The Plant Cell, Vol. 18, 2172–2181, September 2006, www.plantcell.org

GA_4 Is the Active Gibberellin in the Regulation of *LEAFY* Transcription and *Arabidopsis* Floral Initiation [W]

Sven Eriksson, Henrik Böhlenius, Thomas Moritz, and Ove Nilsson[1]

Umeå Plant Science Centre, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-90183 Umeå, Sweden

Flower initiation in *Arabidopsis thaliana* under noninductive short-day conditions is dependent on the biosynthesis of the plant hormone gibberellin (GA). This dependency can be explained, at least partly, by GA regulation of the flower meristem identity gene *LEAFY* (*LFY*) and the flowering time gene *SUPPRESSOR OF CONSTANS1*. Although it is well established that GA_4 is the active GA in the regulation of *Arabidopsis* shoot elongation, the identity of the GA responsible for the regulation of *Arabidopsis* flowering has not been established. Through a combination of GA quantifications and sensitivity assays, we show that GA_4 is the active GA in the regulation of *LFY* transcription and *Arabidopsis* flowering time under short-day conditions. The levels of GA_4 and sucrose increase dramatically in the shoot apex shortly before floral initiation, and the regulation of genes involved in GA metabolism suggests that this increase is possibly due to transport of GAs and sucrose from outside sources to the shoot apex. Our results demonstrate that in the dicot *Arabidopsis*, in contrast with the monocot *Lolium temulentum*, GA_4 is the active GA in the regulation of both shoot elongation and flower initiation.

INTRODUCTION

Arabidopsis thaliana is a facultative long-day plant, meaning that it flowers more rapidly under long-day conditions than under short days. During the last decade, we have gained detailed knowledge about the molecular mechanisms whereby *Arabidopsis* senses long days and how this perception is translated into floral initiation (Yanovsky and Kay, 2003; Searle and Coupland, 2004). In comparison, we have much poorer knowledge about how flowering time is controlled under noninductive short-day conditions.

According to current models, *Arabidopsis* flowering is controlled by the interplay between three different pathways: the long-day pathway, which is responsible for the induction of flowering as a response to long days; the autonomous pathway, which controls flowering time under both long- and short-day conditions; and the gibberellin pathway, which is the most important for floral induction under short days (Mouradov et al., 2002; Boss et al., 2004; Putterill et al., 2004; Simpson, 2004). Under long days, the CONSTANS (CO) protein is stabilized by light, and this leads to induction of the floral activators *FLOWERING LOCUS T* (*FT*) and *SUPPRESSOR OF CONSTANS1* (*SOC1*) (Putterill et al., 1995; Samach et al., 2000; Suárez-López et al., 2001; Valverde et al., 2004). By contrast, the autonomous pathway genes mediate their activity through the floral repressor FLOWERING LOCUS C, which represses the transcription of both *FT* and *SOC1* (Michaels and Amasino, 1999, 2001; Sheldon et al., 1999; Samach et al., 2000; Searle et al., 2006).

Arabidopsis plants that are unable to synthesize the growth hormone gibberellic acid (GA), such as the mutant *ga1-3*, fail to flower when grown under short days (Wilson et al., 1992), suggesting that GAs play a central role in the control of flower initiation under short days, a role that is much less important under long days, in which the flowering of *ga1-3* is only marginally delayed (Wilson et al., 1992; Reeves and Coupland, 2001). It has been shown that one of the reasons why *ga1-3* mutants fail to flower under short days is because they cannot upregulate expression of the flower meristem identity gene *LEAFY* (*LFY*) (Blázquez et al., 1998). The GA effect on *LFY* is mediated through a GA-response site in the *LFY* promoter with similarities to a GA-myb binding site, and when this site is mutated, a minimal *LFY* promoter fails to be upregulated in short days but still responds to the long-day signal (Blázquez and Weigel, 2000). Furthermore, *LFY* expression from a constitutive promoter induces flowering in a *ga1-3* mutant background (Blázquez et al., 1998), proving that *LFY* acts downstream of the GA signal. This is also true for *SOC1*, which is also downregulated in a *ga1-3* background and can induce flowering when constitutively expressed (Moon et al., 2003). However, although *soc1* mutants are late flowering in short days, they are not delayed to such a dramatic extent as *ga1-3* mutants (Onouchi et al., 2000). In the absence of data showing the extent to which *SOC1* acts upstream of *LFY*, currently available data suggest that GAs regulate flowering in short days by regulating both *LFY* and *SOC1* and that the regulation of these genes is at least partly independent (Lee et al., 2000; Moon et al., 2003).

LFY is expressed in young leaf primordia and is gradually upregulated during growth under short days until the plant reaches a threshold level when floral initiation is induced (Blázquez et al., 1997). It is not known whether this gradual increase in *LFY*

[1] To whom correspondence should be addressed. E-mail ove.nilsson@genfys.slu.se; fax 46-90-786-8165.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Ove Nilsson (ove.nilsson@genfys.slu.se).
[W] Online version contains Web-only data.
www.plantcell.org/cgi/doi/10.1105/tpc.106.042317

(and *SOC1*) activity is caused by a concomitant increase in the shoot apical levels of active GAs. However, in a *ga1-3* mutant background, this gradual upregulation does not occur (Blázquez et al., 1998).

The later steps in the production of biologically active GAs are catalyzed by a set of 2-oxoglutarate–dependent dioxygenases (Figure 1). The precursors of biologically active GAs GA_{53} and GA_{12} are converted in parallel pathways through three consecutive oxidations on C-20 by GA20-oxidase (GA20OX), leading to the production of GA_9 and GA_{20}, which are further oxidized on C-3 by GA3-oxidase (GA3OX) to form the bioactive GAs GA_4 and GA_1 (Figure 1) (Hedden and Phillips, 2000; Yamaguchi and Kamiya, 2000; Olszewski et al., 2002). Furthermore, the biologically active compounds GA_3, GA_5, and GA_6 can be derived from the precursor GA_{20}. The bioactive GA_1 and GA_4 can be deactivated through oxidation by GA2-oxidase (GA2OX) to the inactive GA_{34} and GA_8 (Figure 1) (Hedden and Phillips, 2000). The *Arabidopsis* genome contains multiple genes of the enzymes involved in these latter stages of GA metabolism. The GA20OX enzymes are encoded by five genes, *GA20OX1* to *GA20OX5* (Phillips et al., 1995; Hedden et al., 2001); the GA3OX by four genes, *GA3OX1* to *GA3OX4* (Hedden and Phillips, 2000), where *GA3OX1* and *GA3OX2* appear to be the most important (Mitchum et al., 2006); and the GA2OX by eight genes, *GA2OX1* to *GA2OX8* (Thomas et al., 1999; Hedden and Phillips, 2000; Schomburg et al., 2003), although *GA2OX5* does not appear to encode any functional protein (Hedden et al., 2001). High levels of bioactive GA can trigger a feedback mechanism that represses the expression of certain *GA20OX* and *GA3OX* genes and upregulates *GA2OX* (Hedden and Phillips, 2000). However, up until recently, it was unclear how plants perceive GA and how the GA signal is transduced to cause GA-regulated responses. GA signaling has been proposed to be repressed by the action of members in the DELLA subfamily of the GRAS regulatory protein family (Peng et al., 1997; Dill and Sun, 2001; King et al., 2001a). In response to GA, DELLA proteins are targeted for ubiquitination by SCF E3 ubiquitin ligase and subsequent degradation by the 26S proteosome (McGinnis et al., 2003; Sasaki et al., 2003). A recent pivotal discovery from rice (*Oryza sativa*) identified the protein GIBBERELLIN DWARF1 (GID1) to be a soluble receptor of GA that upon GA binding interacts with the rice DELLA protein SLENDER RICE1 (Ueguchi-Tanaka et al., 2005). GID1 exhibits high binding affinities toward biologically active GAs, whereas it has low affinity, or none at all, for biologically inactive GAs. In *Arabidopsis*, there are three orthologs to the rice protein GID1 (Ueguchi-Tanaka et al., 2005), and all these orthologs display a much higher binding activity to GA_4 than to any other bioactive GA (Nakajima et al., 2006). This finding corresponds well to previous data showing that GA_4 is the active GA in the regulation of *Arabidopsis* cell elongation and shoot growth (Talon et al., 1990; Xu et al., 1997; Cowling et al., 1998). However, there is still no proof that GA_4 is the active GA in the regulation of flowering. Indeed, in other species, such as the monocot *Lolium temulentum*, it has been shown that GA_5 and GA_6 are the active GAs in the induction of flowering, but they have very little effect on the regulation of stem elongation (King et al., 2001b, 2003), where instead, GA_4 shows high activity (Evans et al., 1990; King et al., 2001b). Therefore, it could be speculated that in *Arabidopsis*, GAs other than GA_4, such as GA_1, GA_3, GA_5, or GA_6, could be responsible for the regulation of flowering.

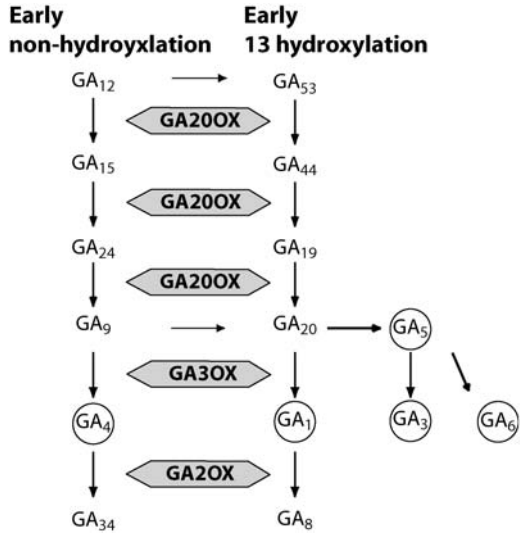

Figure 1. Overview of Gibberellin Metabolism in Higher Plants.

Bioactive GAs (circled) are biosynthesized from GA_{12} by oxidation of C-20 by GA20ox to GA_{20} and GA_9 followed by 3β oxidation by GA3ox. Bioactive GA_1 and GA_4 are inactivated by GA2 oxidation to GA_{34} and GA_8 by GA2ox.

Here, we show, by a combination of GA quantifications and sensitivity assays, that GA_4 is the active GA in the regulation of *LFY* transcription and, thus, in the regulation of *Arabidopsis* floral initiation under short-day conditions. We also show that during growth in short days, shoot apical levels of GA_4 and sucrose increase dramatically before floral initiation occurs and that the expression patterns of the genes involved in GA metabolism suggest that this increase in GA_4 possibly originates from sources outside the shoot apex.

RESULTS

Levels of GA_4 and Sucrose Increase before Floral Initiation

During growth in short days, *LFY* transcription is gradually upregulated in the young leaf primordia until the time of floral initiation (Blázquez et al., 1997, 1998) (Figure 2A). To investigate whether the levels of certain GAs also gradually increase in the shoot apical regions, we sampled microdissected shoot apices at weekly intervals until flowers could be seen visually. These shoot apical regions were delimited by the oldest leaf primordium expressing *LFY* to include all *LFY*-expressing tissues. In addition, to pinpoint the time of floral initiation, we quantified the levels of *APETALA1* (*AP1*) and *AP3* transcription in parallel samples. *AP1* transcription is one of the earliest markers for floral initiation and can be detected before formation of the floral primordium (Hempel et al., 1997). By contrast, *AP3* transcription is first seen in the developing floral primordium at stage 3 (Jack et al., 1992), approximately 2 d after the beginning of stage 1 (Smyth et al., 1990). *AP1* transcript could first be detected at very low levels on

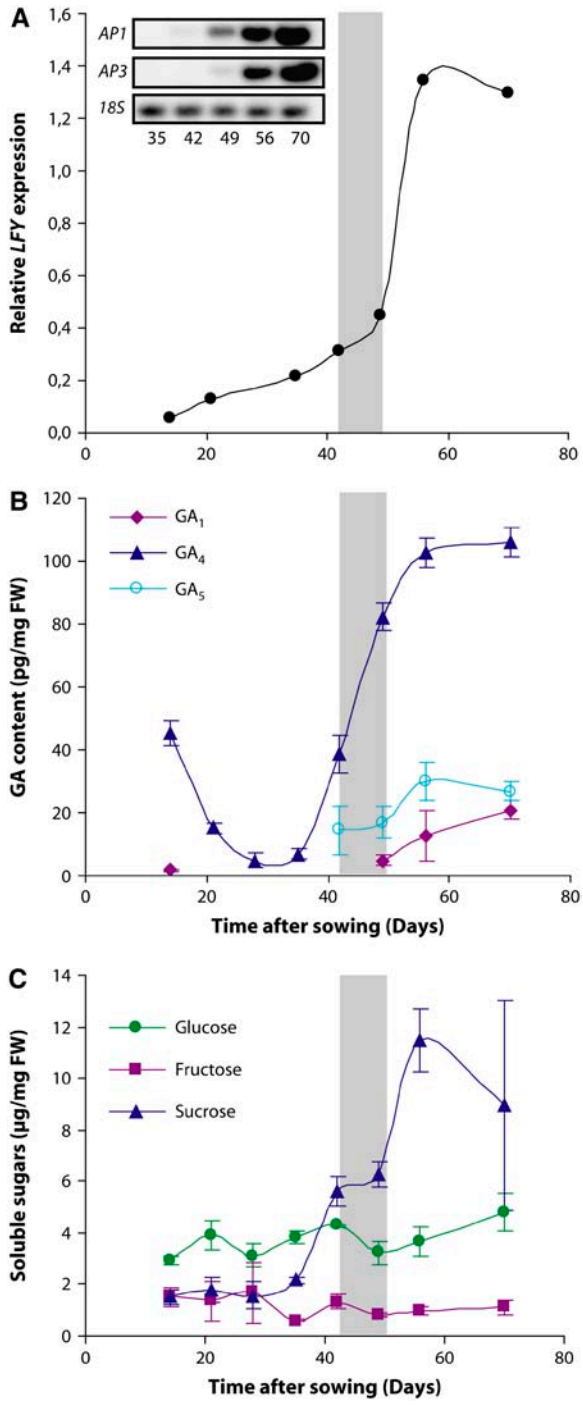

Figure 2. *LFY* Expression and GA and Sugar Quantifications during Flower Initiation.

(A) Real-time RT-PCR analysis of *LFY* expression in excised shoot apices during growth in short days. Estimated time of flower induction is marked by shaded area. Values are expressed relative to 18S rRNA. Inset: semiquantitative RT-PCR analysis of *AP1* and *AP3* expression in excised shoot apices during growth in short days. The 18S rRNA was amplified as an internal control. *AP1* can be detected from day 42 and *AP3* from day 49. Numbers indicate days after sowing.

day 42 and more strongly on day 49, while *AP3* transcription was first seen on day 49 and more clearly on day 56 (Figure 2A). The relative timing of upregulation is consistent with our observations that the first flower primordia could be seen using a stereomicroscope at day 56, and the first flower buds were visible to the naked eye at day 63 (data not shown). From these results, we conclude that floral initiation takes place between days 42 and 49.

The quantification of the GA content in the shoot apices showed that, of all tested GAs, GA_4 was present at the highest level at all time points (Figure 2B). The quantification of GA_4 in the shoot apices showed that young plants had a relatively high level of GA_4, but the levels subsequently dropped to very low levels for 2 to 3 weeks (Figure 2B). The initial high levels of GA_4 could presumably be related to the rapid hypocotyl elongation that ends at about this time. Interestingly, just before floral initiation, between days 35 and 42, the shoot apical levels of GA_4 increased dramatically and continued to rise until they reached ~100-fold higher levels by day 56. Thereafter, the levels of GA_4 stayed at constantly high levels. The levels of the equally bioactive GA_3 were nondetectable in this investigation but have in an earlier study of the Landsberg *erecta* line of *Arabidopsis* been found to be in the range of 2 to 10 pg/g fresh weight (FW) (T. Moritz, unpublished results). This was at least 1000-fold lower than the levels of GA_4 in that investigation and provides a possible explanation to why Talon et al. (1990) did not identify GA_3 in shoots of *Arabidopsis* in their large-scale identification of GAs. The shoot apical levels of sucrose followed a similar pattern to the levels of GA_4 (Figure 2C). The concentration of sucrose in the shoot apex stayed constant at ~1 to 2 μg/mg FW during most of the vegetative growth period. However, between days 35 and 42, the levels started to rise strongly and rose almost 10-fold to 12 μg/mg FW at day 56 (Figure 2C). By contrast, the levels of glucose and fructose remained unchanged during the floral transition (Figure 2C). This shows that the levels of both GA_4 and sucrose increase dramatically in the shoot apices, in the same tissues that express *LFY*, just before floral initiation under short-day conditions.

GA_4 Is the Relevant Endogenous GA Regulating *LFY* Transcription

Although GA_4 is the GA present at the highest level, it does not have to mean that GA_4 is the relevant endogenous GA inducing flowering in *Arabidopsis*. Previously it has been shown that GA_4 is more effective then GA_1 at shortening the time to visible flower buds of *Arabidopsis* in short days (Xu et al., 1997). In

(B) Gas chromatography–mass spectrometry (GC-MS) quantifications of the levels of GA_1, GA_4, and GA_5 in excised shoot apices during growth in short days. Microdissected shoot apices from 20 to 30 plants were pooled for each time point. Values are expressed as means ± SE of the mean ($n = 3$).

(C) GC-MS quantifications of the levels of soluble sugars in excised shoot apices during growth in short days. Microdissected shoot apices from 20 to 30 plants were pooled for each time point. Values are expressed as means ± SE of the mean ($n = 3$). Shaded area indicates estimated time for flower initiation.

L. temulentum, GA_5 and GA_6 have been proposed to be the GAs responsible for the induction of flowering (King et al., 2001b, 2003). To check if GA_5 and GA_6 or other GAs with biological activity could be involved in the regulation of flowering in *Arabidopsis*, we treated wild-type and GA-deficient *ga1-13* plants with GA during growth in short days. Only GA_3 and GA_4 significantly decreased the total number of leaves formed before flowering of the wild type (Figure 3) and induced flowering in the *ga1-13* mutant (Table 1). The effect of GA_4 on the wild type was the same as previously shown (Xu et al., 1997). To further investigate the effect of other GAs on flowering in short days, we chose to analyze the effect of application of GA on *LFY* transcription. Using this system, we determined dose–response curves for all the potentially bioactive GAs to evaluate their ability to induce a *LFY:*β*-glucuronidase* (*GUS*) transgene (Figure 4A). While increasing levels of the inactive GA_8 did not lead to increased GUS activities, all the potentially bioactive GAs were able to increase *LFY* transcription. GA_3 and GA_4 were the most active, followed by GA_1, GA_5, and GA_6 with the lowest activity. To be able to get better resolution on the activity of the different GAs we chose to repeat the dose–response experiment with a previously described in vitro assay (Blázquez et al., 1998). This in vitro assay is based on plant seedlings submerged in a medium and therefore allows a better control over the uptake and concentration dependence of the various added GAs. In spite of the different developmental stages of the plants between these two experiments, the result from the in vitro experiment (Figure 4B) was similar to the result from application of GA to shoot apices (Figure 4A), suggesting that GA regulation of *LFY* expression uses a similar mechanism in seedlings as in 4- to 8-week-old plants grown in short days. There were dramatic differences in the sensitivity to the various GAs. The most active GA was GA_4, which induced a half-maximum response at ~4 nM, while GA_1, GA_5, and GA_6 induced the same response at ~4 μM—a 1000-fold difference. GA_3 displayed an intermediate dose response with a half-maximum response at ~0.3 μM (Figure 4B). These data suggest that GA_4 and GA_3 are the most active endogenous GAs in terms of being able to induce *LFY* transcription. Taken together with the fact that GA_4 is present at significantly higher levels in the shoot apices during floral initiation than GA_3 (Figure 2B), we conclude that GA_4 is the relevant endogenous GA in the regulation of *LFY* transcription and the control of flowering time of short-day-grown *Arabidopsis* plants.

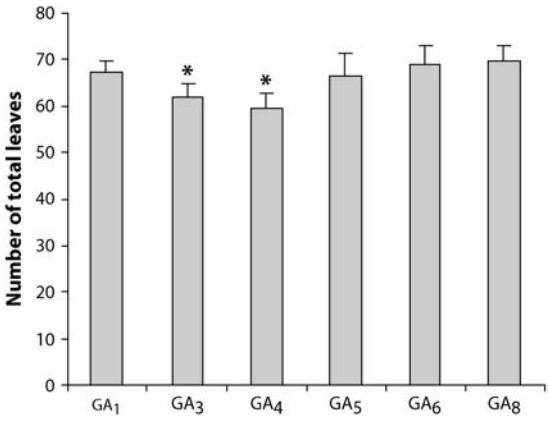

Figure 3. Flowering Time of Short-Day-Grown Plants after Treatment with Various GAs to the Shoot Apex.

Treatment was done from day 28, twice per week, until day 56. Time to flowering was determined as the total number of leaves in the primary shoot. Values are shown as the means ± 2 × SE of the mean ($n = 9$). Asterisks indicate values that are significantly different from the control (Student's t test, $P < 0.05$)

Table 1. Flowering Time of *ga1-13* Plants after GA Treatment

GA	Days
GA_1	>120[a]
GA_3	94
GA_4	90
GA_5	120
GA_6	>130[a]
GA_8	>130[a]

A single leaf was treated every third or fourth day with 5 μL of a 10 μM GA solution. Flowering time was determined as the time when 50% of the plants had visible flower buds.

[a] No plant had started to flower before the experiment was terminated.

Transcriptional Activity of Genes Involved in GA Metabolism

An interesting issue raised by the strong increases observed in the levels of GA_4 in the shoot apex is whether they are due to local changes in GA metabolism or if GAs are transported into the shoot apex from outside sources. Since transcription of the genes controlling GA metabolism is also subject to regulation by active GAs (Hedden and Phillips, 2000), the expression patterns of these genes can give valuable insights into the cause of changes in GA concentrations. While several of the *GA20OX* and *GA3OX* genes are negatively feedback regulated by active GAs, several of the *GA2OX* genes are positively regulated. When the transcriptional activity of the *GA20OX* genes was determined in parallel samples to those used to quantify GAs and sugars, it was found that the expression of these genes remained unchanged from day 35 to day 42 when the highest relative increase in GA levels was detected (Figure 5A). After day 42, the *GA20OX* expression started to gradually increase (Figure 5A). The same pattern was seen for the *GA3OX1* gene, the activity of which decreased at day 42 and then gradually increased until day 56 (Figure 5B). By contrast, the *GA3OX2* gene, which has been shown not to be feedback regulated by high levels of GAs (Yamaguchi et al., 1998), showed increased expression only at day 49 (Figure 5B). The activity of the *GA2OX* genes gradually increased from day 35 to day 56, with no significant down-regulation at day 42 (Figure 5C). Taken together, these data show that the increase in the shoot apical levels of GA_4 at day 42 can be explained neither by a local induction of *GA20OX* nor by a decrease in the activity of the *GA2OX*genes, suggesting that the dramatically increased amounts of GA_4 may be derived from sources outside the plant apex. It has already been demonstrated that tetradeuterated GA_5 can be transported from leaf to

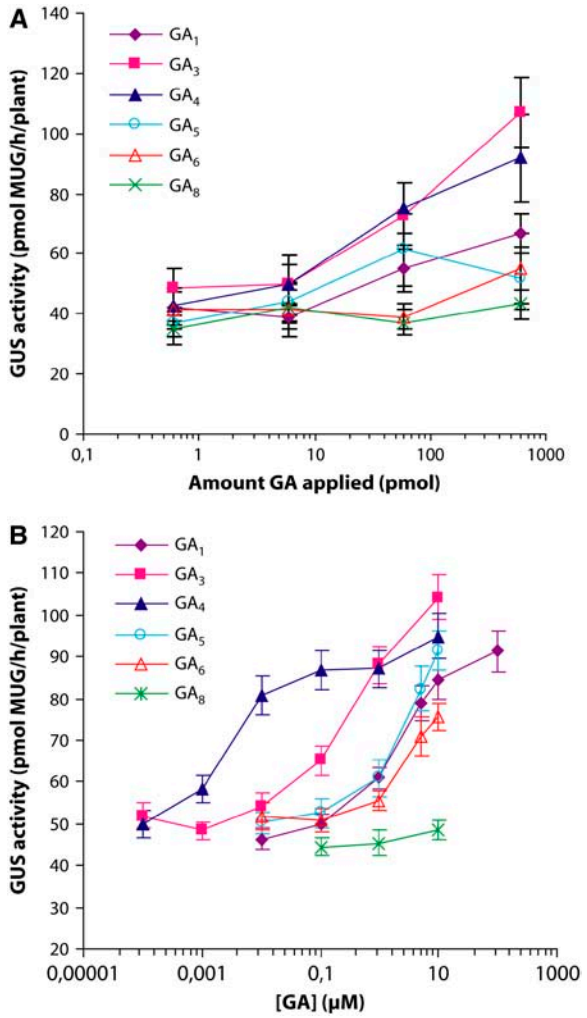

Figure 4. Dose–Response Curves for the Activation of *LFY:GUS* Expression by Different GAs.

(A) Dose–response curves for the activation of *LFY* by different GAs in short-day-grown plants. Twenty-four-day-old plants were treated three times, at 2-d intervals, by application of 20 μL of different concentrations of various GAs to the shoot apical part of the plant. Plants were then assayed for GUS activity. Values are shown as the means ± 2 × SE of the mean ($n = 24$).

(B) Three-day-old seedlings grown in half-strength Murashige and Skoog medium supplemented with 0.5% sucrose were treated with different concentrations of GA_1, GA_3, GA_4, GA_5, GA_6, and GA_8 for 3 d and then assayed for GUS activity. Values are shown as means ± 2 × SE of the mean ($n = 24$). MUG, 4-methylumbelliferyl β-D-glucuronide.

shoot apex in the grass *L. temulentum* (King et al., 2001b). To test the possibility of transport of GA from *Arabidopsis* leaves, we applied the bioactive GA_4 on a single leaf of wild-type plants and analyzed the effect on the total number of leaves formed by the plant. This reduced the number of leaves formed before flowering from 73.8 ± 4.9 to 63.6 ± 3.7 (mean ± SD, $n = 10$). Furthermore, after application of deuterium-labeled GA_4 to a single leaf, labeled GA_4 could be detected at the shoot apex (Table 2). Although not conclusive, given our poor knowledge about the location of GA biosynthesis and the nature of GA transport, these observations suggest that GAs can be transported from leaf to shoot apex to induce flowering both in *Lolium* and *Arabidopsis*.

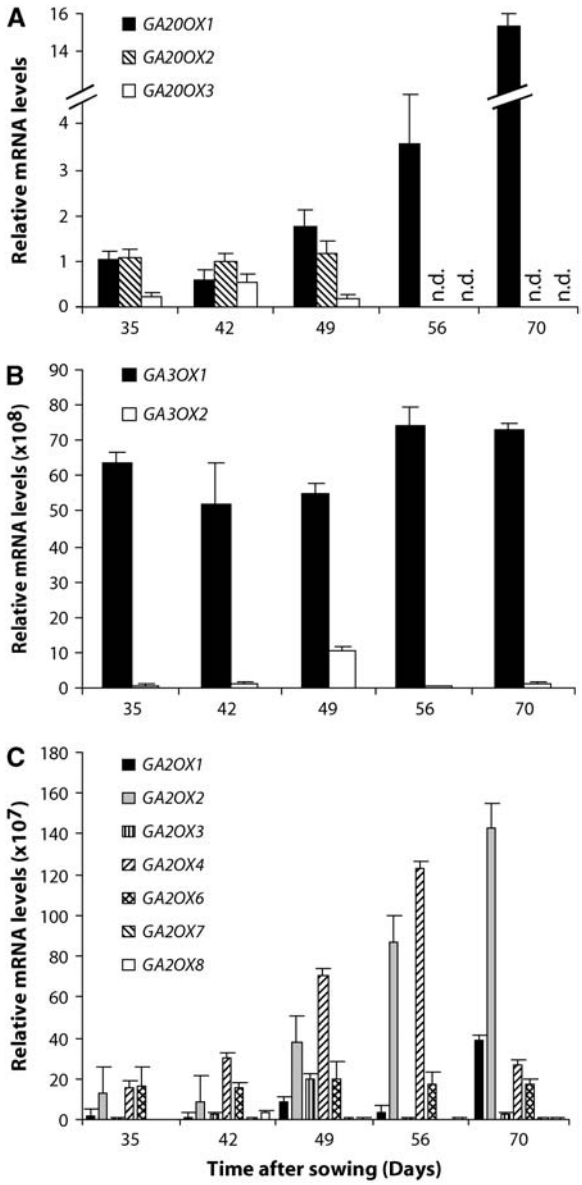

Figure 5. Expression of Genes Involved in GA Metabolism in Shoot Apices.

RT-PCR **(A)** and real-time RT-PCR (**[B]** and **[C]**) analyses of gene expression in excised shoot apices during growth in short days. Values are expressed relative to 18S rRNA.

(A) Expression of *GA20OX1*, *GA20OX2*, and *GA20OX3*. n.d., not determined.

(B) Expression of *GA3OX1* and *GA3OX2*.

(C) Expression of the *GA2OX* genes. Values are expressed as means ± SE of the mean ($n = 3$).

Table 2. Identification of Leaf-Fed Deuterated GA_4 in *Arabidopsis* Shoot Apices

Sample	Ratio (*m/z* 420→392)/(*m/z* 418→390)
Nonlabeled GA_4 standard	0.13
Shoot apices in $^2H_2$-GA_4 leaf-fed plants	0.21

$^2H_2$-GA_4 transport was detected by measuring endogenous and labeled GA_4 in apex samples and GA_4 standards with GC-MS in selected reaction monitoring mode and comparing the ratio of endogenous and labeled GA_4 in the sample and GA_4 standard. *m/z*, mass-to-charge ratio.

DISCUSSION

A hormonal response is always triggered by a combination of the concentration of a particular hormone and the plant's sensitivity to it. The concentration of a given hormone is determined by the balance between its biosynthesis, inactivation through catabolism or conjugation, and its transport in and out of the tissue concerned (Davies, 2004). The sensitivity to a hormone is determined by the concentration and activity of proteins involved in hormone reception and signal transduction. In order to identify the endogenous GA that is relevant for the regulation of *LFY* transcription (and thus flowering) in the *Arabidopsis* plants examined here, we have determined their sensitivity to, and endogenous concentrations of, various GAs that have been suggested to be biologically active.

Bioactive GAs Regulating Flowering

The result of the in vitro dose–response experiment (Figure 4B) clearly shows that in terms of the regulation of *LFY* expression, the plant is >100 times more sensitive to GA_4, with a half-maximal response at a concentration of ~4 nM, than to any other putatively active GA. GA_4 and GA_3 are clearly also the most active GAs in inducing flowering (Figure 3) and activating *LFY* transcription (Figure 4A) when applied to apices of short-day-grown plants. The reason why GA_3 displays the same relative activity as GA_4 in the experiment with the older plants could be that in this experiment the uptake and endogenous concentration of the applied GA is likely to be much less controlled. GA_3 is also much more stable than GA_4 since it is not the subject of degradation by GA2 oxidases, which could also result in a difference in endogenous cellular concentrations between the experiments. Nevertheless, the two experiments, although using very different experimental setups and with plants at different developmental stages, still show that GA_4 and GA_3 are more effective than any of the other GAs. It should be noted that the GA levels used in the dose–response experiment (0.1 nM to 10 μM) fall within the physiological range of GA_4 concentrations. We found that the shoot apical content of GA_4 varied between 2 and 110 pg/mg FW during growth in short days (Figure 2B). If one assumes that 90% of the fresh weight consists of water, this corresponds to an aqueous concentration of GA_4 in the range of 3 to 400 nM. Therefore, it is likely that the effects caused by the exogenous supply of GAs closely reflect those caused by changes in the endogenous levels.

At the time of flowering, the levels of GA_4 were found to be higher than those of any of the other bioactive GAs (Figure 2B). Taken together, the findings that GA_4 is the most potent activator of *LFY* transcription and that GA_4 is present at higher levels than any other bioactive GA at the time of flowering strongly suggest that GA_4 is the relevant active endogenous GA in the regulation of *LFY* transcription and, thus, *Arabidopsis* flowering during growth in short days. It is interesting to compare the situation in *Arabidopsis* with that described for grasses. In the grass *L. temulentum*, it has been shown that while GA_4 appears to be the active GA in the regulation of elongation growth, GA_5 and GA_6 are active in the regulation of flowering, and the concentrations of these GAs in the shoot apices double within 8 h of an inductive long-day treatment (King et al., 2001b, 2003). By contrast, our results show that GA_4 is the active GA in the regulation of both flower initiation and elongation growth in *Arabidopsis*. Therefore, it will be interesting to see if this represents a general distinction between monocot and dicot plants.

GA_4 and Sucrose as Mobile Signals Inducing Flowering

Our data show that the levels of GA_4 increase dramatically in the shoot apices of *Arabidopsis* plants just before flowering is initiated in short days (Figure 2B), just as GA_5 levels rise strongly after a short-day to long-day shift in *Lolium*. Given our findings here that GA_4 is the most active GA in the regulation of *LFY* transcription, it is likely that this increase in the levels of GA_4 is necessary for floral initiation to occur since a *ga1-3* GA biosynthesis mutant containing very low levels of GA_4, and also of other bioactive GAs (King et al., 2001a), fails to flower when grown in short days (Wilson et al., 1992). We also know that this failure to flower is, at least partially, caused by a failure to upregulate the expression of the GA-regulated genes *LFY* and *SOC1* (Blázquez et al., 1998; Moon et al., 2003). This sharp increase in the shoot apical levels of GA_4 is surprising since the plants were growing under constant short-day conditions with no environmental trigger for flower initiation. It seems that as the plants grow older they reach a critical age or size at which a rapid increase in the levels of GA in the shoot apex is triggered, regardless of the daylength. A correlation between a sharp rise in the shoot apical content of GAs and floral induction in response to a short-day to long-day shift has also been show in *Silene armeria* (Talon and Zeevaart, 1990).

It should be pointed out that our data show that there is no simple correlation between GA levels and the transcriptional activity of *LFY*. For instance, relatively high levels of GA_4 could be detected in the shoot apices of 14-d-old seedlings (Figure 2B), while the expression of *LFY* at this time is still very low (Figure 2A). This indicates that the young seedling is not as competent to respond to the same level of GA_4 as an older plant. This is further corroborated by the finding that even when short-day-grown wild-type or *ga1-3* plants are treated with GA throughout development, they still display a gradual increase in the expression of a *LFY:GUS* construct (Blázquez et al., 1998). However, since a *ga1-3* mutant in short days requires this GA treatment in order to display any signs of *LFY* upregulation, and the *LFY* upregulation is necessary for flowering (Blázquez et al., 1998), our data still strongly suggest that the dramatic increase in GA_4 levels that is

correlated with the *LFY* upregulation before floral initiation is relevant for the timing of flowering.

An interesting issue raised by the observed increases in active GA_4 is whether the additional amounts are synthesized locally in the shoot apex or are caused by a transport of GAs to the apex from other sources. The fact that transcription of the feedback-regulated *GA20OX* and *GA3OX* GA biosynthesis genes appears to be unaffected or reduced at the same time as the GA levels in the shoot apex start to rise (Figures 2B, 5A, and 5B), while expression of the feed-forward-activated *GA2OX* genes is increased (Figures 2B and 5C), suggests that the increased amounts of GA_4 detected in the shoot apex could be derived from the import of GAs from outside sources. This is further supported by the finding that GA_4 applications to a single leaf can induce early flowering of short-day-grown plants and that labeled GA_4 can move from leaf to shoot apex (Table 2). It is also interesting to note that at the same time that the levels of GA_4 started to increase, the shoot apical levels of sucrose also increased markedly (Figures 2B and 2C). Since the shoot apex is a very strong sink for nutrients and has very limited photosynthetic capacity compared with developed source leaves, the increased amounts of sucrose are most likely derived from outside sources. It is possible that the GAs, like sucrose, are transported from source leaves to the shoot apical meristem sink via the phloem (Bernier and Périlleux, 2005).

Recent data suggest that the classic florigen signal moving from leaf to shoot apex to induce flowering can be explained by a movement of the *FT* mRNA through the phloem (Abe et al., 2005; Huang et al., 2005; Wigge et al., 2005). This movement is responsible for the long-day-induced flowering in *Arabidopsis*.

Interestingly, it has been suggested that sucrose, like GAs, may also act as a signaling molecule in flowering regulation. There is a synergistic interaction between GAs and sucrose in the activation of *LFY* transcription (Blázquez et al., 1998), and sucrose supplied to the shoot apex has been shown to complement the late-flowering phenotype of the *Arabidopsis* mutants *co*, *gi*, *fca*, *fpa*, and *fve* (Roldán et al., 1999). The only late-flowering mutant that could not be rescued by sucrose in the cited study was *ft* (Roldán et al., 1999). Taken together, these results suggest that *Arabidopsis* flowering time in noninductive short-day conditions is determined by sharp increases in the shoot apical levels of GA_4 and sucrose just before flower initiation. These increases could possibly be caused by increased transport of GAs and sucrose from the source leaves coupled to an opening of the plasmodesmatal connections between the end of the phloem and the shoot apical meristem, as demonstrated for long-day-induced flowering (Ormenese et al., 2000). According to this hypothesis, GAs and sucrose can be seen as part of a short-day florigenic signal moving from the leaf to the shoot preceding flower induction.

Functional Redundancy between GAs and CO/FT

Another interesting issue to consider is the relevance of these findings to long-day-induced flowering in *Arabidopsis*. In spinach (*Spinacia oleracea*) shoots, it has been shown that the levels of GAs increase dramatically after a short-day to long-day shift (Talon et al., 1991). However, this shift, in both *Arabidopsis* and spinach, induces very rapid stem elongation (bolting), which is clearly a GA-regulated process. Since this occurs very close in time to flower initiation, it is impossible to tell if the increase in GAs is associated with flower initiation, bolting, or both. In short days, flower initiation and bolting is separated by several weeks (Figure 2A), and we can therefore say that the pronounced increase in GAs seen under short days is much more closely correlated to flower initiation than to bolting. In fact, GAs seem to have a very marginal role in the regulation of flowering time in long days since flowering is only slightly delayed in GA biosynthesis and signal transduction mutants under these conditions (Wilson et al., 1992). Instead, it seems that the role of GAs in short days is taken over by the flowering activators CO and FT in long days (Searle and Coupland, 2004) since in a *co* mutant, GAs are necessary for flowering and *LFY* regulation in long days (Blázquez and Weigel, 2000). This functional redundancy between GAs and CO/FT is interesting from many perspectives. Both GAs and CO/FT regulate *SOC1*, and both GAs and CO/FT seem to be associated with a mobile flower-inducing signal that moves from the leaf to the shoot apex. Further investigations will help to determine the molecular basis of this functional redundancy and help to establish the relationship between the activities of GAs and CO/FT in the regulation of flower initiation.

METHODS

Plant Growth and Material

The *Arabidopsis thaliana LFY:GUS* line DW150.209 (Blázquez et al., 1997) of the Columbia-0 (Col-0) ecotype was used as wild-type control in this study. The *ga1-13* mutant was isolated by searching the SALK institute Genomic Analysis Laboratory T-DNA express database for *ga1* T-DNA insertion mutants in the Col-0 background (Alonso et al., 2003). T3 seeds corresponding to SALK_109115 were requested from the Nottingham Arabidopsis Stock Centre.

Growth conditions consisted of short days (9 h of light from fluorescent lamps at 130 μmol m^{-2} s^{-1} and 15 h of darkness) and long days (16 h of light and 8 h of darkness) at 23°C.

Seeds were stratified in 0.1% agarose for 2 d before planting. Plants for GA, sugar, and RNA extraction were grown on a 3:1 mixture of soil and vermiculite in short days. For sampling of shoot apices, samples were collected once per week, in the middle of the photoperiod, from 2 weeks after planting until a majority of the plants had started to bolt. Shoot apices carrying leaves smaller than 1 mm were collected from 20 to 30 plants for each sample. Four equal samples, weighing from 4 to 10 mg, were collected per time point. Three samples were randomly selected for GA/sugar quantification and one sample for mRNA extraction. The whole growth experiment was repeated once with similar results.

GA Activity Measurements

The in vitro seedling assay for *LFY:GUS* activity was performed essentially as previously described (Blázquez et al., 1998), with the exception that seedlings were grown, 20 to 25 per well, in 12-well cell culture plates, in half-strength Murashige and Skoog medium supplemented with 0.5% sucrose. Three-day-old seedlings grown under long-day conditions were challenged with the addition of various amounts of different GAs (purchased from Lew Mander). *LFY:GUS* activity was analyzed 3 d later on 24 seedlings from each treatment.

Activity measurements on plants grown on soil in short day were done by application of 20 μL of various concentrations of different GAs three

times, with 2-d intervals, to the apical region of the plant. GUS activity was analyzed the day after the last application on 24 shoot apices from each treatment.

GA applications for determination of the effect on flowering time were done by applying a solution of GA in 20% ethanol to the shoot apical region, or a single leaf, of individual wild-type Col plants or *ga1-13* plants every third or fourth day. For *ga1-13* plants, a single leaf was treated with 5 μL of a 10 μM GA solution from day 25, until the first flowers buds could be seen. Flowering time was determined as the time when 50% of the plants had visible flower buds. Wild-type Col plants were treated with 20 μL of a 10 μM GA solution from day 22 until day 49 either on leaves or on the shoot apex. Flowering time was scored as the total number of leaves formed on the primary shoot. Calculation of statistical significance was done with a Student's *t* test assuming equal means.

GA and Sugar Quantifications

Three replicate samples were analyzed for each time point. Samples (4 to 10 mg) were homogenized and extracted for 2 h in 500 μL 80% methanol, including $^{2}H_{2}$-GAs (purchased from Lew Mander), D-Sucrose-$^{13}C_{12}$ (Larodan Fine Chemicals), D-glucose-1,2-$^{13}C_{2}$, and D-fructose-2-$^{13}C_{1}$ (Cambridge Isotope Laboratories) as internal standards. Fifty microliters of each extract was removed, evaporated to dryness, and methoxymated and trimethylsilylated for quantification of soluble sugars by GC-MS (Uggla et al., 2001).

The remaining extract was evaporated to dryness in vacuo. The residue was dissolved in 50 μL 80% methanol, mixed with 500 μL hexane, and loaded onto a pre-equilibrated Si ISOLUTE cartridge (Sorbent). The column was washed with 2 mL hexane and 2 mL ethyl acetate prior to elution with 2 mL methanol (1% HOAc). The eluate was evaporated to dryness, methylated, purified by HPLC, and analyzed by GC-MS in selected reaction monitoring mode using a JEOL JMS MStation as described earlier (Peng et al., 1999).

RNA Isolation and Analysis by PCR

Total RNA was isolated from plant shoot apices using RNaqueous-4PCR (Ambion) and treated with DNA-free DNase treatment and removal reagents (Ambion) to remove genomic DNA. Absence of genomic DNA contamination in the DNase I–treated RNA samples was verified by PCR using *LFY* primers. Two micrograms of total RNA was subjected to reverse transcription with a first-strand cDNA synthesis kit (Amersham Biosciences) with 200 pg of random hexamer primers. *GA20OX1*, *GA20OX2*, *GA20OX3*, *AP1*, *AP3*, and *LFY* transcription was analyzed with RT-PCR using QuantumRNA 18S internal standard as control (Ambion). The PCR program used was 94°C for 1 min, 94°C for 10 s, 55°C for 30 s, and 72°C for 30 s. Steps 2 to 4 were repeated 33 times. Products were labeled during PCR with DIG (Roche), and fragments were separated on agarose gels and blotted onto Hybond N^{+} membranes (Amersham Biosciences). Membranes were probed with Anti-Digoxigenein-AP Fab fragment (Roche) incubated with ECF substrate (Amersham Biosciences), and the resulting signals were detected using a Typhoon 9410 workstation (Amersham Biosciences). All amplifications resulted in a single product of the expected size, except for the *GA20ox* genes, where two splice variants are amplified. All quantifications were performed on the shorter, completely spliced product.

Expression of the *GA3OX* and *GA2OX* genes was analyzed with real-time RT-PCR in a BIO-RAD Mycycler using Syber Green Supermix (Bio-Rad) as previously described (Norberg et al., 2005). The 18S rRNA was amplified as a loading control. All amplifications generated a single product. Relative expression levels were calculated with Q-Gene software (Muller et al., 2002). Primer sequences can be found in Supplemental Table 1 online.

Accession Numbers

Arabidopsis Genome Initiative locus identifiers for the genes mentioned in this article are as follows: *AP1* (At1g69120); *AP3* (At3g54340); *GA1* (At4g02780); *GA2OX1* (At1g78440); *GA2OX2* (At1g30040); *GA2OX3* (At2g34555); *GA2OX4* (At1g47990); *GA2OX6* (At1g02400); *GA2OX7* (At1g50960); *GA2OX8* (At4g21200); *GA3OX1* (At1g15550); *GA3OX2* (At1g80340); *GA20OX1* (At4g25420); *GA20OX2* (At5g51810); *GA20OX3* (At5g07200); and *LFY* (At5g61850).

Supplemental Data

The following material is available in the online version of this article.

Supplemental Table 1. Sequence of Forward and Reverse Primers Used for Quantification of mRNA by RT-PCR.

ACKNOWLEDGMENTS

We thank Inga-Britt Carlsson for excellent technical assistance and Andy Phillips for providing gene sequence information. This work was supported by grants to O.N. and T.M. from the Swedish Natural Science Research Council and the Swedish Foundation for Strategic Research.

Received March 7, 2006; revised July 11, 2006; accepted July 26, 2006; published August 18, 2006.

REFERENCES

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.

Alonso, J.M., et al. (2003). Genome-wide insertional mutagenesis of *Arabidopsis thaliana*. Science **301,** 653–657.

Bernier, G., and Périlleux, C. (2005). A physiological overview of the genetics of flowering time control. Plant Biotechnol. J. **3,** 3–16.

Blázquez, 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.

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

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

Boss, P.K., Bastow, R.M., Mylne, J.S., and Dean, C. (2004). Multiple pathways in the decision to flower: Enabling, promoting, and resetting. Plant Cell **16,** S18–S31.

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.

Davies, P.J. (2004). Plant Hormones: Biosynthesis, Signal Transduction, Action! (Berlin: Springer).

Dill, A., and Sun, T.-p. (2001). Synergistic derepression of gibberellin signaling by removing RGA and GAI function in *Arabidopsis thaliana*. Genetics **159,** 777–785.

Evans, L.T., King, R.W., Chu, A., Mander, L.N., and Pharis, R.P. (1990). Gibberellin structure and florigenic activity in *Lolium temulentum*, a long-day plant. Planta **182,** 97–106.

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

Hedden, P., Phillips, A.L., Rojas, M.C., Carrera, E., and Tudzynski, B. (2001). Gibberellin biosynthesis in plants and fungi: A case of convergent evolution? J. Plant Growth Regul. **20,** 319–331.

Hempel, F.D., Weigel, D., Mandel, M.A., Ditta, G., Zambryski, P.C., Feldman, L.J., and Yanofsky, M.F. (1997). Floral determination and expression of floral regulatory genes in *Arabidopsis*. Development **124,** 3845–3853.

Huang, T., Böhlenius, H., Eriksson, S., Parcy, F., and Nilsson, O. (2005). The mRNA of the *Arabidopsis* gene *FT* moves from leaf to shoot apex and induces flowering. Science **309,** 1694–1696.

Jack, T., Brockman, L.L., and Meyerowitz, E.M. (1992). The homeotic gene *APETALA3* of *Arabidopsis thaliana* encodes a MADS box and is expressed in petals and stamens. Cell **68,** 683–697.

King, K.E., Moritz, T., and Harberd, N.P. (2001a). Gibberellins are not required for normal stem growth in *Arabidopsis thaliana* in the absence of GAI and RGA. Genetics **159,** 767–776.

King, R.W., Evans, L.T., Mander, L.N., Moritz, T., Pharis, R.P., and Twitchin, B. (2003). Synthesis of gibberellin GA_6 and its role in flowering of *Lolium temulentum*. Phytochemistry **62,** 77–82.

King, R.W., Moritz, T., Evans, L.T., Junttila, O., and Herlt, A.J. (2001b). Long-day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. Plant Physiol. **127,** 624–632.

Lee, H., Suh, S.S., Park, E., Cho, E., Ahn, J.H., Kim, S.G., Lee, J.S., Kwon, Y.M., and Lee, I. (2000). The AGAMOUS-LIKE 20 MADS domain protein integrates floral inductive pathways in *Arabidopsis*. Genes Dev. **14,** 2366–2376.

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., 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.

Michaels, S.D., and Amasino, R.M. (2001). Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. Plant Cell **13,** 935–941.

Mitchum, M.G., Yamaguchi, S., Hanada, A., Kuwahara, A., Yoshioka, Y., Kato, T., Tabata, S., Kamiya, Y., and Sun, T.-p. (2006). Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J. **45,** 804–818.

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.

Mouradov, A., Cremer, F., and Coupland, G. (2002). Control of flowering time: Interacting pathways as a basis for diversity. Plant Cell **14,** S111–S130.

Muller, P.Y., Janovjak, H., Miserez, A.R., and Dobbie, Z. (2002). Processing of gene expression data generated by quantitative real-time RT-PCR. Biotechniques **32,** 1372–1379.

Nakajima, M., et al. (2006). Identification and characterization of Arabidopsis gibberellin receptors. Plant J. **46,** 880–889.

Norberg, M., Holmlund, M., and Nilsson, O. (2005). The *BLADE ON PETIOLE* genes act redundantly to control the growth and development of lateral organs. Development **132,** 2203–2213.

Olszewski, N., Sun, T.-p., and Gubler, F. (2002). Gibberellin signaling: Biosynthesis, catabolism, and response pathways. Plant Cell **14,** S61–S80.

Onouchi, H., Igeno, M.I., Périlleux, C., Graves, K., and Coupland, G. (2000). Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among Arabidopsis flowering-time genes. Plant Cell **12,** 885–900.

Ormenese, S., Havelange, A., Deltour, R., and Bernier, G. (2000). The frequency of plasmodesmata increases early in the whole shoot apical meristem of *Sinapis alba* L. during floral transition. Planta **211,** 370–375.

Peng, J., 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 Dev. **11,** 3194–3205.

Peng, J., Richards, D.E., Moritz, T., Cano-Delgado, A., and Harberd, N.P. (1999). Extragenic suppressors of the Arabidopsis *gai* mutation alter the dose–response relationship of diverse gibberellin responses. Plant Physiol. **119,** 1199–1208.

Phillips, A.L., Ward, D.A., Uknes, S., Appleford, N.E., Lange, T., Huttly, A.K., Gaskin, P., Graebe, J.E., and Hedden, P. (1995). Isolation and expression of three gibberellin 20-oxidase cDNA clones from Arabidopsis. Plant Physiol. **108,** 1049–1057.

Putterill, J., Laurie, R., and Macknight, R. (2004). It's time to flower: The genetic control of flowering time. Bioessays **26,** 363–373.

Putterill, J., Robson, F., Lee, K., Simon, R., and Coupland, G. (1995). The *CONSTANS* gene of Arabidopsis promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell **80,** 847–857.

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.

Roldán, M., Gómez-Mena, C., Ruiz-García, L., Salinas, J., and Martínez-Zapater, J.M. (1999). Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering of *Arabidopsis* in the dark. Plant J. **20,** 581–590.

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.

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

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.

Searle, I., and Coupland, G. (2004). Induction of flowering by seasonal changes in photoperiod. EMBO J. **23,** 1217–1222.

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 Dev. **20,** 898–912.

Sheldon, C.C., Burn, J.E., Perez, P.P., Metzger, J., Edwards, J.A., Peacock, W.J., and Dennis, E.S. (1999). The *FLF* MADS box gene: A repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell **11,** 445–458.

Simpson, G.G. (2004). The autonomous pathway: Epigenetic and post-transcriptional gene regulation in the control of *Arabidopsis* flowering time. Curr. Opin. Plant Biol. **7,** 570–574.

Smyth, D.R., Bowman, J.L., and Meyerowitz, E.M. (1990). Early flower development in *Arabidopsis*. Plant Cell **2,** 755–767.

Suárez-López, 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.

Talon, M., Koornneef, M., and Zeevaart, J.A. (1990). Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the

biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc. Natl. Acad. Sci. USA **87,** 7983–7987.

Talon, M., and Zeevaart, J.A.D. (1990). Gibberellins and stem growth as related to photoperiod in *Silene armeria* L. Plant Physiol. **92,** 1094–1100.

Talon, M., Zeevaart, J.A.D., and Gage, D.A. (1991). Identification of gibberellins in spinach and effects of light and darkness on their levels. Plant Physiol. **97,** 1521–1526.

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. Proc. Natl. Acad. Sci. USA **96,** 4698–4703.

Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.-y., Hsing, Y.-i., Kitano, H., Yamaguchi, I., and Matsuoka, M. (2005). *GIBBERELLIN INSENSITIVE DWARF1* encodes a soluble receptor for gibberellin. Nature **437,** 693–698.

Uggla, C., Magel, E., Moritz, T., and Sundberg, B. (2001). Function and dynamics of auxin and carbohydrates during earlywood/latewood transition in scots pine. Plant Physiol. **125,** 2029–2039.

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

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.

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.

Xu, Y.L., Gage, D.A., and Zeevaart, J. (1997). Gibberellins and stem growth in *Arabidopsis thaliana*. Plant Physiol. **114,** 1471–1476.

Yamaguchi, S., and Kamiya, Y. (2000). Gibberellin biosynthesis: Its regulation by endogenous and environmental signals. Plant Cell Physiol. **41,** 251–257.

Yamaguchi, S., Smith, M.W., Brown, R.G.S., Kamiya, Y., and Sun, T.-p. (1998). Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating Arabidopsis seeds. Plant Cell **10,** 2115–2126.

Yanovsky, M.J., and Kay, S.A. (2003). Living by the calendar: How plants know when to flower. Nat. Rev. Mol. Cell Biol. **4,** 265–276.

GA_4 Is the Active Gibberellin in the Regulation of *LEAFY* Transcription and *Arabidopsis* Floral Initiation

Sven Eriksson, Henrik Böhlenius, Thomas Moritz and Ove Nilsson

Plant Cell 2006;18;2172-2181; originally published online August 18, 2006;
DOI 10.1105/tpc.106.042317