Journal of Experimental Botany, Vol. 59, No. 14, pp. 3821–3829, 2008
doi:10.1093/jxb/ern232
This paper is available online free of all access charges (see http://jxb.oxfordjournals.org/open_access.html for further details)

Journal of Experimental Botany
www.jxb.oxfordjournals.org

RESEARCH PAPER

The nature of floral signals in *Arabidopsis*. II. Roles for *FLOWERING LOCUS T* (*FT*) and gibberellin

Tamotsu Hisamatsu* and Rod W. King†

CSIRO Plant Industry, GPO Box 1600, Canberra, ACT 2600, Australia

Received 9 June 2008; Revised 14 July 2008; Accepted 15 August 2008

Abstract

Signals produced in leaves are transported to the shoot apex where they cause flowering. Protein of the gene *FLOWERING LOCUS T* (*FT*) is probably a long day (LD) signal in *Arabidopsis*. In the companion paper, rapid LD increases in *FT* expression associated with flowering driven photosynthetically in red light were documented. In a far red (FR)-rich LD, along with FT there was a potential role for gibberellin (GA). Here, with the GA biosynthesis dwarf mutant *ga1-3*, GA_4-treated plants flowered after 26 d in short days (SD) but untreated plants were still vegetative after 6 months. Not only was *FT* expression low in SD but applied GA bypassed some of the block to flowering in *ft-1*. On transfer to LD, *ga1-3* only flowered when treated simultaneously with GA, and *FT* expression increased rapidly (<19.5 h) and dramatically (15-fold). In contrast, in the wild type in LD there was little requirement for GA for *FT* increase and flowering so its endogenous GA content was near to saturating. Despite this permissive role for endogenous GA in Columbia, RNA interference (RNAi) silencing of the GA biosynthesis gene, *GA 20-OXIDASE2*, revealed an additional, direct role for GA in LD. Flowering took twice as long after silencing the LD-regulated gene, *GA 20-OXIDASE2*. Such independent LD input by *FT* and GA reflects their non-sympatric expression (*FT* in the leaf blade and *GA 20-OXIDASE2* in the petiole). Overall, *FT* acts as the main LD floral signal in Columbia and GA acts on flowering both via and independently of *FT*.

Key words: *Arabidopsis*, far-red light, flowering, FT, gibberellin, c long day.l.

Introduction

Recent studies of floral signalling in *Arabidopsis* (Corbesier *et al*., 2007; Jaeger and Wigge, 2007; Mathieu *et al*., 2007), *Cucumis* spp (Lin *et al*. 2007), and rice (Tamaki *et al*., 2007) have indicated that *FLOWERING LOCUS T* (*FT*) could be involved in long day (LD) floral signalling, its protein acting as a signal transported from the photoinduced leaves to the shoot apex where it evokes flowering (see reviews in Kobayashi and Weigel, 2007; Turck *et al*., 2008).

The nature of the LD photoresponse(s) can be critical for understanding *FT* regulation of flowering. As documented in the companion paper (King *et al*., 2008), in a high light intensity LD from red light (R)-rich lamps, photosynthesis up-regulates *FT* expression and causes flowering of *Arabidopsis*. In contrast, an LD from far-red-rich lamps (LD-*FR*) up-regulates *FT*, causes flowering, and, in addition, increases biosynthesis of the gibberellin (GA) class of plant growth regulator (Xu *et al*., 1997; Gocal *et al*., 2001; Hisamatsu *et al*., 2005). Comparable FR-regulated LD increases in GA content have been widely reported for other species (see reviews by García-Martínez and Gil, 2002; King and Evans, 2003) so GA could act as an additional LD signal.

For the LD grass, *Lolium temulentum*, both GA and FT may regulate its flowering (King *et al*., 2006), but genetic analysis has not been possible. For *Arabidopsis*, in contrast, genetic studies do not implicate GA in the LD response but show that it is needed for flowering in short days (SD) (see reviews in Boss *et al*., 2004; Searle and Coupland, 2004; Imaizumi and Kay, 2006). The evidence of large increases in shoot tip GA during the transition to flowering in SD (Eriksson *et al*., 2006) is consistent with the genetic evidence, but none of these studies rule out a role for GA in LD flowering.

* Present address: National Institute of Floricultural Science (NIFS), Tsukuba, 305-8519 Japan
† To whom correspondence should be addressed. E-mail: rod.king@csiro.au
Abbreviations: *CO*, *CONSTANS*; FR, far red light; *FT*, *FLOWERING LOCUS T*; GA, gibberellin; LD, long day conditions; R, red light; RNAi, RNA interference; SD, short day conditions.

Here the contribution of *FT* and GA to LD flowering of *Arabidopsis* has been examined. Using genetic and molecular approaches, the potential for *FT* and GA to act both independently and interactively in LD floral signalling is documented.

Materials and methods

Plant material, growing conditions, and LD treatment

Plants of *Arabidopsis thaliana* (L.) Heynh., ecotype Columbia, mutants and RNA interference (RNAi) silencing lines were grown vegetatively for 5 weeks in 8 h SD at 22 °C under an irradiance of 100 μmol m^{-2} s^{-1} from fluorescent lamps. In the case of the *ga1-3* mutant, it was grown in these SD conditions for 3 months. When exposed to an LD for floral induction, the main 8 h light period was extended by 16 h to give a total of 24 h light. This single LD was at a low intensity (10 μmol m^{-2} s^{-1}) from incandescent bulbs (FR-enriched light; LD-*FR*) or from R-rich fluorescent lamps (LD-*lR*). In a few instances the LD exposure was for two cycles and involved a low intensity FR-rich LD or an LD from R-rich fluorescent lamps at 100 μmol m^{-2} s^{-1} (LD-*R*). Treatments where LD was for more than one cycle enhanced the response somewhat. More details of such LD treatments and responses are given in the companion paper. Plants of Columbia retained in SD remained vegetative for at least another 6 weeks, whereas those of *ga1-3* were still vegetative 3 months later.

Mutants and gene silencing lines were all in ecotype Columbia. The *ga1-3* mutant from Landsberg *erecta* had been backcrossed six times into Columbia (Tyler *et al*., 2004). The *GA 20-OXIDASE1* T-DNA insert null mutant, *ga5-3*, and RNAi silencing lines for *GA 20-OXIDASE2* were described in Hisamatsu *et al.* (2005). Subsequently this *ga5-3* mutant has been renamed by Rieu and co-workers (2007) as *ga20ox1-3* but, for continuity, the original terminology has been retained here. The *ft-1* mutant is described in the companion paper.

Chemical treatments

GA_4 (1 mM) was applied either as a 10 μl drop to three leaves or as a spray to run off. Response was similar in these treatments, and this is in accordance with the known transport of GA_4 in *Arabidopsis* (Ericksson *et al*., 2006). Control plants were treated with the same aqueous solvent containing 20% ethanol and 0.02% Tween-20. A commercially available GA biosynthesis inhibitor, paclobutrazol ([2S,3S; 2R,3R]-1-[4-chlorophenyl]-4,4-dimethyl-2-[1,2,4-triazol-l-yl] pentan-3-ol), was applied as a 6 ml pot drench at a dose of 0.05 mg ml^{-1} in water.

Errors are shown as means ±SE. In many instances the error was smaller than the symbol and is not evident in the figures. All experiments reported here were repeated at least once.

Quantitative real-time PCR analysis of gene expression

Conditions, primers, and materials for gene expression studies were as documented in the companion paper and previously by Hisamatsu *et al.* (2005).

Results

In the companion paper we detailed distinct LD light responses which trigger rapid and obligate flowering in *Arabidopsis*, ecotype Columbia. Briefly, in a high light intensity, R-rich LD, photosynthesis up-regulated *FT* expression and flowering while at a 10-fold lower intensity, an FR-rich LD acting independently of photosynthesis rapidly up-regulated *FT* and induced flowering. Plants in a low light intensity R-rich LD or in SD showed weak *FT* expression and remained vegetative for ⩾6 weeks.

Because an FR-rich LD activates GA biosynthesis in the petioles of Columbia (Gocal *et al*., 2001; Hisamatsu *et al*., 2005), three approaches have been used to examine potential GA/*FT* regulation of flowering. First, to determine if *FT* and endogenous GA might act in concert, GA biosynthesis has been blocked in a mutant or with a GA biosynthesis inhibitor. Secondly, GA regulation of flowering has been examined in application studies with Columbia and the *ft-1* mutant. Lastly, the role of GA biosynthesis in LD flowering has been examined by silencing a *GA 20-OXIDASE* genes along with analysis of tissue specificity of gene expression patterns.

Inhibition of GA synthesis, LD flowering, and a role for FT

The *GA1* gene of *Arabidopsis* regulates an early step of GA biosynthesis (Zeevaart and Talon, 1992), and the *ga1-3* mutant is dwarfed and flowers late in SD unless treated with GA over many weeks (Koorneef and van der Veen, 1980; Wilson *et al*., 1992; Putterill *et al*., 1995; Reeves and Coupland, 2001; Ericksson *et al*., 2006; Rieu *et al*., 2008). In LD, *ga1-3* can flower reasonably rapidly although with some delay relative to GA-treated LD plants (Koorneef and van der Veen, 1980; Wilson *et al*., 1992; Putterill *et al*., 1995; Reeves and Coupland, 2001; Ericksson *et al*., 2006; Rieu *et al*., 2008).

In the present studies, *ga1-3* in Columbia was vegetative and severely dwarfed after 6 months in 8 h SD (cf. Fig 1). It also failed to flower when exposed at 3 months to 30 LD either from FR-rich incandescent lamps or at a high intensity from R-rich fluorescent lamps (data not shown). However, for plants grown for 3 months in SD, the non-flowering, dwarf phenotype of *ga1-3* was completely and rapidly reversed by applying GA_4 twice over consecutive days with plants both held in SD and transferred to one or two LD (Fig 1). The same response was obtained after a single GA_4 application (not shown). Within 16 h of the first GA applications, the stem, petioles, and leaf blades began to elongate (not shown) and flower buds were visible within 7–9 d in LD and at 15 d in SD (photographed at 10 d in Fig. 1). This rapid response contrasts with findings with a *ga1* T-DNA mutant in Columbia which took 90 d to flower in SD when treated twice weekly with GA (Ericksson *et al*., 2006).

The present results also contrast with the rapid, GA-independent flowering of *ga1-3* exposed to LD from germination (Koorneef and van der Veen 1980; Wilson *et al*., 1992; Putterill *et al*., 1995; Reeves and Coupland,

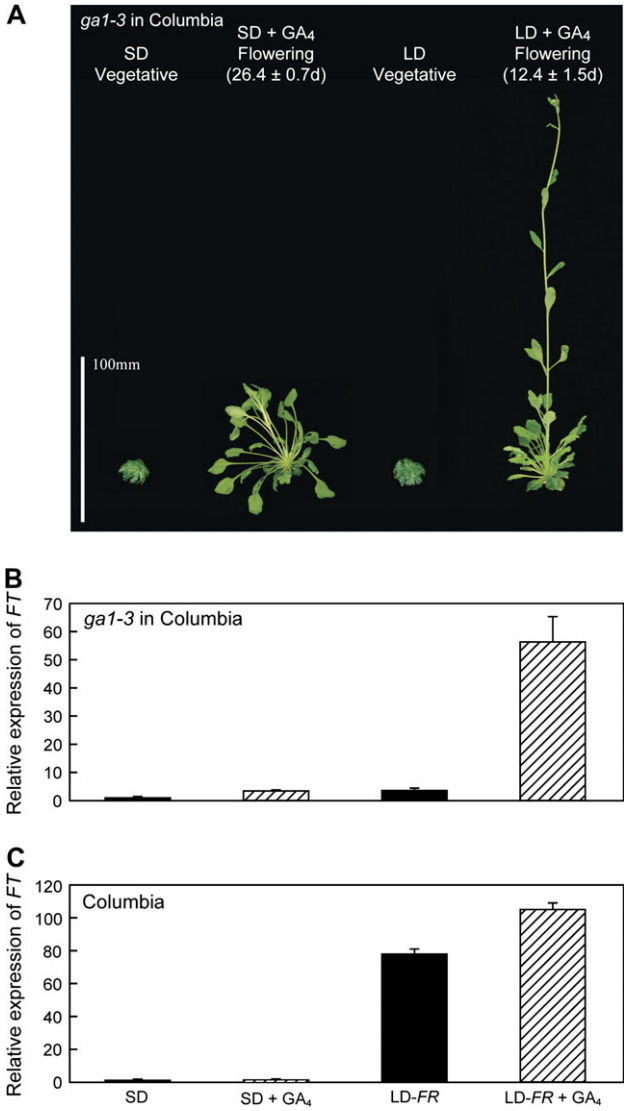

Fig. 1. GA_4 applied to *ga1-3* shows an *FT*-independent effect on flowering in SD and a permissive effect involving *FT* expression in LD. A 10 µl drop of GA_4 [1 mM in 20% ethanol:water (v:v)] was applied to each of three leaves on consecutive days either in SD or at the start of a far-red-rich LD (LD-*FR*). Plants of *ga1-3* flowered, bolted, and leaves grew (A). Its *FT* expression increased most after GA treatment in LD (B), and (C) shows the effect of GA_4 on *FT* expression in Columbia. Prior to treatment, the plants of *ga1-3* had been grown in SD for 12 weeks and those of Columbia for 5 weeks. The low intensity FR-rich LD exposure was for 2 d. GA_4 was applied 8 h after starting the day, and leaf blades were harvested 19.5 h later for assays of *FT* expression (leaves harvested at 16 h showed similar increases; not shown). There was no effect of solvent application on flowering or gene expression (not shown). All *FT* expression was normalized to the value in SD without GA application. The means and SE were based on three replicates for *FT* assays and 10 replicates for flowering time.

2001; Rieu *et al.*, 2008). GA_3 applied for germination of *ga1-3* may carry over to the plant (Y Kamiya, Riken, Kanagawa, Japan, personal communication) but probably not for the less stable GA_4 used here for germination. Furthermore, Reeves and Coupland (2001) and Rieu *et al.* (2008) showed that carryover was not important when they used seed coat removal, not GA, for germination. All the responses reported here for *ga1-3* were completely reproducible and there does not appear to be an explanation for the non-flowering in LD, but this may relate to environmental differences and the age of plants when first treated with GA or exposed to LD.

To examine the effect of GA on *FT* expression, leaf blades were harvested 19.5 h after GA_4 application, a time which matches high LD expression of *FT* in Columbia (see the companion paper). In LD, GA treatment increased *FT* expression 15-fold (Fig. 1B) and the plants flowered. In SD, flowering induced by GA was associated with a much smaller increase in *FT* (3.5-fold). Comparable responses were found for harvests at 16 h (not shown)

A crucial clue to explaining the GA effects on *FT* in LD is provided by comparison of its expression in *ga1-3* with that in Columbia (Fig. 1B versus C). LD up-regulation of *FT* in *ga1-3* required GA application, but an LD alone was sufficient for Columbia. The quite small increase in *FT* when GA was applied to Columbia in LD (35% increase) contrasts with the large increase in *ga1-3* (15-fold). Apparently, the high endogenous GA level in Columbia (>10 times that in *ga1*-3; Zeevaart and Talon, 1992; Xu *et al.*, 1997) permits *FT* expression in LD whereas the low GA level in *ga1-3* almost completely blocks *FT* expression.

In further support of a permissive role for GA in LD-regulated *FT* expression in Columbia (Fig. 1), a single application of paclobutrazol, an inhibitor of GA biosynthesis (Rademacher, 2000), completely blocked flowering in an FR-rich LD (Fig. 2). Paclobutrazol action was GA specific as its inhibition of flowering was completely reversed by a simultaneous application of GA_4 (data not shown).

Flowering was only inhibited when paclobutrazol was applied before the LD (Fig. 2) so GA is required for flowering; however, this evidence does not imply an LD increase in GA biosynthesis. Of the two LD light conditions used in this experiment, only the FR-rich LD increases GA biosynthesis (Hisamatsu *et al.*, 2005); however, flowering in high light, R-rich LD was also substantially inhibited by paclobutrazol yet this LD does not increase GA biosynthesis (Hisamastsu *et al.*, 2005) but acts by photosynthetic amplification of *FT* expression in the leaf blade (cf. companion paper).

As an aside, for a harvest of *ga1-3* at the same time that it was found that GA_4 increased FT expression (Fig. 1), there was no promotion of *SOC1* expression in the leaf blade (Fig. 3). Compared with the substantial GA/LD effect on *FT*, there were only small GA-dependent increases in *CONSTANS* (CO) expression and they were similar across all daylength and light quality conditions (Fig. 3 and data not shown). Nevertheless, the positive GA responsiveness of CO is consistent with its role in activation of *FT*. Circadian regulation of CO message and protein

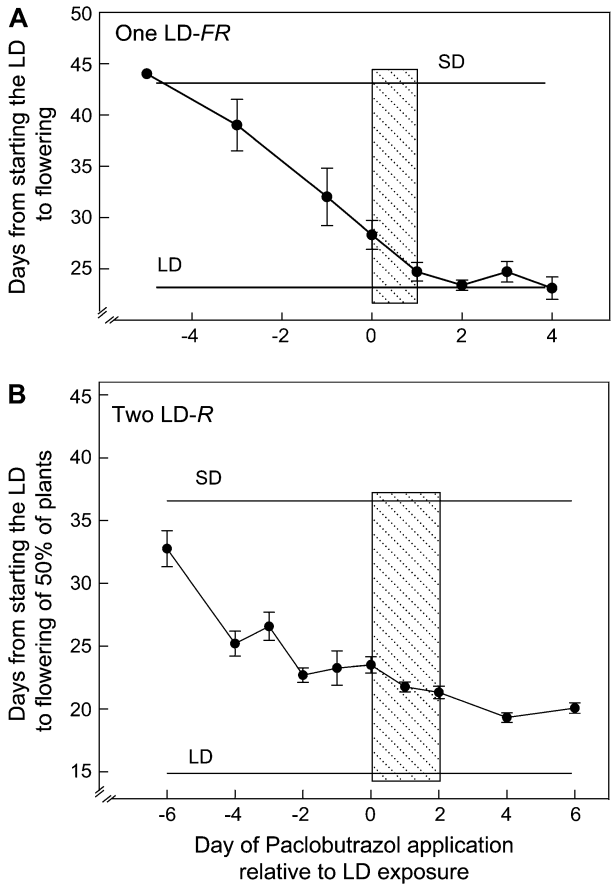

Fig. 2. Flowering is blocked by a GA biosynthesis inhibitor, paclobutrazol (PAC), if it is applied prior to an LD exposure. PAC was applied once as a soil drench at various times before or after the plants were exposed to: (A) a single FR-rich LD from incandescent lamps (*LD-FR*); or (B) two LD at high intensity from fluorescent lamps (LD-*R*). The shaded bar shows the LD exposure. The horizontal lines indicate flowering times of untreated plants exposed to one or two LD. The SD plants were vegetative when the experiment was terminated. The means and SE were based on 14 replicates in (A) and 16 in (B).

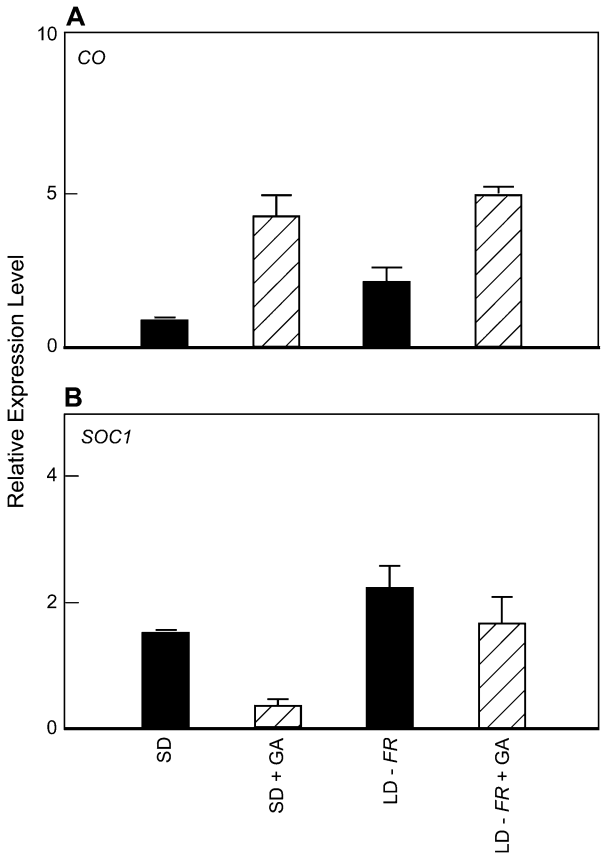

Fig. 3. Effect of daylength and GA on expression of *CO* and *SOC1* in the leaf blade of the *Arabidopsis ga1-3* mutant. A 1 mM solution of GA_4 was applied to the leaf blade of *ga1-3*. These assays were from the same experiment reported in Fig. 1. Comparable results were obtained in a second sample harvested at 16 h (not shown).

abundance may influence the extent of this GA regulation,c but such a study was beyond the scope of this work.

FT-independent regulation of flowering by GA

GA_4 promoted flowering of Columbia in all daylengths (Fig. 4). In SD, whereas 50% of the GA_4-treated plants had flowered after 42 d, only 12% of untreated plants had flowered by 52 d (Fig. 4A).This GA_4-reguated flowering in SD should be independent of *FT* because *FT* levels are low and GA_4 had little immediate effect on *FT* expression (Fig. 1C). The enhanced flowering with GA_4 treatment in LD (Fig. 4) might also be *FT* independent because there was only a small GA-induced *FT* increase (35%; Fig. 1). A more compelling argument for *FT*-independent action of GA is seen in the GA_4 reversal of the block to flowering in *ft-1*. In SD, 17% of GA_4-treated *ft-1* plants had flowered by 33 d, whereas all untreated plants were still vegetative (Fig. 4B). In LD, the effect of GA_4 on flowering of *ft-1* was even more dramatic; all treated plants had flowered by 15 d but none of the untreated *ft-1* controls in LD had flowered by 33 d. It is not clear why in SD GA_4-treated *ft-1* plants flowered earlier than GA_4 - treated plants of Columbia.

Taken together, these studies along with those with *ga1-3* (Fig. 1) highlight a complex coupling between daylength, GA, *FT*, and flowering. Below, to examine this coupling further, lines with restricted GA biosynthesis were used to examine LD-specific GA input.

Endogenous GA contributes to flowering in LD

To define the link between GA biosynthesis and flowering in an FR-rich LD, two LD-specific *GA 20-OXIDASE2* gene silencing lines were used and, as a negative control, a daylength-insensitive *GA 20-OXIDASE1* T-DNA mutant was used. These 20-oxidases control an important step in GA biosynthesis (Thomas and Hedden, 2006).

In the two *GA 20-OXIDASE2* RNAi silencing lines, flowering was delayed on exposure to two LD from FR-rich incandescent lamps (Fig. 5A, B). These two lines

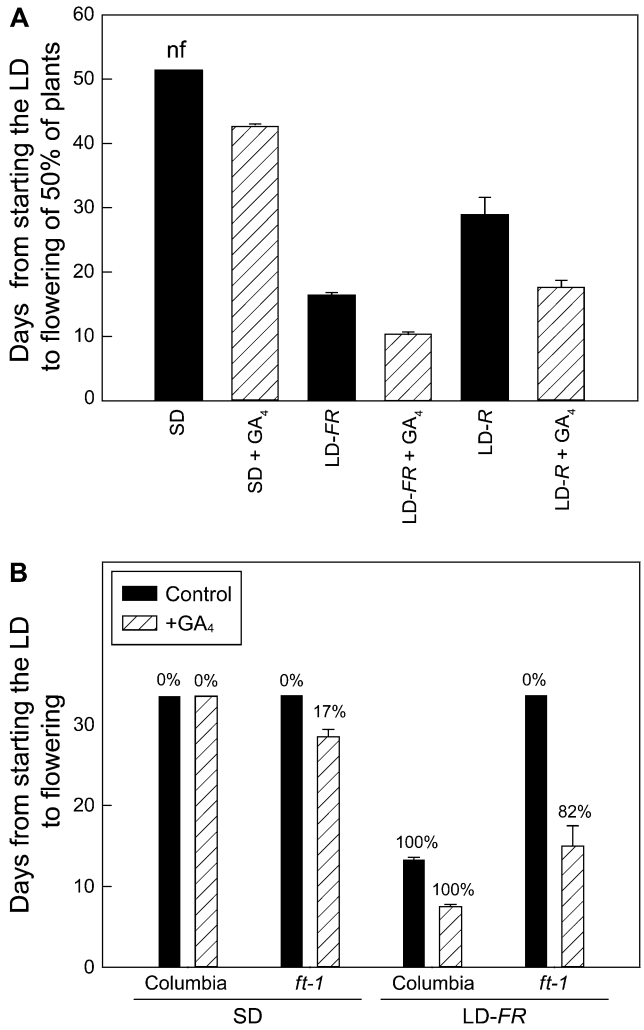

Fig. 4. Flowering of Columbia or the *ft-1* mutant after a single GA treatment to the leaves of plants in SD or exposed to LD. GA_4 (1 mM in 20% ethanol) or the solvent alone was applied as a spray to run off. There were 12–17 plants per treatment. In (A) only 12% of the Columbia plants had flowered in SD at 52 d when the experiment was terminated compared with 50% flowering 42 d after GA treatment. In LD, all Columbia plants flowered after exposure to a single low-intensity, FR-rich LD (LD-*FR*) or two cycles of a high intensity R-rich LD (LD-*R*). In (B) the experiment was terminated at 3s d when the only flowering in SD was 17% for GA-treated *ft-1* plants. In LD, all Columbia plants had flowered after 33 d, 82% of the GA-treated *ft-1* plants, and none of the untreated *ft-1* plants.

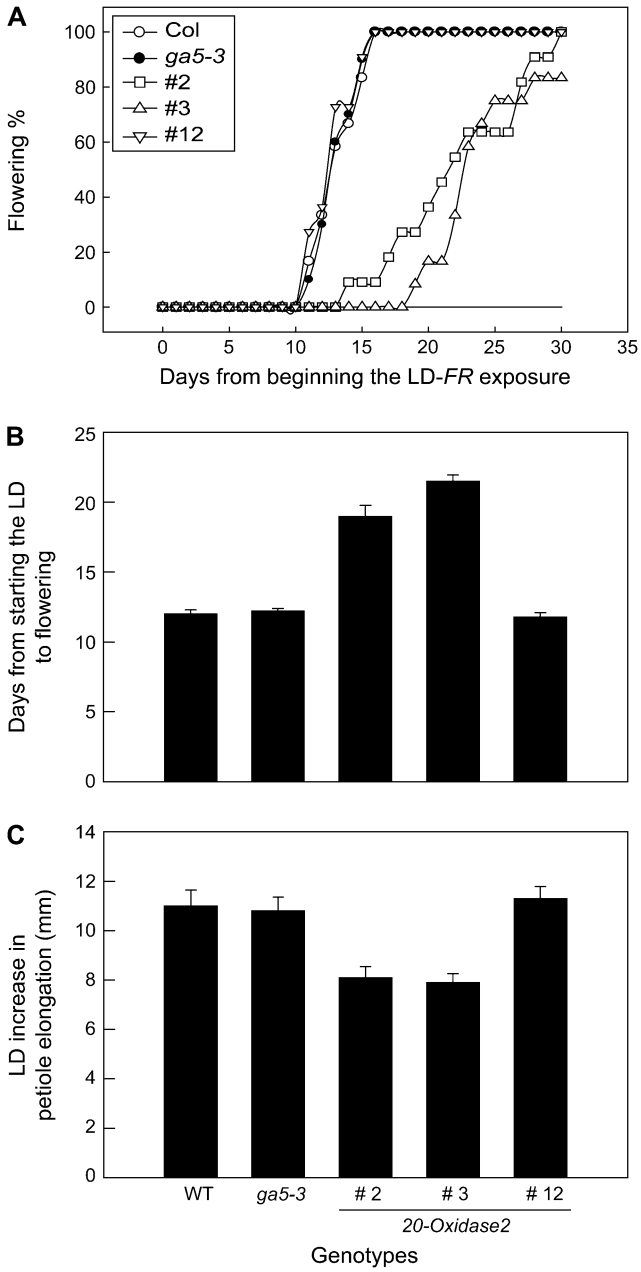

Fig. 5. Silencing *GA 20-OXIDASE2* expression delays flowering of *Arabidopsis* exposed to a single FR-enriched LD from incandescent lamps. Comparisons involve two *GA 20-OXIDASE2* silencing lines, *hpAtGA20ox2#2* (open square) and *#3* (open triangle); the wild type, Columbia (open circle); a non-silenced transgenic line #12 (inverted open triangle); and *ga5*-3 (filled circle), a null mutant recently renamed as *ga20ox1-3*. There was no flowering in SD plants at 30 d as indicated by the horizontal line. In (B) days to flowering is shown as the mean and SE at 50% flowering (n=10–14). The LD effect on petiole elongation of the same plants is redrawn with the permission of Hisamatsu *et al.* (2005).

show ~55% (*hpAtGA20ox2#2*) and 90% (*hpAtGA20ox2#3*) reduction in *20-OXIDASE2* expression (Hisamatsu *et al.*, 2005) and, in parallel, they inhibit a GA-regulated, LD increase in petiole elongation (Fig. 4C; Hisamatsu *et al.*, 2005). An additional RNAi line (*hpAtGA20ox2#12*) was included as a control for transformation effects; it showed normal *20-OXIDASE2* expression (Hisamatsu *et al.*, 2005) and there was neither a delay in its flowering nor a reduction in LD promotion of its petiole elongation (Fig. 5). The *GA 20-OXIDASE1* null mutant, *ga5-3*, although dwarfed in its growth (not shown, but see Rieu *et al.*, 2008), showed normal LD flowering and LD increase in petiole elongation (Fig. 5).

A repeat study with T_4 progeny of the most effective RNAi silencing line (*hpAtGA20ox2#3-6*) confirmed the

delay of flowering in LD-*FR*. Columbia flowered after 16.8±0.6 d but *hpAtGA20ox2#3-6* flowered significantly later at 20.1±0.6 d (P <0.001). As a negative test for up-regulation of GA biosynthesis, a high light intensity R-rich LD does not increase GA biosynthesis (Hisamatsu *et al*., 2005), and LD flowering of Columbia and *hpAtGA20ox2#3-6* was not significantly different (Columbia 20.2±1.4 d; *hpAtGA20ox2#3*-6 22.5±0.9 d; *ga5-3* 21.0±0.6 d, and the SD controls were still vegetative at 40 d). Recently Rieu *et al*. (2008) reported a very slight delay of flowering in a T-DNA mutant of *20-OXIDASE2*, and this supports the present findings; however, it is difficult to draw any conclusions from their study. The LD was at a high light intensity from lamps with an R/FR output of ~2.2, so flowering of both the mutant and wild type will be affected by photosynthesis, along with uncertain effects of lamp spectral composition on *GA 20-OXIDASE2* expression in the non-mutant line.

In parallel with delayed flowering and reduced petiole elongation in the *GA 20-OXIDASE2* silencing lines (Fig. 5), expression of this 20-oxidase increased when plants of Columbia were transferred to LD (Fig. 6). In other studies, the increase in gene expression in the petiole was 10-fold to 100-fold over the first 2–3 h of starting the LD, and then expression declined (Hisamatsu *et al*., 2005).

On a point of technique, it can be expected that, in precisely controlled conditions, the oscillation in gene expression over any one diurnal cycle of an SD will be the same as for the next day. This has been confirmed (Hisamatsu *et al*., 2005) in a parallel study where petiole *GA 20-OXIDASE1* expression was followed for 48 h (i.e. over two SD). Therefore, here, the gene expression patterns found over an SD have been extended to indicate the probable oscillation over the following SD.

Surprisingly, the *20-OXIDASE2* gene is only expressed in the petiole and shoot tip and not in the leaf blade

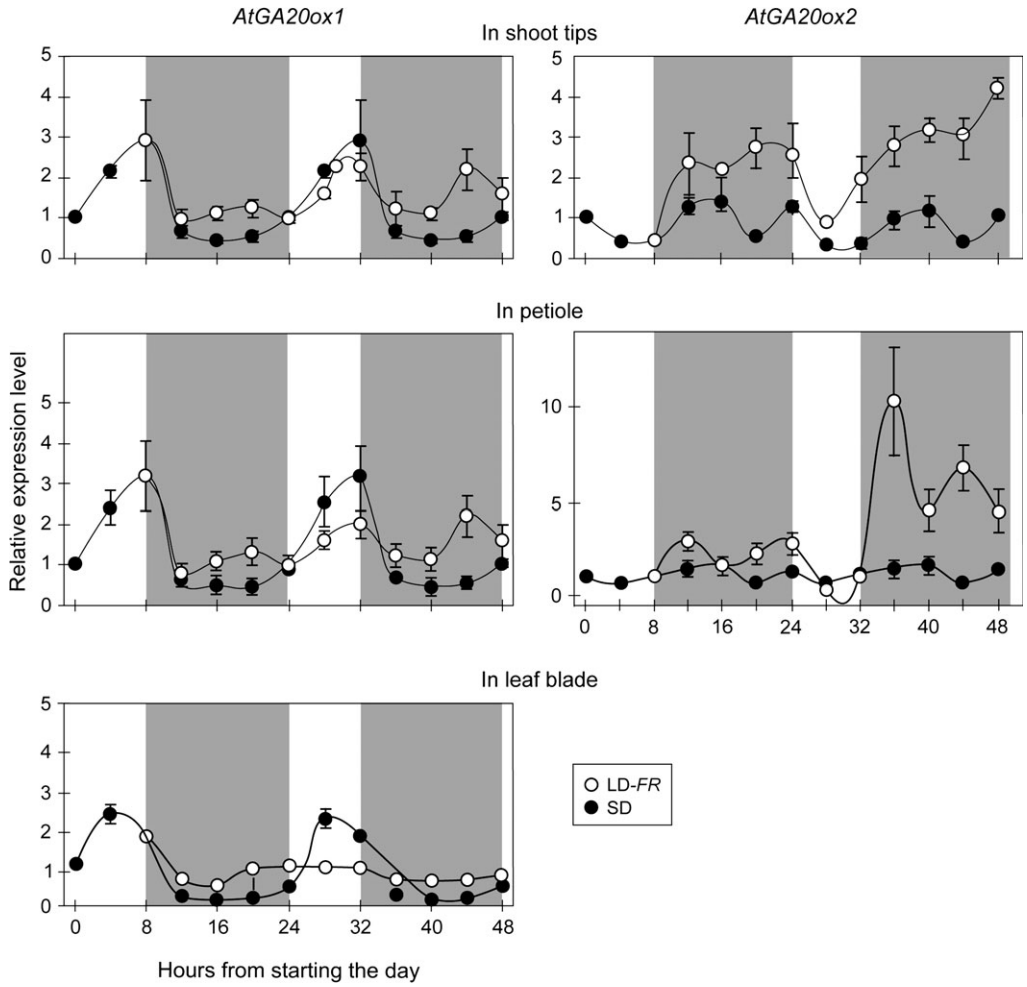

Fig. 6. Effect of LD on expression of two GA 20-oxidase genes in the leaf blade, petiole, and shoot tip of *Arabidopsis*. Gene expression was analysed for plants of Columbia held in SD (filled circle) or shifted to LD (open circle). The values of the second SD cycle are those of the first day as previously very little difference across days was found (Hisamatsu *et al*., 2005). The shaded areas show when the 'overnight' 16 h light or dark treatments were imposed. There was no detectable expression of *GA 20-OXIDASE2* in the leaf blade. All values are means ±SE (n=3). Error bars when not evident were smaller than the symbol.

(Fig. 6). In contrast, all three tissues clearly expressed the closely related *GA 20-OXIDASE1* gene (Fig. 6), and its absolute level of expression was comparable in all three tissues although slightly lower than for *20-OXIDASE2* in the shoot tip and petiole (not shown). In addition to *20-OXIDASE1*, *ACTIN* was effectively detected in all leaf blade samples where *20-OXIDASE2* was not detected. Thus, the possibility of failed assays can be excluded; there is a true lack of expression of the *20-OXIDASE2* gene in the leaf blade. Differences in tissue expression patterns of GA 20-oxidases have been reported previously for rice (Kaneko *et al*., 2003).

The diurnal periodicity shown for *GA 20-OXIDASE1* expression (Fig. 6) reveals circadian regulation based on cycling continuing over 48 h in constant conditions involving high intensity white light (Hisamatsu *et al*., 2005). Thus, in much the same way as the circadian rhythm in *CO* expression (Suarez-Lopez *et al*., 2001) modulates the effect of light on *FT* (Valverde *et al*., 2004), GA synthesis could be regulated by a circadian clock. Specifying how light and rhythms regulate flowering is tangential to the analysis, but the characterization of diurnal changes in gene expression is important for any integrated analysis of responses to a LD.

In contrast to *20-OXIDASE2*, *FT* expresses most in the leaf blade (~70-fold more than in the petiole: data not shown). Previous studies showed a similar pattern, with the highest *FT* promoter::GUS expression in the leaf blade, very little in petioles (Yamaguchi *et al*., 2005; Yoo *et al*., 2005), and, based on *in situ* expression assays, none in the shoot apex (Kardailsky *et al*., 1999).

Overall, because of their non-sympatric expression, GA and *FT* might act as independent LD signals but with a dominant role for *FT*.

Discussion

Floral signalling in LD plants may involve leaf to shoot apex transport of the FT protein (Turck *et al*., 2008) and/or the GA class of plant hormones (King and Evans, 2003). The role of the FT protein as a transported floral signal has been highlighted in a number of recent genetic/molecular studies with *Arabidopsis*, rice, and cucumber (Corbesier *et al*., 2007; Jaeger and Wigge, 2007; Lin *et al*. 2007; Mathieu *et al*., 2007; Tamaki *et al*., 2007). Although the response to *ft* mutants shows that *FT* plays a dominant role in LD flowering, GA contributes to flowering of *Arabidopsis* in LD (Figs 5, 6) and in SD (see Ericksson *et al*., 2006).

Based on evidence presented here and in the companion paper, Fig. 7 summarizes the ways that LD light might regulate *FT*, GA, and flowering. The complete block by *ft-1* of flowering of *Arabidopsis* exposed to a high light intensity, R-rich LD shows the dominance of photosynthesis in *FT* up-regulation. In contrast, at low, non-photosynthetic intensities involving FR-rich LD, phytochrome is the primary step of regulation of flowering and of *FT* expression (Goto *et al*., 1991; Reed *et al*., 1994; Bagnall and King, 2001; Cerdän and Chory 2003; Halliday *et al*., 2003). Interestingly, *ft-1* incompletely blocked flowering in response to an FR-rich LD (see companion paper). Therefore, there could be an additional *FT*-independent LD input and, potentially, via GA since FR-rich conditions up-regulate GA biosynthesis in *Arabidopsis* plants (Xu *et al*., 1997; Gocal *et al*., 2001; Hisamatsu *et al*., 2005) as also in other species (reviewed in García–Martínez and Gil, 2002; King and Evans, 2003).

Two potential actions of GA on flowering are indicated in Fig. 7, namely that GA acts on *FT* signalling in LD and that LD increases GA content in the petiole, this GA acting as a second floral signal. Based on the present findings, the extremely low levels of GA in *ga1-3* (see Zeevaart and Talon, 1992; Xu *et al*., 1997) allowed the demonstration of GA regulation of *FT* expression (Fig. 1) whereas with Columbia its endogenous GA levels were apparently close to sufficient for LD up-regulation of *FT* expression and flowering. Thus, GA plays an important permissive role for *FT* up-regulation and LD flowering.

Previously the possibility of GA induction of flowering by up-regulation of *FT* was discounted because plants of ecotype Landsberg *erecta* flowered early when *ga1-3* was crossed with a line overexpressing *FT* under the control of the 35S promoter (Blázquez *et al*., 2002). However, the

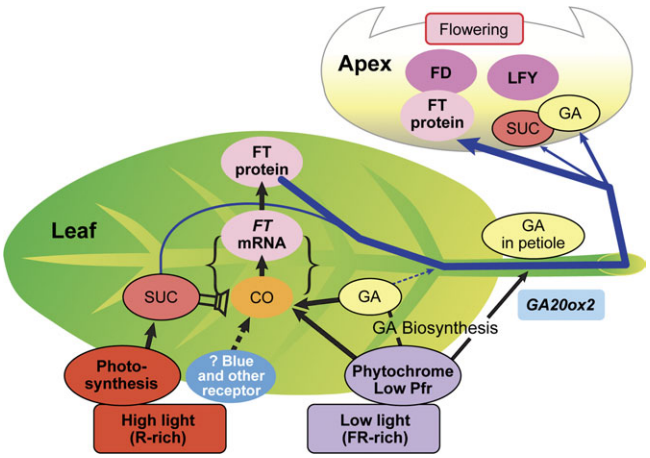

Fig. 7. Summary of findings here and in the companion paper of positive effects (arrows) on flowering and *CO*/*FT* for two commonly used LD photoresponses. This schematic incorporates effects on *FT* and flowering of: mutants; gene silencing; change in light intensity; and a block to photosynthesis. Predominantly, in LD, photosynthetic sucrose amplifies *CO*/*FT* expression (see companion paper) while phytochrome acts directly and also via GA, which plays a permissive and, often, non-limiting role. There is also a direct but lesser LD-mediated increase in GA supply via the petiole response to FR-rich light. A dashed arrow indicates a potential step of regulation, and weaker responses are indicated by thinner arrows. The electronics symbol for a speaker is used to show sucrose amplification of *CO*/*FT* expression.

FT promoter contains three GA response elements and a nearby pyrimidine box which could be sufficient for GA to regulate *FT* transcriptionally. Use of the constitutively expressed 35S promoter to control *FT* would not reveal such potential for GA regulation of *FT*.

Considering the role of GA as a second LD floral signal, flowering was inhibited when GA biosynthesis was blocked in *ga1-3* or by application of paclobutrazol (Figs 1, 2). Conversely, GA application caused rapid flowering and reversed the dwarfing effect of *ga1-3*. Predominantly these responses to applied GA involve FT up-regulation (see above). However, GA enhanced flowering even in SD where *FT* is only weakly expressed (Fig. 1) and, more cogently, GA dependent *FT*-independent flowering was demonstrated by application of GA to the *ft-1* mutant in SD or LD (Fig. 4).

This claim that GA can act endogenously as a floral signal is supported by earlier evidence that an FR-rich LD up-regulates GA biosynthesis in the petiole via a specific *GA 20-OXIDASE2* gene (Hisamatsu *et al*., 2005) and that there are associated increases in endogenous GA content (Gocal *et al*., 2001). Interestingly, this *20-OXIDASE2* is not expressed in the leaf blade (Fig. 6) and, conversely, *FT* is expressed in the leaf blade and not the petiole (Kardailsky *et al*., 1999; Yamaguchi *et al*., 2005; Yoo *et al*., 2005; Hisamatsu unpublished data). Lastly, the inhibition of LD flowering on silencing *GA 20-OXIDASE2* expression confirms that endogenous GA plays a small role in flowering in an FR-rich LD (Fig. 5).

Although the effect of applied GA on flowering in SD or LD is only weak (Figs 1, 4; and see Gocal *et al*., 2001; Ericksson *et al*., 2006), a role for GA is consistent with recent evidence of a large increase in endogenous GAs associated with very late SD flowering (Ericksson *et al*., 2006). However, the site of action of LD-generated GA is unclear. Despite evidence for GA_4 transport from the leaf blade to the shoot tip of *Arabidopsis* (Ericksson *et al*., 2006), GA sourced from the LD petiole could be transported to and act in either or both the leaf blade and the shoot apex.

At the molecular level, in the leaf blade GA acts in an as yet unknown way on *FT* expression. At the shoot apex there is evidence that GA activates a *GAMYB* (Blázquez and Weigel, 2000; Gocal *et al*., 2001) which up-regulates expression of the floral regulator gene, *LEAFY* (Blázquez *et al*., 1998). Although the focus in the present study was on early response to GA, it also enhances later, visible, stem elongation (bolting) of *Arabidopsis* (Xu *et al*., 1997). Such GA action on later steps of floral development/stem bolting might explain the more rapid visible flowering after GA treatment (Fig. 4A; 6–10 d earlier). An equally plausible explanation, but one not generally considered, involves a common action of GA on both floral initiation and later floral development. Some common actions are likely since, within 48 h of exposure of *Arabidopsis* to a single LD, there are large increases in shoot apex height (Gocal *et al*., 2001).

Although direct GA regulation of flowering is weak in *Arabidopsis*, its extent varies across plant species and, possibly, inversely with the role played by *FT*. Unlike *Arabidopsis*, in *L. temulentum*, leaf-applied GA causes substantial and rapid flowering in SD despite the low level of expression of *LtFT* in SD (King *et al*., 2006). More cogently, GA is an important floral signal in *L. temulentum* because it is also effective when supplied directly to isolated shoot tips in culture (reviewed in King and Evans, 2003). Evidence of rapid increases in endogenous GAs first in the LD leaf blade and then in the shoot apex further supports direct GA signalling, as does evidence of a relationship between GA dose, flowering, and transport of intact tetradeuterated GA from the leaf to the apex (King *et al*., 2001, 2006). In these studies there was also little or no effect of increased or decreased GA on the LD increase in *FT* expression (King *et al*., 2006). This latter result contrasts with evidence for *Arabidopsis* where flowering and *FT* expression are restricted when GA synthesis is blocked in *ga1-3* (Fig. 1) or, probably, with the use of paclobutrazol to inhibit flowering (Fig. 2).

Overall, the focus of the present study was on *FT* and GA, but the findings emphasize the importance of treating the photoperiodic regulation of flowering as a complex of interacting responses. As summarized in Fig. 7, *FT* plays a dominant role in floral signalling in *Arabidopsis*, and its protein (An *et al*., 2004; Corbesier *et al*., 2007; Turck *et al*., 2008) or some closely linked factor is the primary leaf-sourced factor transported to the shoot apex where it evokes flowering. LD up-regulate *FT* expression whether by phytochrome or by photosynthesis, but in the latter instance *FT* expression may involve an additional action of a blue or red photoreceptor. However, although it is considered that GA and photosynthetically generated sucrose up-regulate *FT* expression, they may also play direct, albeit small, roles as mobile floral signals.

Acknowledgements

Dr Tai-ping Sun provided seed of *ga1-3*. Drs Lloyd Evans and Masumi Robertson (CSIRO) provided valuable comment on the manuscript.

References

An HL, Roussot C, Suarez-Lopèz P, *et al.* 2004. *CONSTANS* acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. *Development* **131,** 3615–3626.

Bagnall DJ, King RW. 2001. Phytochrome and flowering of *Arabidopsis thaliana*: photophysiological studies using mutants and transgenic lines. *Australian Journal of Plant Physiology* **28,** 401–408.

Blázquez MA, Green R, Nilsson O, Sussman MR, Weigel D. 1998. Gibberellins promote flowering of Arabidopsis by activating the *LEAFY* promoter. *The Plant Cell* **10,** 791–800.

Blázquez MA, Trenor M, Weigel D. 2002. Independent control of gibberellin biosynthesis and flowering time by the circadian clock in Arabidopsis. *Plant Physiology* **130,** 1770–1775.

Blázquez MA, Weigel D. 2000. Integration of floral inductive signals in Arabidopsis. *Nature* **404,** 889–902.

Boss PK, Bastow RM, Mylne JS, Dean C. 2004. Multiple pathways in the decision to flower: enabling, promoting, and resetting. *The Plant Cell* **16,** S18–S31.

Cerdän PD, Chory J. 2003. Regulation of flowering time by light quality. *Nature* **423,** 881–885.

Corbesier L, Vincent C, Jang SH, *et al.* 2007. FT protein movement contributes to long-distance signalling in floral induction of Arabidopsis. *Science* **316,** 1030–1033.

Eriksson S, Bohlenius H, Moritz T, Nilsson O. 2006. GA_4 is the active gibberellin in the regulation of *LEAFY* transcription and Arabidopsis floral initiation. *The Plant Cell* **18,** 2172–2181.

García-Martínez JL, Gil J. 2002. Light regulation of gibberellin biosynthesis and mode of action. *Journal of Plant Growth Regulation* **20,** 354–368.

Gocal GFW, Sheldon C, Gubler F, Moritz T, Bagnall D, Song FL, Parish RW, Dennis ES, Weigel D, King RW. 2001. *GAMYB*-like genes and gibberellin in Arabidopsis. *Plant Physiology* **127,** 1682–1693.

Goto N, Kumagi T, Koornneef M. 1991. Flowering responses to light breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long day plant. *Physiologia Plantarum* **83,** 209–215.

Halliday KJ, Salter MG, Thingnaes E, Whitelam GC. 2003. Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator *FT*. *The Plant Journal* **33,** 875–885.

Hisamatsu T, King RW, Helliwell CA, Koshioka M. 2005. The involvement of gibberellin 20-oxidase genes in phytochrome-regulated petiole elongation of Arabidopsis. *Plant Physiology* **138,** 1106–1116.

Imaizumi T, Kay SA. 2006. Photoperiodic control of flowering not only by coincidence. *Trends in Plant Science* **11,** 550–558.

Jaeger KE, Wigge PA. 2007. FT protein acts as a long-range signal in Arabidopsis. *Current Biology* **17,** 1050–1054.

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

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

King RW, Evans LT. 2003. Gibberellins and flowering of grasses and cereals: prizing open the lid of the 'florigen' black box. *Annual Review of Plant Biology* **54,** 307–328.

King RW, Moritz T, Evans LT, Junttila O, Herlt AJ. 2001. Long day induction of flowering in *Lolium temulentum* involves sequential increases in specific gibberellins at the shoot apex. *Plant Physiology* **127,** 624–632.

King RW, Moritz T, Evans LT, Martin J, Andersen CH, Blundell C, Kardailsky I, Chandler PM. 2006. Regulation of flowering in the long day grass, *Lolium temulentum* L., by gibberellins and the gene, *FLOWERING LOCUS T (FT)*. *Plant Physiology* **141,** 498–507.

King RW, Hisamatsu T, Goldschmidt EE, Blundell C. 2008. The nature of floral signals in *Arabidopsis* I Photosynthesis and a Far-Red independently regulate flowering by increasing expression of *FLOWERING LOCUS T (FT)*. *Journal of Experimental Botany*.

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

Koornneef M, van der Veen JH. 1980. Induction and analysis of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. *Theoretical and Applied Genetics* **58,** 257–263.

Lin MK, Belanger H, Lee YJ, *et al.* 2007. *FLOWERING LOCUS T* protein may act as the long-distance florigenic signal in the Cucurbits. *The Plant Cell* **19,** 488–1506.

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

Putterill J, Robson F, Lee K, Simon R, 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.

Rademacher W. 2000. Growth retardants: effects on gibberellin biosynthesis and other metabolic pathways. *Annual Review of Plant Physiology and Plant Molecular Biology* **51,** 501–531.

Reed JW, Nagatini A, Elich TD, Fagan M, Chory J. 1994. Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. *Plant Physiology* **104,** 1139–1149.

Reeves PH, Coupland G. 2001. Analysis of flowering time control in Arabidopsis by comparison of double and triple mutants. *Plant Physiology* **126,** 1085–1091.

Rieu R, Ruiz-Rivero O, Fernandez Garciaa N, *et al.* 2007. The gibberellin-biosynthetic genes *AtGAa20ox1* and *AtGAa20ox2* act, partially redundantly, to promote growth and development throughout the Arabidopsis life cycle. *The Plant Journal* **53,** 488–504.

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

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

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K. 2007. Hd3a protein is a mobile flowering signal in rice. *Science* **316,** 1033–1036.

Thomas SG, Hedden P. 2006. Gibberellin metabolism and signal transduction. In: Hedden P, Thomas SG, eds. *Plant hormone signalling*. Oxford: Blackwell Publishing, 147–184.l.

Turck F, Formara F, Coupland G. 2008. Regulation and identity of florigen: FLOWERING LOCUS T moves centre stage. *Annual Reviews of Plant Biology* **59,** 573–594.

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP. 2004. DELLA proteins and gibberellin-regulated seed germination and floral development in Arabidopsis. *Plant Physiology* **135,** 1008–1019.

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

Wilson RN, Heckman JW, Somerville CR. 1992. Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. *Plant Physiology* **100,** 403–408.

Xu YL, Gage DA, Zeevaart JAD. 1997. Gibberellins and stem growth in *Arabidopsis thaliana*. Effects of photoperiod on the *GA4* and *GA5* loci. *Plant Physiology* **114,** 1471–1461.

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

Yoo SK, Chung KS, Kim J, Lee JH, Hong SM, Yoo SJ, Yoo SY, Lee JS, Ahn JH. 2005. *CONSTANS* activates *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1* through *FLOWERING LOCUS T* to promote flowering in Arabidopsis. *Plant Physiology* **139,** 770–778.

Zeevaart JAD, Talon M. 1992. Gibberellins and mutants in *Arabidopsis thaliana*. In: Karstens CM, Loon LC, van Vreugdenhil D, eds. *Plant growth regulation*. Amsterdam: Kluwer, 34–42.