GA catabolism and turnover (Rieu et al., 2008a). Depletion of GA in the CC of the leaf had a significant effect on stem elongation. *SUC2:GA2ox7* plants showed a strong reduction in plant height compared to wild-type. The elongation of the stem is promoted by GA in a group of cells that belong to the rib meristem region, which is located at the very base of the SAM (Cowling et al., 1998; Achard et al., 2009). Thus, the effect of S*UC2:GA2ox7* on stem elongation should be mediated by a factor/s that moves from the CC to the SAM, to induce stem growth. GA is required to increase the expression of *FT* in the CC (Hisamatsu and King, 2008; Galvao et al., 2012), therefore one possibility is that the GA effect on stem elongation could be mediated by reduced *FT* expression.

However, *FT* overexpression in the CC in *SUC2:GA2ox7* background, completely suppressed the flowering time defect, but not the reduced plant height. This observation indicates that *FT* is unlikely to be the factor that enables GA in the CC to promote stem elongation. One option is that GA itself could move from the CC to the SAM. This possibility seems to be reasonable, because labelled active GA_4 applied in the

leaf could be detected at the apex of *Arabidopsis* (Eriksson et al., 2006). This result together with our experiments suggests that GA might translocate from the CC to the apex where it contributes to elongate the stem by acting in the rib meristem region. The effect of *SUC2:GA2ox7* could also be observed with regard to leaf expansion and chlorophyll concentration. GA regulates chlorophyll content through the downstream acting genes *GNL* and *GNC*, which encode GATA-type transcription factors that positively regulate protochlorophyllide oxidoreductases (PORs), thus promoting chlorophyll biosynthesis (Richter et al., 2010). In contrast with these data we could not detect decreased levels of *GNC* and *GNL* mRNAs in dissected leaves of *SUC2:GA2ox7* compared to wild-type. One possible explanation is that in wild-type plants *GNL* and *GNC* are expressed throughout the leaf, so that a reduction of these mRNAs only in the CC may be undetectable by q-PCR. In addition, GA might move from the CC to the surrounded mesophyll cells where it could control chlorophyll biosynthesis through genes other than *GNC* and *GNL*.

The effect of *SUC2:GA2ox7* in leaf expansion may be mediated by PIF transcription factors, which are activated by GA through releasing them from interaction with DELLA proteins (de Lucas et al., 2008; Feng et al., 2008). PIF proteins have been shown to control several developmental traits including cell elongation and seed germination (Feng et al., 2008; Leivar and Quail, 2011). We could not detect changes in *PIF4* or *PIF5* mRNA abundance in *SUC2:GA2ox7* compared to wild-type plants (data not shown), but further genetic and protein analysis would be necessary to test whether more PIF protein was sequestered in complexes with DELLAs in these plants.

The effect of GA in the SAM on stem elongation and chlorophyll content was also assessed. *KNAT1:GA2ox7* plants showed a strong impairment in stem growth, which also resulted in no internode formation, suggesting that GA in the rib meristem is essential to promote plant height. In this sense, *KNAT1:GA2ox7* may deplete not only GA synthesized *in situ*, but also that transported from the leaf to the meristem, which could partly explain the stronger effect observed in *KNAT1:GA2ox7* compared to *SUC2:GA2ox7* plants. In wild-type plants *GA2ox7* and *GA2ox8* expression was detected in the SAM of *Arabidopsis*, rice and maize. (Sakamoto et al., 2001; Bolduc and Hake, 2009), and ectopic expression of *GA2ox6* in rice recreated semi-dwarf phenotypes (Huang et al., 2010). Conversely, exogenous application of active GA_3 and GA_4 in wild-tiype *Oryza sativa* could

promote shoot growth and leaf sheath length (Huang et al., 2010). Our results along with these published data corroborate the importance of GA in promoting stem elongation at the SAM and suggest that GA2ox enzymes may play important roles in defining growth under natural conditions by controlling GA levels throughout development.

Reduced levels of chlorophyll were not detected in the leaf of *KNAT1:GA2ox7* in agreement with the *KNAT1* promoter being active specifically in the SAM. The phenotypes of *KNAT1:GA2ox7* and *SUC2:GA2ox7* plants are similar to those observed in *ga20ox1 ga20ox2*, a mutant containing low GA levels (Rieu et al., 2008b).

Overall our genetic approaches defined the tissue requirement for GA to control several developmental phenotypes.

GA effect on the transcriptional activation of *FT* in the companion cell of the leaf

Misexpression of *GA2ox7* in the CC caused a significant delay in flowering under LDs but not SDs. This observation suggests that GA might act to regulate factor/s with predominant roles during the LD photoperiodic flowering response. In agreement with this hypothesis, the mRNA levels of the photoperiodic gene *FT* was decreased in *SUC2:GA2ox7* plants, and it was negatively correlated with *GA2ox7* mRNA transcript abundance. Therefore, GA is required for the activation of transcription of *FT* in the CC in a dosage dependent manner. Previously, a GA dependent effect on *FT* transcription was detected by other means (Hisamatsu and King, 2008). These authors showed that *ga1-3* mutants growing under enriched far-red conditions contained lower levels of *FT* mRNA, demonstrating a significant role for GA upstream of an important component of the photoperiod pathway. Here we identified the tissue in which GA is required to induce expression of *FT*, which complements the findings of Hisamatsu and King. In addition, using marker gene fusions we demonstrated that GA controls *FT* expression through responsive elements contained in the *FT* promoter. In agreement with our results, inactivation of GA signalling caused phenotypes similar to those observed in *SUC2:GA2ox7* transgenic plants, including low *FT* mRNA levels (Galvao et al., 2012). Moreover, GA signalling acts to increase *FT* mRNA levels through the promoter

sequences, and exogenous application of active GA_3 resulted in increased GUS signal in *pFT:GUS* plants (Galvao et al., 2012) . The mechanism by which GA regulates *FT* transcript abundance is unlikely to be mediated by the *FT* transcriptional activators *CO* and *GI*, whose expression was unaffected in *SUC2:GA2ox7* plants. Therefore GA and GA signalling may act in parallel to the photoperiod pathway to ensure high levels of *FT* under LDs. On the other hand, mutations in both the *GI* and *CO* genes, lead to strong downregulation of *FT* mRNAs, which are almost undetectable in *gi* and *co* mutant background (Suarez-Lopez et al., 2001; Corbesier et al., 2007) . Therefore although GA seems to act in parallel with *GI* and *CO*, the loss of function of these two genes is sufficient to mask the GA contribution to *FT* expression. One possibility is that GA is a facilitator of *FT* activation that exerts its function only in the presence of the activator *CO* and *GI* genes. In this sense we believe that the function of GA is more a permissive role that enhances *FT* transcription only when *GI* and *CO* are functional. The *FT* regulation exerted by GA could be mediated by other important regulators including AP2-like transcription factors such as SMZ, which works as a repressor of *FT* transcription (Mathieu et al., 2009). In transgenic plants, in which GA signalling is impaired, the levels of *SMZ* mRNA are increased in abundance (Yu et al., 2012). Expression of *SMZ* and other *AP2-like* genes is controlled by SPL transcription factors which lead to the activation of transcription of the precursor of miR172, a non-coding RNA that targets AP2-like transcription factor transcripts to prevent their translation (Schmid et al., 2003). Yu et al (2012) showed that RGA binds *in vivo* several SPL proteins, demonstrating that the GA effect on *FT* transcription may be mediated by the *SPL*-miR172 module. SMZ binds the *FT* locus 1.5 Kb downstream of the coding sequence (Mathieu et al., 2009), in contrast to our interpretation and those of Galvao et al (2012) that sequences located in the *FT* promoter are important for GA function. Thus our experiment identified an *SPL* independent effect of GA in the regulation of *FT*, indicating that different mechanisms act cooperatively to modulate *FT* mRNA levels in response to GA (Fig 1). The GA effect on *FT* might be mediated by PIF4, which was recently shown to bind directly to the *FT* promoter under high temperature SD conditions (Kumar et al., 2012). In contrast, we could not detect a significant role of PIF4 on *FT* transcription under normal temperature LDs (data not shown), which might be due to the high degree of redundancy between PIF proteins.

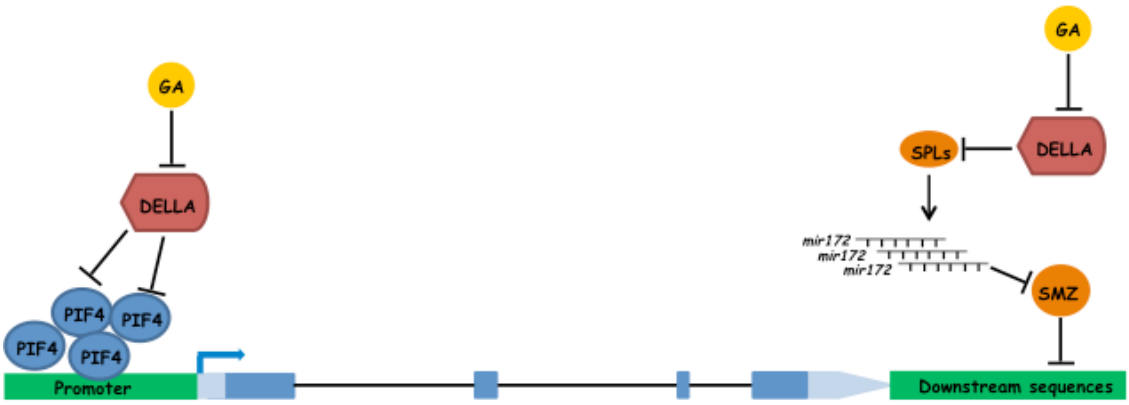

Figure 1. Distinct mechanisms of *FT* regulation by GA.

Under high temperature (28°), PIF4 proteins bind *FT* promoter leading to transcriptional activation. This function of PIF4 in promoting *FT* is not detected under LDs at lower temperature, maybe due to the high redundancy between PIF proteins. The effect of GA on *FT* might also be mediated by the SPL-miR172 module. In the presence of GA the SPLs are released from the DELLA repressors, and cause activation of transcription of miR172 precursor, which consequently reduces *SMZ* and other *AP2-like* mRNAs. Reduced levels of *AP2-like* transcripts would lead to increased activation of *FT*.

GA role in flowering at the shoot apical meristem and the link with *SVP*

The effect of GA in promoting flowering at the SAM was intensively characterised using *KNAT1:GA2ox7,* which decreases GA content specifically in the meristem. Under LDs depletion of GA did not affect *SOC1* expression, in contrast to what was previously reported under SDs (Moon et al., 2003; Achard et al., 2004). Under SDs GA also activates the expression of the floral identity gene *LFY* (Eriksson et al., 2006), perhaps indirectly through SOC1. Genetic and molecular experiments corroborate the importance of GA in promoting flowering through *SOC1* and *LFY*. For example overexpression of *SOC1* in a *ga1-3* mutant background accelerated flowering under SDs supporting the function of

SOC1 downstream of GA (Moon et al., 2003). Blazquez et al (1998) introduced a *LFY:GUS* reporter gene in the GA constitutive response mutant *spy-5* and demonstrated that *LFY* mRNA abundance increases in response to functional GA signalling. Transcriptional activation of *LFY* is also dependent on *SOC1* function, indicating that GA activates *LFY* upstream and in parallel to *SOC1.* Here we report that under LDs GA is not required to activate transcription of *SOC1*, but for later steps in floral induction, including the transcriptional activation of *SPL* genes.

The expression dynamics of *SOC1* were unaffected when *KNAT1:GA2ox7* plants were shifted from SDs to inductive LDs. However, the *KNAT1:GA2ox7* transgenic plants showed a significant delay in flowering under LDs compared to wild-type plants. This result suggests that GA acts mainly through a *SOC1* independent pathway to regulate flowering under LDs. The late flowering of *KNAT1:GA2ox7* plants was associated with delayed expression of *SPL3, SPL4, SPL5* and *SPL9* at the SAM after shifting to LDs.

Under inductive LDs the photoperiod pathway acts through *FT* and *TSF* to promote expression of *SOC1* at the meristem, presumably by directly interacting with FD. In agreement with these data wild-type plants transferred to LDs showed *SOC1* expression 1 LD after transfer, whereas no *SOC1* mRNA could be detected at the SAM of similarly treated *ft tsf* double mutants (Torti et al., 2012). These results together with our experiments suggest that GA is likely to act downstream or in parallel to the photoperiodic genes *FT* and *TSF*, between *SOC1* and *SPL* activity.

Levels of active GA such as GA_4, increase at the SAM of *Arabidopsis* during flowering under SDs, and *ga1-3* mutants strongly delay flowering under these conditions, indicating an important regulatory function of this hormone during this phase transition (Eriksson et al., 2006). However, expression of genes encoding GA biosynthetic enzymes was mostly unchanged in the meristem, indicating that GA synthesised in tissues other than the SAM, could migrate to the apex or that genes encoding catabolic enzymes could be reduced in expression. Our results indicate that under LDs the photoperiod pathway through *FT*, *TSF* and the downstream target *SOC1*, contribute to increasing GA content by repressing the expression of the floral repressor *SVP*. First of all, we identified a novel function of *SVP* in repressing *GA20ox2*, which encodes an enzyme involved in GA_4 biosynthesis. Mutation in *SVP* resulted in higher levels of *GA20ox2* mRNAs and GA_4, and, conversely, *SVP* gain of function created a GA deficient

environment with lower GA_4 levels. SVP is a repressor of *FT* and *TSF* in the leaf, and of *SOC1* in the meristem (Lee et al., 2007). Our experiment showed that once the photoperiod pathway is activated in response to LDs, *FT* and *TSF* activate *SOC1,* which in turn repressed *SVP* expression leading to the upregulation of *GA20ox2* mRNA. SOC1 directly binds *SVP* (Immink et al., 2012), providing a direct mechanism by which the photoperiod pathway represses *SVP* expression and increases GA content at the SAM. The upregulation of *GA20ox2* is likely to occur at the base of the shoot apex in the rib meristem, a group of cells were FT proteins seem to accumulate after their movement through the phloem (Liu et al., 2012).

The increase in active GA_4 in the meristem ultimately leads to flowering through *SPL* genes (Galvao et al., 2012). Recently, the DELLA protein RGA was shown to bind *in vivo* several members of the SPL family, including SPL9 and SPL4 (Yu et al., 2012). Therefore GA is produced in the meristem in response to LDs, and ensures the activation at both the transcriptional and post-transcriptional levels of the *SPL* floral integrators.

Our current model suggests that under SDs in the absence of *FT* and *TSF*, the floral repressor *SVP* acts at the SAM to prevent GA biosynthesis and flowering by repressing transcription of *GA20ox2*. In contrast, under LDs the activation of *SOC1* by FT TSF provides a mechanism that ensures direct downregulation of *SVP* and derepression of *GA20ox2*. This novel mechanism could play a relevant function in Nature (Fig.2), when plants are exposed to long photoperiods characteristic of spring-summer seasons, and ensures high GA levels required to induce flowering at the appropriate time of the year.

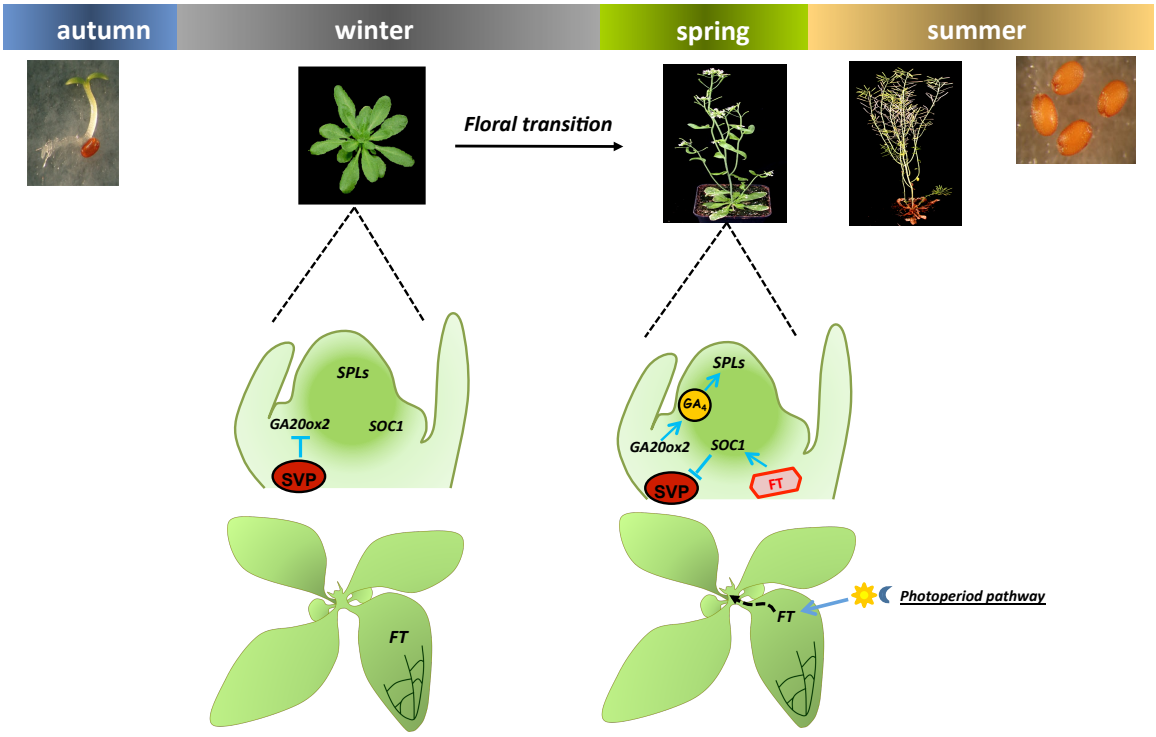

Figure 2. Role of GA in the life cycle of winter annual *Arabidopsis thaliana.*

After germination, *Arabidopsis* seeds give rise to seedlings that stay vegetative during the winter. SVP represses expression of *GA20ox2* during non-inductive short days of winter. In spring-summer conditions the increasing number of sunlight hours leads to the activation of transcription of *FT* and *TSF*, whose proteins then move to the meristem and activate *SOC1*. The floral repressor *SVP* is directly repressed by SOC1 resulting in increased *GA20ox2* mRNA and GA_4 levels. GAs then promote flowering by activating *SPL* genes at both the transcriptional and post-transcriptional levels. (Plant pictures, courtesy of Maria Albani and Fernando Andrés)

Link between GA and chromatin remodelling complexes

Active GA contributes to many development traits. The GA contribution to hypocotyl growth is mediated by the bHLH transcription factors PIF4 and PIF3, which integrate light and hormone signals in *Arabidopsis* (de Lucas et al., 2008; Feng et al., 2008). In the presence of GA PIFs are released from the DELLA repressors, and directly activate genes that promote growth (Feng et al., 2008). In addition, GA controls chlorophyll content and leaf expansion through *GNC* and *GNL*, downstream of the DELLAs-PIFs module (Richter et al., 2010).

ATP-dependent chromatin remodelling complexes (CRCs) are involved in the regulation of transcription, cell cycle and DNA replication (Clapier and Cairns, 2009). Our results indicate that the core SWI3C subunit of SWI/SNF CRCs is required for the proper regulation of GA biosynthesis, and GA signalling to control many aspects of plant development, including leaf expansion, hypocotyl growth and plant size. Mutants of the *SWI3C* gene displayed some phenotypic traits similar to those observed in *ga1-3* mutant plants, suggesting an interaction between *SWI3C* and GA. Furthermore GA treatments corrected some of the developmental defects of *swi3c* mutants (e.g. root structure), indicating that *swi3c* does reduce GA levels. Direct measurements demonstrated that levels of active GA_4 were indeed lower in *swi3c* plants compared to wild-type, supporting the idea that reduced GA may be the basis of some of the *swi3c* mutant phenotypes.

The transcript abundance of *GA3ox2* was significantly reduced in *swi3c* mutants, consistent with the decreased levels of active GA detected in those plants. In addition *swi3c* showed lower *GIDa* expression in leaves, which may explain the insensitivity of the curly leaf phenotype to exogenous GA treatment. SWI3C protein also interacts *in vivo* with the some of the DELLA repressors RGA, RGL1, RGL2, RGL3 and O-GlcNAc transferase SPY. Support for the significance of such protein interactions came from genetic interactions, which demonstrated that the phenotypes of *swi3c* could be substantially suppressed by introducing the *spy* mutation. These data provide genetic evidence for the interaction between *SWI3C* and GA signalling pathways. Previously, mutation of *BRM*, which encodes a key subunit of CRCs, was described to have effects on plant development similar to the *swi3c* mutation (Archacki et al., 2009). In addition, *brm* also influences the GA signalling pathway in Arabidopsis (Archacki et al., 2013). This indicates that both *BRM* and *SWI3C* control development by modulating GA levels and perception. Observed interactions of SWI/SNF complexes with components of the GA signalling pathway are in agreement with data available for mammals in which SWI/SNF complexes bind hormone receptors (DiRenzo et al., 2000; John et al., 2008). These results are in agreement with our idea that CRCs influence GA signalling by interacting directly with signalling components. Overall, our studies provide new information on the function of CRCs, and demonstrate a tight correlation between CRCs and the GA pathway in the context of plant development.

FUTURE CONSIDERATIONS

This research project demonstrates the importance of the plant growth regulator gibberellin in the promotion of flowering at the tissue-specific level, and shows how its biosynthesis is modulated in response to the external cue of day length. Under LDs the floral integrator *SOC1* increases gibberellin content at the shoot apex downstream of *FT* and *TSF*. The levels of *GA20ox2* mRNA rise in a specific area of the shoot apical meristem (rib meristem), where gibberellins have important functions in promoting stem elongation. Our results suggest that the increase in gibberellin in the rib meristem region is also crucial for flowering through the transcriptional activation of several *SPL* genes including *SPL3*, *SPL4*, *SPL5*, and *SPL9*. The expression pattern of *GA20ox2* showed a clear overlap with those of *SPL3*, *SPL4* and *SPL5*, whose mRNAs are also detectable in the rib area. Since GA20ox2 produces a precursor of active gibberellin, it might be expected that GA3ox enzymes, which convert the precursor into active forms, also act in the rib meristem region to ensure sufficient levels of the hormone required to promote *SPL3*, *SPL4* and *SPL5* transcription.

On the other hand, *SPL9* that is regulated by gibberellin at both the transcriptional and protein levels (Yu et al., 2012), is mostly expressed on the flanks of the shoot apical meristem. These results imply that precursors of gibberellin produced in the rib meristem by GA20ox2 could spread through the meristem and be converted to active forms by GA3ox at the site where *SPL9* transcription is activated. Alternately gibberellin might migrate from the rib meristem to the flanks, after being converted to the active molecule by GA3ox in the rib meristem. Therefore a deeper cell biological approach will be necessary to fully understand how the biosynthesis of gibberellin is linked to the spatially controlled expression dynamics of *SPL* genes.

Transgenic plants expressing different *GA20ox2* and *GA3ox* genes fused to the *GUS* reporter sequence will clarify whether the rib meristem is the primary site for active gibberellin biosynthesis and action. In addition, the use of *pDELLA:DELLA:GFP* plants will be useful to assess in which part of the shoot apical meristem gibberellin signaling is initiated to control gene expression. Therefore, the future perspective of this project includes the study of gibberellin biosynthesis and signaling at the cellular level in the shoot apex in the context of flowering.

Another important issue that will be crucial in the study of gibberellin function is whether long-distance movement of the hormone occurs in plants. Several experiments suggest that gibberellin moves between plant tissues. For instance, exogenous GA_4 applied to the leaf can be detected at the apex of *Arabidopsis* (Eriksson et al., 2006). Although these results provide a first suggestion for gibberellin movement, they do not reveal whether this process occurs under wild-type conditions at endogenous expression levels of GA, nor does it show how much GA movement contributes to flowering and development. On the other hand, the dwarf phenotype observed in the *SUC2:GA2ox7* plants described here, suggests that the hormone might move through the phloem system to the shoot apex to contribute to stem elongation. Whether this is also significant to activate flowering is not yet clear. Gibberellins are required in the companion cell to trigger expression of *FT* but an additional contribution requiring movement to activate flowering at the apex cannot be excluded. Nevertheless, *SUC2:gai-D* transgenic plants, in which gibberellin signaling is impaired but in which GA levels are not reduced, also showed reduced levels of *FT* transcript and late flowering. However, in contrast to *SUC2:GA2ox7*, the height of *SUC2:gai-D* plants is largely unaffected (data not shown), and this might be due to reduced movement of GA from the companion cells. Moreover, our preliminary results indicate that the double transgenic *SUC2:GA2ox7 SUC2:gai-D* has an additive effect in delaying flowering compared to either single transgenic, but not on the downregulation of *FT* mRNA. These data also suggest that gibberellin has a non-cell autonomous function in the companion cell of the leaf to activate flowering, which cannot depend entirely on the signaling pathway in the companion cells. The above experiments lead to the conclusion that gibberellin might move from the companion cells to the shoot apex to promote stem elongation and flowering.

Understanding whether the hormone is able to travel from the leaf to the apex through the phloem system, and whether it can move locally from one cell to another in the meristem region will be a major challenge for the future of this research project.

REFERENCES (Chapter 1)

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G., and Turck, F. (2010). cis-Regulatory Elements and Chromatin State Coordinately Control Temporal and Spatial Expression of FLOWERING LOCUS T in Arabidopsis. Plant Cell 22, 1425-1440.

An, H., Roussot, C., Suarez-Lopez, P., Corbesier, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., and Coupland, G. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131, 3615-3626.

Andres, F., and Coupland, G. (2012). The genetic basis of flowering responses to seasonal cues. Nature Reviews Genetics 13, 627-639.

Bastow, R., Mylne, J.S., Lister, C., Lippman, Z., Martienssen, R.A., and Dean, C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. Nature 427, 164-167.

Bergonzi, S., Albani, M.C., Ver Loren van Themaat, E., Nordstrom, K.J., Wang, R., Schneeberger, K., Moerland, P.D., and Coupland, G. (2013). Mechanisms of age-dependent response to winter temperature in perennial flowering of Arabis alpina. Science 340, 1094-1097.

Birkenbihl, R.P., Jach, G., Saedler, H., and Huijser, P. (2005). Functional dissection of the plant-specific SBP-domain: overlap of the DNA-binding and nuclear localization domains. Journal of molecular biology 352, 585-596.

Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K., and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in Arabidopsis. Plant Journal 24, 591-599.

Cardon, G., Hohmann, S., Klein, J., Nettesheim, K., Saedler, H., and Huijser, P. (1999). Molecular characterisation of the Arabidopsis SBP-box genes. Gene 237, 91-104.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., and Coupland, G. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030-1033.

de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blazquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480-U411.

de Montaigu, A., Toth, R., and Coupland, G. (2010). Plant development goes like clockwork. Trends in genetics : TIG 26, 296-306.

Eriksson, S., Bohlenius, H., Moritz, T., and Nilsson, O. (2006). GA(4) is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell 18, 2172-2181.

Feng, S.H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J.L., Wang, F., Chen, L.Y., Yu, L., Iglesias-Pedraz, J.M., Kircher, S., Schafer, E., Fu, X.D., Fan, L.M., and Deng, X.W. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 451, 475-U479.

Fornara, F., Panigrahi, K.C., Gissot, L., Sauerbrunn, N., Ruhl, M., Jarillo, J.A., and Coupland, G. (2009). Arabidopsis DOF transcription factors act redundantly to reduce CONSTANS expression and are essential for a photoperiodic flowering response. Dev Cell 17, 75-86.

Gandikota, M., Birkenbihl, R.P., Hohmann, S., Cardon, G.H., Saedler, H., and Huijser, P. (2007). The miRNA156/157 recognition element in the 3' UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. Plant J 49, 683-693.

Gendall, A.R., Levy, Y.Y., Wilson, A., and Dean, C. (2001). The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in Arabidopsis. Cell 107, 525-535.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z.L., Powers, S.J., Gong, F., Phillips, A.L., Hedden, P., Sun, T.P., and Thomas, S.G. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell 18, 3399-3414.

Helliwell, C.A., Sullivan, J.A., Mould, R.M., Gray, J.C., Peacock, W.J., and Dennis, E.S. (2001). A plastid envelope location of Arabidopsis ent-kaurene oxidase links the plastid and endoplasmic reticulum steps of the gibberellin biosynthesis pathway. Plant Journal 28, 201-208.

Heo, J.B., and Sung, S. (2011). Vernalization-mediated epigenetic silencing by a long intronic noncoding RNA. Science 331, 76-79.

Itoh, H., Tanaka-Ueguchi, M., Kawaide, H., Chen, X.B., Kamiya, Y., and Matsuoka, M. (1999). The gene encoding tobacco gibberellin 3 beta-hydroxylase is expressed at the

site of GA action during stem elongation and flower organ development. Plant Journal 20, 15-24.

Jaeger, K.E., and Wigge, P.A. (2007). FT protein acts as a long-range signal in Arabidopsis. Current biology : CB 17, 1050-1054.

Jang, S., Torti, S., and Coupland, G. (2009). Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. Plant J 60, 614-625.

Jang, S., Marchal, V., Panigrahi, K.C.S., Wenkel, S., Soppe, W., Deng, X.W., Valverde, F., and Coupland, G. (2008). Arabidopsis COP1 shapes the temporal pattern of CO accumulation conferring a photoperiodic flowering response. Embo J 27, 1277-1288.

Jung, J.H., Ju, Y., Seo, P.J., Lee, J.H., and Park, C.M. (2011). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. Plant J.

Kaneko, M., Itoh, H., Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Ashikari, M., and Matsuoka, M. (2003). Where do gibberellin biosynthesis and gibberellin signaling occur in rice plants? Plant Journal 35, 104-115.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Activation tagging of the floral inducer FT. Science 286, 1962-1965.

Kobayashi, Y., and Weigel, D. (2007). Move on up, it's time for change--mobile signals controlling photoperiod-dependent flowering. Genes Dev 21, 2371-2384.

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

Koornneef, M., and Vanderveen, J.H. (1980). Induction and Analysis of Gibberellin Sensitive Mutants in Arabidopsis-Thaliana (L) Heynh. Theoretical and Applied Genetics 58, 257-263.

Laubinger, S., Marchal, V., Gentilhomme, J., Wenkel, S., Adrian, J., Jang, S., Kulajta, C., Braun, H., Coupland, G., and Hoecker, U. (2006). Arabidopsis SPA proteins regulate photoperiodic flowering and interact with the. oral inducer CONSTANS to regulate its stability (vol 133, pg 3213, 2006). Development 133, 4391-4391.

Lee, J., Oh, M., Park, H., and Lee, I. (2008). SOC1 translocated to the nucleus by interaction with AGL24 directly regulates leafy. Plant J 55, 832-843.

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev 21, 397-402.

Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y., and Yu, H. (2012). FTIP1 is an essential regulator required for florigen transport. PLoS biology 10, e1001313.

Mathieu, J., Warthmann, N., Kuttner, F., and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Current biology : CB 17, 1055-1060.

Michaels, S.D., and Amasino, R.M. (1999). FLOWERING LOCUS C encodes a novel MADS domain protein that acts as a repressor of flowering. Plant Cell 11, 949-956.

Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J 35, 613-623.

Murase, K., Hirano, Y., Sun, T.P., and Hakoshima, T. (2008). Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456, 459-463.

Rhoades, M.W., Reinhart, B.J., Lim, L.P., Burge, C.B., Bartel, B., and Bartel, D.P. (2002). Prediction of plant microRNA targets. Cell 110, 513-520.

Samach, A., and Coupland, G. (2000). Time measurement and the control of flowering in plants. BioEssays : news and reviews in molecular, cellular and developmental biology 22, 38-47.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science 288, 1613-1616.

Sawa, M., Nusinow, D.A., Kay, S.A., and Imaizumi, T. (2007). FKF1 and GIGANTEA complex formation is required for day-length measurement in Arabidopsis. Science 318, 261-265.

Searle, I., He, Y., Turck, F., Vincent, C., Fornara, F., Krober, S., Amasino, R.A., and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in *Arabidopsis* Genes & development 20.

Sheldon, C.C., Finnegan, E.J., Rouse, D.T., Tadege, M., Bagnall, D.J., Helliwell, C.A., Peacock, W.J., and Dennis, E.S. (2000). The control of flowering by vernalization. Current opinion in plant biology 3, 418-422.

Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H., Kato, H., and Matsuoka, M. (2008). Structural basis for gibberellin recognition by its receptor GID1. Nature 456, 520-523.

Silverstone, A.L., Chang, C.W., Krol, E., and Sun, T.P. (1997). Developmental regulation of the gibberellin biosynthetic gene GA1 in Arabidopsis thaliana. Plant Journal 12, 9-19.

Sung, S., Schmitz, R.J., and Amasino, R.M. (2006). A PHD finger protein involved in both the vernalization and photoperiod pathways in Arabidopsis. Genes & development 20, 3244-3248.

Swiezewski, S., Liu, F., Magusin, A., and Dean, C. (2009). Cold-induced silencing by long antisense transcripts of an Arabidopsis Polycomb target. Nature 462, 799-802.

Tamaki, S., Matsuo, S., Wong, H.L., Yokoi, S., and Shimamoto, K. (2007). Hd3a protein is a mobile flowering signal in rice. Science 316, 1033-1036.

Taoka, K., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwestri, Y.A., Tamaki, S., Ogaki, Y., Shimada, C., Nakagawa, A., Kojima, C., and Shimamoto, K. (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature 476, 332-335.

Thomas, S.G., Phillips, A.L., and Hedden, P. (1999). Molecular cloning and functional expression of gibberellin 2-oxidases, multifunctional enzymes involved in gibberellin deactivation. P Natl Acad Sci USA 96, 4698-4703.

Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. (2012). Analysis of the Arabidopsis Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering. The Plant Cell Online.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol 59, 573-594.

Usami, T., Horiguchi, G., Yano, S., and Tsukaya, H. (2009). The more and smaller cells mutants of Arabidopsis thaliana identify novel roles for SQUAMOSA PROMOTER BINDING PROTEIN-LIKE genes in the control of heteroblasty. Development 136, 955-964.

Valverde, F., Mouradov, A., Soppe, W., Ravenscroft, D., Samach, A., and Coupland, G. (2004). Photoreceptor regulation of CONSTANS protein and the mechanism of photoperiodic flowering. Science 303, 1003-1006.

Wang, J.W., Czech, B., and Weigel, D. (2009). miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. Cell 138, 738-749.

Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U., and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. Science 309, 1056-1059.

Wu, G., Park, M.Y., Conway, S.R., Wang, J.W., Weigel, D., and Poethig, R.S. (2009). The Sequential Action of miR156 and miR172 Regulates Developmental Timing in Arabidopsis. Cell 138, 750-759.

Yamaguchi, A., Wu, M.F., Yang, L., Wu, G., Poethig, R.S., and Wagner, D. (2009). The MicroRNA-Regulated SBP-Box Transcription Factor SPL3 Is a Direct Upstream Activator of LEAFY, FRUITFULL, and APETALA1. Developmental Cell 17, 268-278.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annual Review of Plant Biology 59, 225-251.

Yu, N., Cai, W.J., Wang, S., Shan, C.M., Wang, L.J., and Chen, X.Y. (2010). Temporal control of trichome distribution by microRNA156-targeted SPL genes in Arabidopsis thaliana. Plant Cell 22, 2322-2335.

REFERENCES (Chapter 2)

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

Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T.S., and Genschik, P. (2009). Gibberellin Signaling Controls Cell Proliferation Rate in Arabidopsis. Current Biology *19*, 1188-1193.

Achard, P., Herr, A., Baulcombe, D.C., and Harberd, N.P. (2004). Modulation of floral development by a gibberellin-regulated microRNA. Development *131*, 3357-3365.

Adrian, J., Farrona, S., Reimer, J.J., Albani, M.C., Coupland, G., and Turck, F. (2010). cis-Regulatory Elements and Chromatin State Coordinately Control Temporal and Spatial Expression of FLOWERING LOCUS T in Arabidopsis. Plant Cell *22*, 1425-1440.

An, H.L., Roussot, C., Suarez-Lopez, P., Corbesler, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C*., et al.* (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development *131*, 3615-3626.

Blazquez, M.A., Green, R., Nilsson, O., Sussman, M.R., and Weigel, D. (1998). Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell *10*, 791-800.

Blazquez, M.A., Soowal, L.N., Lee, I., and Weigel, D. (1997). LEAFY expression and flower initiation in Arabidopsis. Development *124*, 3835-3844.

Blazquez, M.A., and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. Nature *404*, 889-892.

Bolduc, N., and Hake, S. (2009). The Maize Transcription Factor KNOTTED1 Directly Regulates the Gibberellin Catabolism Gene ga2ox1. Plant Cell *21*, 1647-1658.

Borner, R., Kampmann, G., Chandler, J., Gleissner, R., Wisman, E., Apel, K., and Melzer, S. (2000). A MADS domain gene involved in the transition to flowering in Arabidopsis. Plant Journal *24*, 591-599.

Bowman, J.L., Alvarez, J., Weigel, D., Meyerowitz, E.M., and Smyth, D.R. (1993). Control of Flower Development in Arabidopsis-Thaliana by Apetala1 and Interacting Genes. Development *119*, 721-743.

Cardon, G., Hohmann, S., Klein, J., Nettesheim, K., Saedler, H., and Huijser, P. (1999). Molecular characterisation of the Arabidopsis SBP-box genes. Gene *237*, 91-104.

Cardon, G.H., Hohmann, S., Nettesheim, K., Saedler, H., and Huijser, P. (1997). Functional analysis of the Arabidopsis thaliana SBP-box gene SPL3: a novel gene involved in the floral transition. Plant Journal *12*, 367-377.

Castillejo, C., and Pelaz, S. (2008). The balance between CONSTANS and TEMPRANILLO activities determines FT expression to trigger flowering. Current Biology *18*, 1338-1343.

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

Cowling, R.J., and Harberd, N.P. (1999). Gibberellins control Arabidopsis hypocotyl growth via regulation of cellular elongation. J Exp Bot *50*, 1351-1357.

Daykin, A., Scott, I.M., Francis, D., and Causton, D.R. (1997). Effects of gibberellin on the cellular dynamics of dwarf pea internode development. Planta *203*, 526-535.

de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blazquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature *451*, 480-U411.

Dill, A., Thomas, S.G., Hu, J.H., Steber, C.M., and Sun, T.P. (2004). The Arabidopsis F-box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell *16*, 1392-1405.

Endo, M., Nakamura, S., Araki, T., Mochizuki, N., and Nagatani, A. (2005). Phytochrome B in the mesophyll delays flowering by suppressing FLOWERING LOCUS T expression in Arabidopsis vascular bundles. Plant Cell *17*, 1941-1952.

Eriksson, S., Bohlenius, H., Moritz, T., and Nilsson, O. (2006). GA(4) is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell *18*, 2172-2181.

Feng, S.H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J.L., Wang, F., Chen, L.Y., Yu, L., Iglesias-Pedraz, J.M., Kircher, S*., et al.* (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature *451*, 475-U479.

Fornara, F., de Montaigu, A., and Coupland, G. (2010). SnapShot: Control of Flowering in Arabidopsis. Cell *141*, -.

Fu, X.D., Richards, D.E., Fleck, B., Xie, D.X., Burton, N., and Harberd, N.P. (2004). The Arabidopsis mutant sleepy1(gar2-1) protein promotes plant growth by increasing the affinity of the SCFSLY1 E3 ubiquitin ligase for DELLA protein substrates. Plant Cell *16*, 1406-1418.

Gandikota, M., Birkenbihl, R.P., Hohmann, S., Cardon, G.H., Saedler, H., and Huijser, P. (2007). The miRNA156/157 recognition element in the 3' UTR of the Arabidopsis SBP box gene SPL3 prevents early flowering by translational inhibition in seedlings. Plant Journal *49*, 683-693.

Gocal, G.F.W., Sheldon, C.C., Gubler, F., Moritz, T., Bagnall, D.J., MacMillan, C.P., Li, S.F., Parish, R.W., Dennis, E.S., Weigel, D*., et al.* (2001). GAMYB-like genes, flowering, and gibberellin signaling in Arabidopsis. Plant Physiol *127*, 1682-1693.

Harberd, N.P., Belfield, E., and Yasumura, Y. (2009). The Angiosperm Gibberellin-GID1-DELLA Growth Regulatory Mechanism: How an "Inhibitor of an Inhibitor" Enables Flexible Response to Fluctuating Environments. Plant Cell *21*, 1328-1339.

Haritatos, E., Ayre, B.G., and Turgeon, R. (2000). Identification of phloem involved in assimilate loading in leaves by the activity of the galactinol synthase promoter. Plant Physiol *123*, 929-937.

Hisamatsu, T., and King, R.W. (2008). The nature of floral signals in Arabidopsis. II. Roles for FLOWERING LOCUS T (FT) and gibberellin. J Exp Bot *59*, 3821-3829.

Jaeger, K.E., and Wigge, P.A. (2007). FT protein acts as a long-range signal in Arabidopsis. Current Biology *17*, 1050-1054.

Jang, S., Torti, S., and Coupland, G. (2009). Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. Plant Journal *60*, 614-625.

Jasinski, S., Piazza, P., Craft, J., Hay, A., Woolley, L., Rieu, I., Phillips, A., Hedden, P., and Tsiantis, M. (2005). KNOX action in Arabidopsis is mediated by coordinate regulation of cytokinin and gibberellin activities. Current Biology *15*, 1560-1565.

Jung, J.-H., Ju, Y., Seo, P.J., Lee, J.-H., and Park, C.-M. (2011). The SOC1-SPL module integrates photoperiod and gibberellic acid signals to control flowering time in Arabidopsis. The Plant Journal *Accepted 6th October*.

Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Activation tagging of the floral inducer FT. Science *286*, 1962-1965.

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

Kobayashi, Y., and Weigel, D. (2007). Move on up, it's time for change - mobile signals controlling photoperiod-dependent flowering. Genes & Development *21*, 2371-2384.

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes & Development *21*, 397-402.

Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C.A., Ito, T., Meyerowitz, E., and Yu, H. (2008). A repressor complex governs the integration of flowering signals in Arabidopsis. Developmental Cell *15*, 110-120.

Markwell, J., Osterman, J.C., and Mitchell, J.L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynth Res *46*, 467-472.

Mathieu, J., Warthmann, N., Kuttner, F., and Schmid, M. (2007). Export of FT protein from phloem companion cells is sufficient for floral induction in Arabidopsis. Current Biology *17*, 1055-1060.

McGinnis, K.M., Thomas, S.G., Soule, J.D., Strader, L.C., Zale, J.M., Sun, T.P., and Steber, C.M. (2003). The Arabidopsis SLEEPY1 gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. Plant Cell *15*, 1120-1130.

Michaels, S.D., Himelblau, E., Kim, S.Y., Schomburg, F.M., and Amasino, R.M. (2005). Integration of flowering signals in winter-annual Arabidopsis. Plant Physiol *137*, 149-156.

Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant Journal *35*, 613-623.

Nakajima, M., Shimada, A., Takashi, Y., Kim, Y.C., Park, S.H., Ueguchi-Tanaka, M., Suzuki, H., Katoh, E., Iuchi, S., Kobayashi, M.*, et al.* (2006). Identification and characterization of Arabidopsis gibberellin receptors. Plant Journal *46*, 880-889.

Peng, J.R., Carol, P., Richards, D.E., King, K.E., Cowling, R.J., Murphy, G.P., and Harberd, N.P. (1997). The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes & Development *11*, 3194-3205.

Phillips, A.L., Ward, D.A., Uknes, S., Appleford, N.E.J., Lange, T., Huttly, A.K., Gaskin, P., Graebe, J.E., and Hedden, P. (1995). Isolation and Expression of 3 Gibberellin 20-Oxidase Cdna Clones from Arabidopsis. Plant Physiol *108*, 1049-1057.

Pnueli, L., Gutfinger, T., Hareven, D., Ben-Naim, O., Ron, N., Adir, N., and Lifschitz, E. (2001). Tomato SP-interacting proteins define a conserved signaling system that regulates shoot architecture and flowering. Plant Cell *13*, 2687-2702.

Ranjan, A., Fiene, G., Fackendahl, P., and Hoecker, U. (2011). The Arabidopsis repressor of light signaling SPA1 acts in the phloem to regulate seedling de-etiolation, leaf expansion and flowering time. Development *138*, 1851-1862.

Reeves, P.H., and Coupland, G. (2001). Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. Plant Physiol *126*, 1085-1091.

Richter, R., Behringer, C., Muller, I.K., and Schwechheimer, C. (2010). The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. Genes & Development *24*, 2093-2104.

Rieu, I., Eriksson, S., Powers, S.J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S.G., Hedden, P*., et al.* (2008a). Genetic Analysis Reveals That C(19)-GA 2-Oxidation Is a Major Gibberellin Inactivation Pathway in Arabidopsis. Plant Cell *20*, 2420-2436.

Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S.J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S.G*., et al.* (2008b). The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. Plant Journal *53*, 488-504.

Sakamoto, T., Kobayashi, M., Itoh, H., Tagiri, A., Kayano, T., Tanaka, H., Iwahori, S., and Matsuoka, M. (2001). Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. Plant Physiol *125*, 1508-1516.

Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science *288*, 1613-1616.

Schmid, M., Uhlenhaut, N.H., Godard, F., Demar, M., Bressan, R., Weigel, D., and Lohmann, J.U. (2003). Dissection of floral induction pathways using global expression analysis. Development *130*, 6001-6012.

Schomburg, F.M., Bizzell, C.M., Lee, D.J., Zeevaart, J.A.D., and Amasino, R.M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell *15*, 151-163.

Schwab, R., Palatnik, J.F., Riester, M., Schommer, C., Schmid, M., and Weigel, D. (2005). Specific effects of MicroRNAs on the plant transcriptome. Developmental Cell *8*, 517-527.

Schwarz, S., Grande, A.V., Bujdoso, N., Saedler, H., and Huijser, P. (2008). The microRNA regulated SBP-box genes SPL9 and SPL15 control shoot maturation in Arabidopsis. Plant Mol Biol *67*, 183-195.

Searle, I., He, Y.H., Turck, F., Vincent, C., Fornara, F., Krober, S., Amasino, R.A., and Coupland, G. (2006). The transcription factor FLC confers a flowering response to vernalization by repressing meristem competence and systemic signaling in Arabidopsis. Genes & Development *20*, 898-912.

Takada, S., and Goto, K. (2003). TERMINAL FLOWER2, an Arabidopsis homolog of HETEROCHROMATIN PROTEIN1, counteracts the activation of FLOWERING LOCUS T by CONSTANS in the vascular tissues of leaves to regulate flowering time. Plant Cell *15*, 2856-2865.

Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. (2012). Analysis of the Arabidopsis Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering. The Plant Cell Online.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annual Review of Plant Biology *59*, 573-594.

Wang, J.W., Czech, B., and Weigel, D. (2009). miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. Cell *138*, 738-749.

Wigge, P.A., Kim, M.C., Jaeger, K.E., Busch, W., Schmid, M., Lohmann, J.U., and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. Science *309*, 1056-1059.

Willige, B.C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E.M.N., Maier, A., and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. Plant Cell *19*, 1209-1220.

Wilson, R.N., Heckman, J.W., and Somerville, C.R. (1992). Gibberellin Is Required for Flowering in Arabidopsis-Thaliana under Short Days. Plant Physiol *100*, 403-408.

Woodger, F.J., Millar, A., Murray, F., Jacobsen, J.V., and Gubler, F. (2003). The role of GAMYB transcription factors in GA-regulated gene expression. Journal of Plant Growth Regulation *22*, 176-184.

Wu, G., Park, M.Y., Conway, S.R., Wang, J.W., Weigel, D., and Poethig, R.S. (2009). The Sequential Action of miR156 and miR172 Regulates Developmental Timing in Arabidopsis. Cell *138*, 750-759.

Wu, G., and Poethig, R.S. (2006). Temporal regulation of shoot development in Arabidopsis thaliana by miR156 and its target SPL3. Development *133*, 3539-3547.

Xu, Y.L., Li, L., Wu, K.Q., Peeters, A.J.M., Gage, D.A., and Zeevaart, J.A.D. (1995). The GA5 locus of Arabidopsis-thaliana encodes a multifunctional Gibberellin 20-Oxidase - molecular cloning and functional expression. P Natl Acad Sci USA *92*, 6640-6644.

Yamaguchi, A., Kobayashi, Y., Goto, K., Abe, M., and Araki, T. (2005). TWIN SISTER OF FT (TSF) acts as a floral pathway integrator redundantly with FT. Plant and Cell Physiology *46*, 1175-1189.

Yamaguchi, A., Wu, M.F., Yang, L., Wu, G., Poethig, R.S., and Wagner, D. (2009). The MicroRNA-Regulated SBP-Box Transcription Factor SPL3 Is a Direct Upstream Activator of LEAFY, FRUITFULL, and APETALA1. Developmental Cell *17*, 268-278.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annual Review of Plant Biology *59*, 225-251.

Yang, H.P., Schmuke, J.J., Flagg, L.M., Roberts, J.K., Allen, E.M., Ivashuta, S., Gilbertson, L.A., Armstrong, T.A., and Christian, A.T. (2009). A novel real-time polymerase chain reaction method for high throughput quantification of small regulatory RNAs. Plant Biotechnol J *7*, 621-630.

REFERENCES (Chapter 3)

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

Adrian, J., Farrona, S., Reimer, J. J., Albani, M. C., Coupland, G., and Turck, F. (2010). cis-Regulatory Elements and Chromatin State Coordinately Control Temporal and Spatial Expression of FLOWERING LOCUS T in Arabidopsis. Plant Cell *22*, 1425-1440.

An, H., Roussot, C., Suarez-Lopez, P., Corbesier, L., Vincent, C., Pineiro, M., Hepworth, S., Mouradov, A., Justin, S., Turnbull, C., and Coupland, G. (2004). CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development *131*, 3615-3626.

Andres, F., and Coupland, G. (2012). The genetic basis of flowering responses to seasonal cues. Nature Reviews Genetics *13*, 627-639.

Blazquez, M. A., Green, R., Nilsson, O., Sussman, M. R., and Weigel, D. (1998). Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell *10*, 791-800.

Blazquez, M. A., and Weigel, D. (2000). Integration of floral inductive signals in Arabidopsis. Nature *404*, 889-892.

Bolduc, N., and Hake, S. (2009). The Maize Transcription Factor KNOTTED1 Directly Regulates the Gibberellin Catabolism Gene ga2ox1. Plant Cell *21*, 1647-1658.

Bradley, D., Carpenter, R., Sommer, H., Hartley, N., and Coen, E. (1993). Complementary floral homeotic phenotypes result from opposite orientations of a transposon at the plena locus of *Antirrhinum*. Cell *72*, 85-95.

Clough, S. J., and Bent, A. F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant Journal *16*, 735-743.

Coles, J. P., Phillips, A. L., Croker, S. J., Garcia-Lepe, R., Lewis, M. J., and Hedden, P. (1999). Modification of gibberellin production and plant development in Arabidopsis by sense and antisense expression of gibberellin 20-oxidase genes. Plant J *17*, 547-556.

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

de Lucas, M., Daviere, J. M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J. M., Lorrain, S., Fankhauser, C., Blazquez, M. A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature *451*, 480-U411.

Eriksson, S., Bohlenius, H., Moritz, T., and Nilsson, O. (2006). GA(4) is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell *18*, 2172-2181.

Feng, S. H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J. L., Wang, F., Chen, L. Y., Yu, L., Iglesias-Pedraz, J. M., Kircher, S*., et al.* (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature *451*, 475-U479.

Fornara, F., de Montaigu, A., and Coupland, G. (2010). SnapShot: Control of Flowering in Arabidopsis. Cell *141*, -.

Fujiwara, S., Oda, A., Yoshida, R., Niinuma, K., Miyata, K., Tomozoe, Y., Tajima, T., Nakagawa, M., Hayashi, K., Coupland, G., and Mizoguchi, T. (2008). Circadian Clock Proteins LHY and CCA1 Regulate SVP Protein Accumulation to Control Flowering in Arabidopsis. Plant Cell *20*, 2960-2971.

Galvao, V. C., Horrer, D., Kuttner, F., and Schmid, M. (2012). Spatial control of flowering by DELLA proteins in Arabidopsis thaliana. Development *139*, 4072-4082.

Gregis, V., Andrés, F., Sessa, A., Guerra, R. F., Simonini, S., Mateos, J. L., Torti, S., Zambelli, F., Prazzoli, G. M., Bjerkan, K. N*., et al.* (2013). Identification of pathways directly regulated by SHORT VEGETATIVE PHASE during vegetative and reproductive development in Arabidopsis. Genome Biology *In press*.

Gregis, V., Sessa, A., Colombo, L., and Kater, M. M. (2008). AGAMOUS-LIKE24 and SHORT VEGETATIVE PHASE determine floral meristem identity in Arabidopsis. Plant J *56*, 891-902.

Gregis, V., Sessa, A., Dorca-Fornell, C., and Kater, M. M. (2009). The Arabidopsis floral meristem identity genes AP1, AGL24 and SVP directly repress class B and C floral homeotic genes. Plant J *60*, 626-637.

Griffiths, J., Murase, K., Rieu, I., Zentella, R., Zhang, Z. L., Powers, S. J., Gong, F., Phillips, A. L., Hedden, P., Sun, T. P., and Thomas, S. G. (2006). Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell *18*, 3399-3414.

Hartmann, U., Hohmann, S., Nettesheim, K., Wisman, E., Saedler, H., and Huijser, P. (2000). Molecular cloning of SVP: a negative regulator of the floral transition in Arabidopsis. Plant Journal *21*, 351-360.

Hay, A., Kaur, H., Phillips, A., Hedden, P., Hake, S., and Tsiantis, M. (2002). The gibberellin pathway mediates KNOTTED1-type homeobox function in plants with different body plans. Curr Biol *12*, 1557-1565.

Hedden, P., and Phillips, A. L. (2000). Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci *5*, 523-530.

Huang, S., Raman, A. S., Ream, J. E., Fujiwara, H., Cerny, R. E., and Brown, S. M. (1998). Overexpression of 20-oxidase confers a gibberellin-overproduction phenotype in Arabidopsis. Plant Physiology *118*, 773-781.

Immink, R. G., Pose, D., Ferrario, S., Ott, F., Kaufmann, K., Valentim, F. L., de Folter, S., van der Wal, F., van Dijk, A. D., Schmid, M., and Angenent, G. C. (2012). Characterization of SOC1's central role in flowering by the identification of its upstream and downstream regulators. Plant Physiology *160*, 433-449.

Jacqmard, A., Gadisseur, I., and Bernier, G. (2003). Cell division and morphological changes in the shoot apex of Arabidopsis thaliana during floral transition. Annals of Botany *91*, 571-576.

Jaeger, K. E., and Wigge, P. A. (2007). FT protein acts as a long-range signal in Arabidopsis. Curr Biol *17*, 1050-1054.

Jang, S., Torti, S., and Coupland, G. (2009). Genetic and spatial interactions between FT, TSF and SVP during the early stages of floral induction in Arabidopsis. Plant J *60*, 614-625.

Lee, D. J., and Zeevaart, J. A. (2007). Regulation of gibberellin 20-oxidase1 expression in spinach by photoperiod. Planta *226*, 35-44.

Lee, J. H., Yoo, S. J., Park, S. H., Hwang, I., Lee, J. S., and Ahn, J. H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev *21*, 397-402.

Li, D., Liu, C., Shen, L., Wu, Y., Chen, H., Robertson, M., Helliwell, C. A., Ito, T., Meyerowitz, E., and Yu, H. (2008). A repressor complex governs the integration of flowering signals in Arabidopsis. Dev Cell *15*, 110-120.

Liu, C., Xi, W., Shen, L., Tan, C., and Yu, H. (2009). Regulation of floral patterning by flowering time genes. Dev Cell *16*, 711-722.

Markwell, J., Osterman, J. C., and Mitchell, J. L. (1995). Calibration of the Minolta SPAD-502 leaf chlorophyll meter. Photosynthesis Research *46*, 467-472.

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

Melzer, S., Lens, F., Gennen, J., Vanneste, S., Rohde, A., and Beeckman, T. (2008). Flowering-time genes modulate meristem determinacy and growth form in Arabidopsis thaliana. Nat Genet *40*, 1489-1492.

Mendez-Vigo, B., Martinez-Zapater, J. M., and Alonso-Blanco, C. (2013). The flowering repressor SVP underlies a novel Arabidopsis thaliana QTL interacting with the genetic background. PLoS Genet *9*, e1003289.

Moon, J., Suh, S. S., Lee, H., Choi, K. R., Hong, C. B., Paek, N. C., Kim, S. G., and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J *35*, 613-623.

Mutasa-Gottgens, E., and Hedden, P. (2009). Gibberellin as a factor in floral regulatory networks. Journal of Experimental Botany *60*, 1979-1989.

Navarro, C., Abelenda, J. A., Cruz-Oro, E., Cuellar, C. A., Tamaki, S., Silva, J., Shimamoto, K., and Prat, S. (2011). Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. Nature *478*, 119-U132.

Osnato, M., Castillejo, C., Matias-Hernandez, L., and Pelaz, S. (2012). TEMPRANILLO genes link photoperiod and gibberellin pathways to control flowering in Arabidopsis. Nat Commun *3*, 808.

Phillips, A. L., Ward, D. A., Uknes, S., Appleford, N. E. J., Lange, T., Huttly, A. K., Gaskin, P., Graebe, J. E., and Hedden, P. (1995). Isolation and Expression of 3 Gibberellin 20-Oxidase Cdna Clones from Arabidopsis. Plant Physiology *108*, 1049-1057.

Plackett, A. R., Powers, S. J., Fernandez-Garcia, N., Urbanova, T., Takebayashi, Y., Seo, M., Jikumaru, Y., Benlloch, R., Nilsson, O., Ruiz-Rivero, O*., et al.* (2012). Analysis of the developmental roles of the Arabidopsis gibberellin 20-oxidases demonstrates that GA20ox1, -2, and -3 are the dominant paralogs. Plant Cell *24*, 941-960.

Porri, A., Torti, S., Romera-Branchat, M., and Coupland, G. (2012). Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development *139*, 2198-2209.

Reeves, P. H., and Coupland, G. (2001). Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. Plant Physiology *126*, 1085-1091.

Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S. J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S. G.*, et al.* (2008). The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. Plant J *53*, 488-504.

Schmid, M., Uhlenhaut, N. H., Godard, F., Demar, M., Bressan, R., Weigel, D., and Lohmann, J. U. (2003). Dissection of floral induction pathways using global expression analysis. Development *130*, 6001-6012.

Seo, M., Jikumaru, Y., and Kamiya, Y. (2011). Profiling of hormones and related metabolites in seed dormancy and germination studies. Methods Mol Biol *773*, 99-111.

Tamaki, S., Matsuo, S., Wong, H. L., Yokoi, S., and Shimamoto, K. (2007). Hd3a protein is a mobile flowering signal in rice. Science *316*, 1033-1036.

Tao, Z., Shen, L., Liu, C., Liu, L., Yan, Y., and Yu, H. (2012). Genome-wide identification of SOC1 and SVP targets during the floral transition in Arabidopsis. Plant J *70*, 549-561.

Taoka, K., Ohki, I., Tsuji, H., Furuita, K., Hayashi, K., Yanase, T., Yamaguchi, M., Nakashima, C., Purwestri, Y. A., Tamaki, S.*, et al.* (2011). 14-3-3 proteins act as intracellular receptors for rice Hd3a florigen. Nature *476*, 332-335.

Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. (2012). Analysis of the Arabidopsis Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering. The Plant Cell Online.

Turck, F., Fornara, F., and Coupland, G. (2008). Regulation and identity of florigen: FLOWERING LOCUS T moves center stage. Annu Rev Plant Biol *59*, 573-594.

Wang, J. W., Czech, B., and Weigel, D. (2009). miR156-Regulated SPL Transcription Factors Define an Endogenous Flowering Pathway in Arabidopsis thaliana. Cell *138*, 738-749.

Wigge, P. A., Kim, M. C., Jaeger, K. E., Busch, W., Schmid, M., Lohmann, J. U., and Weigel, D. (2005). Integration of spatial and temporal information during floral induction in Arabidopsis. Science *309*, 1056-1059.

Willige, B. C., Ghosh, S., Nill, C., Zourelidou, M., Dohmann, E. M. N., Maier, A., and Schwechheimer, C. (2007). The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis. Plant Cell *19*, 1209-1220.

Wilson, R. N., Heckman, J. W., and Somerville, C. R. (1992). Gibberellin Is Required for Flowering in Arabidopsis-Thaliana under Short Days. Plant Physiology *100*, 403-408.

Wu, G., Park, M. Y., Conway, S. R., Wang, J. W., Weigel, D., and Poethig, R. S. (2009). The Sequential Action of miR156 and miR172 Regulates Developmental Timing in Arabidopsis. Cell *138*, 750-759.

Xu, Y. L., Gage, D. A., and Zeevaart, J. A. D. (1997). Gibberellins and stem growth in Arabidopsis thaliana - Effects of photoperiod on expression of the GA4 and GA5 loci. Plant Physiology *114*, 1471-1476.

Xu, Y. L., Li, L., Wu, K. Q., Peeters, A. J. M., Gage, D. A., and Zeevaart, J. A. D. (1995). The GA5 locus of Arabidopsis-thaliana encodes a multifunctional Gibberellin 20-Oxidase - molecular cloning and functional expression. Proceedings of the National Academy of Sciences of the United States of America *92*, 6640-6644.

Yamaguchi, A., Wu, M. F., Yang, L., Wu, G., Poethig, R. S., and Wagner, D. (2009). The MicroRNA-Regulated SBP-Box Transcription Factor SPL3 Is a Direct Upstream Activator of LEAFY, FRUITFULL, and APETALA1. Developmental Cell *17*, 268-278.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annual Review of Plant Biology *59*, 225-251.

Yant, L., Mathieu, J., and Schmid, M. (2009). Just say no: floral repressors help Arabidopsis bide the time. Curr Opin Plant Biol *12*, 580-586.

Yu, S., Galvao, V. C., Zhang, Y. C., Horrer, D., Zhang, T. Q., Hao, Y. H., Feng, Y. Q., Wang, S., Schmid, M., and Wang, J. W. (2012). Gibberellin Regulates the Arabidopsis Floral Transition through miR156-Targeted SQUAMOSA PROMOTER BINDING-LIKE Transcription Factors. Plant Cell *24*, 3320-3332.

REFERENCES (Chapter 4)

Achard P, Gusti A, Cheminant S, Alioua C, Dhondt S, Coppens F, Beemster GT, Genschik P (2009) Gibberellin Signaling Controls Cell Proliferation Rate in Arabidopsis. Current Biology 19: 1188–1193.

Archacki R, Sarnowski TJ, Halibart-Puzio JU, Brzeska K, Buszewicz D, Prymakowska-Bosak M, Koncz C, Jerzmanowski A (2009) Genetic analysis of functional redundancy of BRM ATPase and ATSWI3C subunits of Arabidopsis SWI/SNF chromatin remodelling complexes. Planta 229: 1281-1292.

Archacki R, Buszewicz D, Sarnowski TJ, Sarnowska E, Rolicka AT, Tohge T, Fernie AR, Jikumaru Y, Kotlinski M, Iwanicka-Nowicka R, Kalisiak K, Patryn J, Halibart-Puzio J, Kamiya Y, Davis SJ, Koblowska MK, Jerzmanowski A (2013) BRAHMA ATPase of the SWI/SNF Chromatin Remodeling Complex Acts as a Positive Regulator of Gibberellin – Mediated Responses in Arabidopsis PLoS ONE 8:e58588.

Belandia B, Parker MG (2003) Nuclear receptors: a rendezvous for chromatin remodeling factors. Cell 114: 277-280.

Belda-Palazón B, Ruiz L, Martí E, Tárraga S, Tiburcio AF, Culiáñez F, Farràs R, Carrasco P, Ferrando A (2012) Aminopropyltransferases involved in polyamine biosynthesis localize preferentially in the nucleus of plant cells. PLoS ONE 7: e46907. doi:10.1371/journal.pone.0046907.

Bezhani S, Winter C, Hershman S, Wagner JD, Kennedy JF, Kwon CS, Pfluger J, Su Y, Wagner D (2007) Unique, shared, and redundant roles for the Arabidopsis SWI/SNF chromatin remodeling ATPases BRAHMA and SPLAYED. Plant Cell 19: 403-416.

Curaba J, Moritz T, Blervaque R, Parcy F, Raz V, Herzog M, Vachon G (2004) AtGA3ox2, a key gene responsible for bioactive gibberellin biosynthesis, is regulated during embryogenesis by LEAFY COTYLEDON2 and FUSCA3 in Arabidopsis. Plant Physiol. 136: 3660-3669.

de Lucas M, Daviere JM, Rodriguez-Falcon M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E, Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. Nature 451: 480-484.

Dill AS, Hu TJ, Steber C, Sun T-P (2004) The Arabidopsis F-Box protein SLEEPY1 targets gibberellin signaling repressors for gibberellin-induced degradation. Plant Cell 16: 1392-1405.

DiRenzo J, Shang Y, Phelan M, Sif S, Myers M, Kingston R, Brown M (2000) BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. Mol. Cell. Biol. 20: 7541-7549.

Euskirchen GM, Auerbach, RK, Davidov, E, Gianoulis, TA, Zhong, G, Rozowsky J, Bhardwaj N, Gerstein MB, Snyder M (2011) Diverse roles and interactions of the SWI/SNF chromatin remodeling complex revealed using global approaches. PLoS Genet 7(3): e1002008.

Farrona S, Hurtado L, Bowman JL, Reyes JC (2004) The Arabidopsis thaliana SNF2 homolog AtBRM controls shoot development and flowering. Development 131: 4965-4975.

Feng S, Martinez C, Gusmaroli G, Wang Y, Zhou J, Wang F, Chen L, Yu L, Iglesias-Pedraz JM, Kircher S, Schäfer E, Fu X, Fan LM, Deng XW (2008) Coordinated regulation of *Arabidopsis thaliana* development by light and gibberellins. Nature 451: 475–479.

Fleet CM and Sun TP (2005) *A DELLAcate balance: the role of gibberellin in plant morphogenesis*, Curr. Opin. Plant Biol. 8: 77-85.

Fu X, Harberd NP (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature 421: 740-743.

Griffiths J, Murase K, Rieu I, Zentella R, Zhang ZL, Powers SJ, Gong F, Phillips AL, Hedden P, Sun TP, Thomas SG (2006) Genetic characterization and functional analysis of the GID1 gibberellin receptors in Arabidopsis. Plant Cell 18: 3399–3414.

Han S, Kim D (2006) AtRTPrimer: database for Arabidopsis genome-wide homogeneous and specific RT-PCR primer-pairs. BMC Bioinformatics 7: 179.

Han SK, Sang Y, Rodrigues A, BIOL425 F2010, Wu MF, Rodriguez PL, Wagner D (2013) The SWI2/SNF2 chromatin remodeling ATPase BRAHMA represses abscisic acid responses in the absence of the stress stimulus in Arabidopsis. Plant Cell. 24: 4892-906.

Hartweck LM (2008) Gibberellin signaling. Planta 229: 1–13

Hedden P, Phillips AL (2000) Gibberellin metabolism: new insights revealed by the genes. Trends Plant Sci. 5: 523–530.

Hu CD, Chinenov Y, Kerppola TK (2002) Visualization of interactions among bZIP and Rel family proteins in living cells using bimolecular fluorescence complementation. Mol. Cell 9: 789–798.

Iuchi S, Hiroyuki Suzuki H, Young-Cheon Kim Y-C, Atsuko Iuchi A, Takashi Kuromori T, Ueguchi-Tanaka M, Asami T, Yamaguchi I, Matsuoka M, Kobayashi M, Nakajima M (2007) Multiple loss-of-function of Arabidopsis gibberellin receptor AtGID1s completely shuts down a gibberellin signal. Plant J. 50: 958–966.

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* Locus of Arabidopsis alter gibberellin signal transduction. Plant Cell 5: 887-896.

John S, Sabo PJ, Johnson TA, Sung M-H, Biddie SC, Lightman SL, Voss TC, Davis SR, Meltzer PS, Stamatoyannopoulos JA, Hager GL (2008) Interaction of the glucocorticoid receptor with the chromatin landscape. Mol. Cell 29: 611-624.

Koornneef M, van der Veen JH (1980) Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.). Heynh. Theor. Appl. Genet 58: 257-263.

Marocco K, Bergdoll M, Achard P, Criqui M-C, Genschik P (2010) Selective proteolysis sets the tempo of the cell cycle. Curr Opin in Plant Biol 13: 631-639.

Murase K, Hirano Y, Sun TP, Hakoshima T (2008) Gibberellin-induced DELLA recognition by the gibberellin receptor GID1. Nature 456: 459-463.

Nakajima M, Shimada A, Takashi Y, Kim YC, Park SH, Ueguchi-Tanaka M, Suzuki H, Katoh E, Iuchi S, Kobayashi M, Maeda T, Matsuoka M, Yamaguchi I (2006) Identification and characterization of Arabidopsis gibberellin receptors. Plant J. 46: 880-889.

Plackett AR, Powers SJ, Fernandez-Garcia N, Urbanova T, Takebayashi Y, Seo M, Jikumaru Y, Benlloch R, Nilsson O, Ruiz-Rivero O, Phillips AL, Wilson ZA, Thomas SG, Hedden P (2012) Analysis of the developmental roles of the Arabidopsis gibberellin 20-oxidases demonstrates that GA20ox1, -2, and -3 are the dominant paralogs. Plant Cell 24: 941-960.

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis GAI gene defines a signaling pathway that negatively regulates gibberellin responses. Genes Dev. 11: 3194–3205.

Porri A, Torti S, Romera-Branchat M, Coupland G (2012) Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development 139: 2198–2209.

Richter R, Behringer C, Muller IK, Schwechheimer C (2010) The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. *Genes Dev.* 24: 2093-2104.

Saez A, Rodrigues A, Santiago J, Rubio S, Rodriguez PL (2008) HAB-1-SWI3B interaction reveals a link between abscisic acid signaling and putative SWI/SNF chromatin-remodeling complexes in Arabidopsis. Plant Cell 20: 2972-2988.

Sarnowski T, Swiezewski S, Pawlikowska K, Kaczanowski S, Jerzmanowski A (2002) AtSWI3B, an Arabidopsis homolog of SWI3, a core subunit of yeast Swi/Snf chromatin remodeling complex, interacts with FCA, a regulator of flowering time. Nucleic Acids Res. 30: 3412-3421.

Sarnowski TJ, Rios G, Jasik J, Swiezewski S, Kaczanowski S, Li Y, Kwiatkowska A, Pawlikowska K, Koźbiał M, Koźbiał P, Koncz C, Jerzmanowski A (2005) SWI3 subunits of putative SWI/SNF chromatin remodeling complex play distinct roles during Arabidopsis development. Plant Cell 17: 2454-2472.

Sasaki A, Itoh H, Gomi K, Ueguchi-Tanaka M, Ishiyama K, Kobayashi M, Jeong DH, An G, Kitano H, Ashikari M, Matsuoka M (2003) Accumulation of phosphorylated repressor for gibberellin signaling in an F-box mutant. *Science* 299: 1896–1898

Seo M, Jikumaru Y, Kamiya Y (2011) Profiling of hormones and related metabolites in seed dormancy and germination studies. In Kermode AR, editor, Methods and Protocols. Humana Press, pp. 99-111.

Schwechheimer C, Willige BC (2009) Shedding light on gibberellic acid signalling. Curr. Opin. Plant Biol. 12: 57–62.

Shimada A, Ueguchi-Tanaka M, Sakamoto T, Fujioka S, Takatsuto S, Yoshida S, Sazuka T, Ashikari M, Matsuoka M (2006) The rice *SPINDLY* gene functions as a negative regulator of gibberellin signaling by controlling the suppressive function of the DELLA protein, SLR1, and modulating brassinosteroid synthesis. Plant J. 48: 3390–402

Silverstone AL, Ciampaglio CN, Sun T-P (1998) The Arabidopsis RGA gene encodes a transcriptional regulator repressing the gibberellin signal transduction pathway. Plant Cell 10: 155-169.

Silverstone AL, Tseng TS, Swain SM, Dill A, Jeong SY, Olszewski NE, Sun TP (2007) Functional analysis of SPINDLY in gibberellin signaling in Arabidopsis. Plant Physiol. 143: 987-1000.

Slawson C, Housley MP, Hart GW (2006) O-GlcNAc cycling: how a single sugar post-translational modification is changing the way we think about signaling networks. J Cell Biochem 97: 71–83.

Sun TP (2010) Gibberellin-GID1-DELLA: A Pivotal Regulatory Module for Plant Growth and Development Plant Phys. 154: 567-570.

Torti S, Fornara F, Vincent C, Andrés F, Nordström K, Göbel U, Knoll D, Schoof H, Coupland G (2012) Analysis of the Arabidopsis shoot meristem transcriptome during floral transition identifies distinct regulatory patterns and a leucine-rich repeat protein that promotes flowering. Plant Cell 24: 444-462.

Ueguchi-Tanaka M, Ashikari M, Nakajima M, Itoh H, Katoh E, Kobayashi M, Chow TY, Hsing YI, Kitano H, Yamaguchi I, Matsuoka M (2005) GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. Nature 437: 693-8.

Ueguchi-Tanaka M, Nakajima M, Katoh E, Ohmiya H, Asano K, Saji S, Hongyu X, Ashikari M, Kitano H, Yamaguchi I, Makoto Matsuoka M (2007) Molecular Interactions of a Soluble Gibberellin Receptor, GID1, with a Rice DELLA Protein, SLR1, and Gibberellin. Plant Cell 19: 2140-2155.

Voinnet O, Rivas S, Mestre P, Baulcombe D (2003) An enhanced transient expression system in plants based on suppression of gene silencing by the p19 protein of tomato bushy stunt virus. Plant J. 33: 949–956.

Willige BC, Ghosh S, Nill C, Zourelidou M, Dohmann EM, Maier A, Schwechheimer C (2007) The DELLA domain of GA INSENSITIVE mediates the interaction with the GA INSENSITIVE DWARF1A gibberellin receptor of Arabidopsis.Plant Cell 19:1209-2019.

Zentella R, Zhang ZL, Park M, Thomas SG, Endo A, Murase K, Fleet CM, Jikumaru Y, Nambara E, Kamiya Y, Sun TP (2007) Global analysis of DELLA direct targets in early gibberellin signaling in Arabidopsis. Plant Cell 19: 3037-3057.

Zraly CB, Middleton FA, Dingwall AK (2006) Hormone-response genes are direct in vivo regulatory targets of Brahma (SWI/SNF) complex function. J. Biol. Chem. 281: 35305-35315.

REFERENCES (Chapter 5)

Achard, P., Herr, A., Baulcombe, D.C., and Harberd, N.P. (2004). Modulation of floral development by a gibberellin-regulated microRNA. Development 131, 3357-3365.

Achard, P., Gusti, A., Cheminant, S., Alioua, M., Dhondt, S., Coppens, F., Beemster, G.T., and Genschik, P. (2009). Gibberellin signaling controls cell proliferation rate in Arabidopsis. Current biology : CB 19, 1188-1193.

Archacki, R., Sarnowski, T.J., Halibart-Puzio, J., Brzeska, K., Buszewicz, D., Prymakowska-Bosak, M., Koncz, C., and Jerzmanowski, A. (2009). Genetic analysis of functional redundancy of BRM ATPase and ATSWI3C subunits of Arabidopsis SWI/SNF chromatin remodelling complexes. Planta 229, 1281-1292.

Archacki, R., Buszewicz, D., Sarnowski, T.J., Sarnowska, E., Rolicka, A.T., Tohge, T., Fernie, A.R., Jikumaru, Y., Kotlinski, M., Iwanicka-Nowicka, R., Kalisiak, K., Patryn, J., Halibart-Puzio, J., Kamiya, Y., Davis, S.J., Koblowska, M.K., and Jerzmanowski, A. (2013). BRAHMA ATPase of the SWI/SNF chromatin remodeling complex acts as a positive regulator of gibberellin-mediated responses in arabidopsis. PloS one 8, e58588.

Blazquez, M. A., Green, R., Nilsson, O., Sussman, M. R., and Weigel, D. (1998). Gibberellins promote flowering of Arabidopsis by activating the LEAFY promoter. Plant Cell *10*, 791-800.

Bolduc, N., and Hake, S. (2009). The maize transcription factor KNOTTED1 directly regulates the gibberellin catabolism gene ga2ox1. Plant Cell 21, 1647-1658.

Clapier, C.R., and Cairns, B.R. (2009). The Biology of Chromatin Remodeling Complexes. Annu Rev Biochem 78, 273-304.

Corbesier, L., Vincent, C., Jang, S., Fornara, F., Fan, Q., Searle, I., Giakountis, A., Farrona, S., Gissot, L., Turnbull, C., and Coupland, G. (2007). FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. Science 316, 1030-1033.

Cowling, R.J., Kamiya, Y., Seto, H., and Harberd, N.P. (1998). Gibberellin dose-response regulation of GA4 gene transcript levels in Arabidopsis. Plant Physiol 117, 1195-1203.

de Lucas, M., Daviere, J.M., Rodriguez-Falcon, M., Pontin, M., Iglesias-Pedraz, J.M., Lorrain, S., Fankhauser, C., Blazquez, M.A., Titarenko, E., and Prat, S. (2008). A molecular framework for light and gibberellin control of cell elongation. Nature 451, 480-U411.

DiRenzo, J., Shang, Y., Phelan, M., Sif, S., Myers, M., Kingston, R., and Brown, M. (2000). BRG-1 is recruited to estrogen-responsive promoters and cooperates with factors involved in histone acetylation. Molecular and cellular biology 20, 7541-7549.

Eriksson, S., Bohlenius, H., Moritz, T., and Nilsson, O. (2006). GA(4) is the active gibberellin in the regulation of LEAFY transcription and Arabidopsis floral initiation. Plant Cell 18, 2172-2181.

Feng, S.H., Martinez, C., Gusmaroli, G., Wang, Y., Zhou, J.L., Wang, F., Chen, L.Y., Yu, L., Iglesias-Pedraz, J.M., Kircher, S., Schafer, E., Fu, X.D., Fan, L.M., and Deng, X.W. (2008). Coordinated regulation of Arabidopsis thaliana development by light and gibberellins. Nature 451, 475-U479.

Galvao, V.C., Horrer, D., Kuttner, F., and Schmid, M. (2012). Spatial control of flowering by DELLA proteins in Arabidopsis thaliana. Development 139, 4072-4082.

Hisamatsu, T., and King, R.W. (2008). The nature of floral signals in Arabidopsis. II. Roles for FLOWERING LOCUS T (FT) and gibberellin. Journal of experimental botany 59, 3821-3829.

Huang, J., Tang, D., Shen, Y., Qin, B., Hong, L., You, A., Li, M., Wang, X., Yu, H., Gu, M., and Cheng, Z. (2010). Activation of gibberellin 2-oxidase 6 decreases active gibberellin levels and creates a dominant semi-dwarf phenotype in rice (Oryza sativa L.). Journal of genetics and genomics = Yi chuan xue bao 37, 23-36.

Immink, R.G., Pose, D., Ferrario, S., Ott, F., Kaufmann, K., Valentim, F.L., de Folter, S., van der Wal, F., van Dijk, A.D., Schmid, M., and Angenent, G.C. (2012). Characterization of SOC1's central role in flowering by the identification of its upstream and downstream regulators. Plant Physiol 160, 433-449.

John, S., Sabo, P.J., Johnson, T.A., Sung, M.H., Biddie, S.C., Lightman, S.L., Voss, T.C., Davis, S.R., Meltzer, P.S., Stamatoyannopoulos, J.A., and Hager, G.L. (2008). Interaction of the glucocorticoid receptor with the chromatin landscape. Molecular cell 29, 611-624.

Kumar, S.V., Lucyshyn, D., Jaeger, K.E., Alos, E., Alvey, E., Harberd, N.P., and Wigge, P.A. (2012). Transcription factor PIF4 controls the thermosensory activation of flowering. Nature 484, 242-245.

Lee, J.H., Yoo, S.J., Park, S.H., Hwang, I., Lee, J.S., and Ahn, J.H. (2007). Role of SVP in the control of flowering time by ambient temperature in Arabidopsis. Genes Dev 21, 397-402.

Leivar, P., and Quail, P.H. (2011). PIFs: pivotal components in a cellular signaling hub. Trends in plant science 16, 19-28.

Liu, L., Liu, C., Hou, X., Xi, W., Shen, L., Tao, Z., Wang, Y., and Yu, H. (2012). FTIP1 is an essential regulator required for florigen transport. PLoS biology 10, e1001313.

Mathieu, J., Yant, L.J., Murdter, F., Kuttner, F., and Schmid, M. (2009). Repression of Flowering by the miR172 Target SMZ. Plos Biol 7.

Moon, J., Suh, S.S., Lee, H., Choi, K.R., Hong, C.B., Paek, N.C., Kim, S.G., and Lee, I. (2003). The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in Arabidopsis. Plant J 35, 613-623.

Richter, R., Behringer, C., Muller, I.K., and Schwechheimer, C. (2010). The GATA-type transcription factors GNC and GNL/CGA1 repress gibberellin signaling downstream from DELLA proteins and PHYTOCHROME-INTERACTING FACTORS. Gene Dev 24, 2093-2104.

Rieu, I., Eriksson, S., Powers, S.J., Gong, F., Griffiths, J., Woolley, L., Benlloch, R., Nilsson, O., Thomas, S.G., Hedden, P., and Phillips, A.L. (2008a). Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. Plant Cell 20, 2420-2436.

Rieu, I., Ruiz-Rivero, O., Fernandez-Garcia, N., Griffiths, J., Powers, S.J., Gong, F., Linhartova, T., Eriksson, S., Nilsson, O., Thomas, S.G., Phillips, A.L., and Hedden, P. (2008b). The gibberellin biosynthetic genes AtGA20ox1 and AtGA20ox2 act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. Plant J 53, 488-504.

Sakamoto, T., Kamiya, N., Ueguchi-Tanaka, M., Iwahori, S., and Matsuoka, M. (2001). KNOX homeodomain protein directly suppresses the expression of a gibberellin biosynthetic gene in the tobacco shoot apical meristem. Gene Dev 15, 581-590.

Schmid, M., Uhlenhaut, N.H., Godard, F., Demar, M., Bressan, R., Weigel, D., and Lohmann, J.U. (2003). Dissection of floral induction pathways using global expression analysis. Development 130, 6001-6012.

Schomburg, F.M., Bizzell, C.M., Lee, D.J., Zeevaart, J.A.D., and Amasino, R.M. (2003). Overexpression of a novel class of gibberellin 2-oxidases decreases gibberellin levels and creates dwarf plants. Plant Cell 15, 151-163.

Suarez-Lopez, P., Wheatley, K., Robson, F., Onouchi, H., Valverde, F., and Coupland, G. (2001). *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. Nature 410, 1116-1120.

Torti, S., Fornara, F., Vincent, C., Andrés, F., Nordström, K., Göbel, U., Knoll, D., Schoof, H., and Coupland, G. (2012). Analysis of the Arabidopsis Shoot Meristem Transcriptome during Floral Transition Identifies Distinct Regulatory Patterns and a Leucine-Rich Repeat Protein That Promotes Flowering. The Plant Cell Online.

Yamaguchi, S. (2008). Gibberellin metabolism and its regulation. Annual Review of Plant Biology 59, 225-251.

Yu, S., Galvao, V.C., Zhang, Y.C., Horrer, D., Zhang, T.Q., Hao, Y.H., Feng, Y.Q., Wang, S., Schmid, M., and Wang, J.W. (2012). Gibberellin regulates the Arabidopsis floral transition through miR156-targeted SQUAMOSA promoter binding-like transcription factors. Plant Cell 24, 3320-3332.

Erklärung

“ Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit - einschließlich Tabellen, Karten und Abbildungen -, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie - abgesehen von unten angegebenen Teilpublikationen - noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde. Die Bestimmungen dieser Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. George Coupland betreut worden. ”

Köln den 26. August

Aimone Porri

Teilpublikationen

Porri A, Baroncelli R, Guglielminetti L, Sarrocco S, Guazzelli L, Forti M, et al (2011). Fusarium oxysporum degradation and detoxification of a new textile- glycoconjugate azo dye (GAD). Fungal Biol *115*, 30–37.

Porri, A., Torti, S., Romera-Branchat, M., and Coupland, G. (2012). Spatially distinct regulatory roles for gibberellins in the promotion of flowering of Arabidopsis under long photoperiods. Development *139*, 2198-2209.

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Detailed explanation of my contribution to the experiments presented in the chapter 2, 3 and 4 of this cumulative dissertation

Chapter 2: Spatially distinct regulatory roles for gibberellins in the promotion of flowering of *Arabidopsis* under long photoperiods

As first author of this paper I performed all the experiments presented in the chapter. I developed the experimental design myself and under the supervision of Prof. George Coupland who is present in the manuscript as corresponding author. Stefano Torti, who is present in the manuscript as second author, gave me helpful suggestions to carry out the experiment of figure 6 concerning the study of the temporal and spatial expression patterns of *SPL* genes during floral induction in a gibberellin-deficient transgenic plant.

Chapter 3: Photoperiodic flowering signals increase gibberellin biosynthesis in *Arabidopsis* by repressing transcription of SVP.

As first author of this manuscript I performed most of the experiments presented in the chapter. The figure 1 shows the phenotype of the quintuple mutant *svp ft tsf soc1 ful*. This genetic combination was generated from Stefano Torti who shares with me the first author contribution. In the figure 2F is presented the determination of the concentration of different gibberellin forms. This analytic experiment was performed by José Luis García Martínez, who is an expert on gibberellin measurement. I developed experimental design and concepts together with Prof. George Coupland and Fernando Andres.

Chapter 4: DELLA interacting SWI3C core subunit of SWI/SNF chromatin remodeling complex modulates gibberellin responses and hormonal crosstalk in *Arabidopsis*

I am a coauthor of this third manuscript and, in particular, I provided my expertise on gibberellin biology. I performed the experiments that are presented in the figures 1 and S1. In addition, I constantly gave my support through suggestions and discussions for the experiments presented in the other figures. I often discussed with the corresponding author concepts and experimental design.

The author

Aimone Porri was born on the 8th of November 1984 in Pisa, Italy. Growing up in rural Tuscany, Aimone has always been fascinated by the plant world and agriculture. These passions together with a strong interest for chemistry and biology, prompted him to study *Agro-Industrial Biotechnology* at the University of Pisa, where he obtained a bachelor degree in 2006. For his master degree project he moved to the Max Planck Institute of Cologne in Germany where, under the supervision of Professor George Coupland and Dr. Amaury de Montaigu, he performed a QTL mapping analysis and identified polymorphisms implicated in the circadian clock regulation of *Arabidopsis thaliana*. Aimone defended his master thesis back in Pisa in 2008 and received a Master Degree in *Plant and Microbe Biotechnology* with first class honours. In the same year he cofounded a Biotech company in collaboration with the Plant Pathology Department of the University of Pisa and from 2008 to 2010, he worked as team leader on the project: *Use of microorganisms for the biological remediation of textile effluent wastewater*. He identified the fungus *Fusarium oxysporum* as a microbial agent able to degrade and detoxify a new class of industrial dye with azoic structure employed in the textile industry. Aimone obtained the Italian academic degree of *Doctor in Biotechnology* from the University of Pisa.

In 2010 he moved back to the Max Planck Institute as Ph.D *Marie Curie fellow* where he used genetic and molecular biology approaches to characterize the functions of the plant growth regulator gibberellin in the development and flowering of *Arabidopsis thaliana*.