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The Pea *GIGAS* Gene Is a *FLOWERING LOCUS T* Homolog Necessary for Graft-Transmissible Specification of Flowering but Not for Responsiveness to Photoperiod [C][W]

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Garden pea (*Pisum sativum*) was prominent in early studies investigating the genetic control of flowering and the role of mobile flowering signals. In view of recent evidence that genes in the *FLOWERING LOCUS T* (*FT*) family play an important role in generating mobile flowering signals, we isolated the *FT* gene family in pea and examined the regulation and function of its members. Comparison with *Medicago truncatula* and soybean (*Glycine max*) provides evidence of three ancient subclades (*FTa*, *FTb*, and *FTc*) likely to be common to most crop and model legumes. Pea *FT* genes show distinctly different expression patterns with respect to developmental timing, tissue specificity, and response to photoperiod and differ in their activity in transgenic *Arabidopsis thaliana*, suggesting they may have different functions. We show that the pea *FTa1* gene corresponds to the *GIGAS* locus, which is essential for flowering under long-day conditions and promotes flowering under short-day conditions but is not required for photoperiod responsiveness. Grafting, expression, and double mutant analyses show that *GIGAS*/*FTa1* regulates a mobile flowering stimulus but also provide clear evidence for a second mobile flowering stimulus that is correlated with expression of *FTb2* in leaf tissue. These results suggest that induction of flowering by photoperiod in pea results from interactions among several members of a diversified *FT* family.

INTRODUCTION

In many species, the timing of flowering is regulated by a number of environmental factors, including daylength and temperature, and much recent effort has been directed toward understanding the molecular mechanisms that underlie this regulation. The *Arabidopsis thaliana FLOWERING LOCUS T* (*FT*) gene has an important position within the genetic hierarchy that controls flowering and integrates photoperiod, temperature, vernalization, and light quality signaling. *FT* encodes a small protein with similarities to mammalian phosphatidylethanolamine binding domain protein (PEBP) (Kardailsky et al., 1999; Kobayashi et al., 1999). *Arabidopsis FT* is expressed in leaves under flowering-inductive conditions, and the FT protein moves through the phloem to the shoot apex where it binds to the bZIP transcription factor FD to activate transcription of the floral meristem identity gene *APETALA1* (*AP1*) and possibly other related MADS domain genes (Abe et al., 2005; Wigge et al., 2005; Corbesier et al., 2007; Jaeger and Wigge, 2007; Mathieu et al., 2007). FT-like proteins from several different species have been shown to function in a manner similar to FT with respect to induction of flowering, transport in phloem, and interaction with FD-like proteins (Lifschitz et al., 2006; Lin et al., 2007; Tamaki et al., 2007; Li and Dubcovsky, 2008), suggesting that this general mechanism is likely to be widely conserved across flowering plants.

In *Arabidopsis*, *FT* is part of a small gene family (the so-called PEBP family) with five other members: *TWIN SISTER OF FT* (*TSF*), *TERMINAL FLOWER1* (*TFL1*), *Arabidopsis thaliana CENTRORADIALIS homolog*, *MOTHER OF FT AND TFL1* (*MFT*), and *BROTHER OF FT AND TFL1* (*BFT*) (Bradley et al., 1997; Mimida et al., 2001; Yoo et al., 2004, 2010; Yamaguchi et al., 2005). PEBP gene families in other plant species vary in size, ranging from five genes in tomato (*Solanum lycopersicum*) to around 20 in rice (*Oryza sativa*) and maize (*Zea mays*) (Carmel-Goren et al., 2003; Chardon and Damerval, 2005; Danilevskaya et al., 2008; Igasaki et al., 2008). Phylogenetic analysis resolves three major clades within this family, corresponding to *FT*-like, *TFL1*-like, and *MFT*-like genes. *FT*-like genes promote flowering, whereas *TFL1*-like genes delay flowering and prevent conversion of the shoot apical meristem into a floral meristem (Bradley et al., 1996; Bradley et al., 1997; Pnueli et al., 1998; Foucher et al., 2003). In *Arabidopsis*, the opposing functions of FT and TFL1 have been suggested to derive from a single amino acid difference within a divergent external loop in the fourth exon (Hanzawa et al., 2005; Ahn et al., 2006), and TFL1 has been suggested to act as a competitor of FT, potentially through competitive binding to FD (Ahn et al., 2006). The *Arabidopsis FT*-like clade has two members: *FT* itself and a close paralog, *TSF*. The *FT* and *TSF* genes encode very similar proteins and have a similar proximal promoter and similar patterns of regulation with respect to

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photoperiod and vernalization (Yamaguchi et al., 2005). *TSF* has a small effect on flowering additive with *FT*, and the TSF protein, like FT, can act as a mobile inducer of flowering when expressed in the phloem (Jang et al., 2009). In contrast with the similarity in regulation of *FT* and *TSF*, multiple *FT*-like genes in other species show marked differences in regulation (Faure et al., 2007; Kikuchi et al., 2009; Blackman et al., 2010).

The legumes are a large plant group that are present in most ecosystems and include many important crop species. Most crop and model legumes fall into two distinct clades: the so-called galegoid legumes (including pea [*Pisum sativum*], *Medicago*, *Lotus*, lentil [*Lens culinaris*], and chickpea [*Cicer arietinum*]), which are predominantly vernalization-responsive long-day plants from temperate regions, and the warm season millettioid legumes (including bean [*Phaseolus vulgaris*], soybean [*Glycine max*], and cowpea [*Vigna unguiculata*]) that originate from lower latitudes and are predominantly short-day plants (Cannon et al., 2009).We are investigating the genetic control of flowering in garden pea, a long-day, vernalization-responsive legume that was extensively used in early work on the genetic control of mobile flowering signals (Weller et al., 1997, 2009b). Recently, several pea loci controlling photoperiod responsiveness and mobile flowering signals have been shown to be orthologs of *Arabidopsis* genes involved in circadian clock function. These include *LATE BLOOMER1* (*LATE1*) and *DIE NEUTRALIS* (*DNE*), which are orthologs of *Arabidopsis GIGANTEA* and *EARLY FLOWERING4*, respectively (Hecht et al., 2007; Liew et al., 2009). In view of the potential importance of *FT* genes in mobile floral signaling, we defined the *FT* gene family in pea, *Medicago*, and soybean and investigated the regulation of the pea *FT* family, documenting distinct expression patterns for different members. We also show that one of these genes corresponds to the previously described *GIGAS* locus and examine the role of this gene in photoperiod responsiveness and the induction of flowering. Our results suggest that the role of the *FT* family in induction of flowering is potentially more complex in pea than in *Arabidopsis*, involving transcriptional cross-regulation, multiple mobile signals, and possible functional differentiation of individual members.

RESULTS

The *FT* Gene Family in Legumes

In a previous survey of flowering-related genes in model legumes, we identified five *FT*-like genes in *Medicago truncatula* (*FTLa-FTLe*) and a single gene in pea most similar to *Medicago FTLe* (Hecht et al., 2005; Liew et al., 2009). Four additional pea *FT* genes were identified by library screening and PCR approaches, and isolation of the corresponding full-length cDNAs demonstrated that all five pea *FT* genes are expressed and have similar intron/exon structure to *FT* genes in other species (Figure 1A). For two of the genes, we were unable to easily isolate specific introns, presumably due to their large size. To obtain a broader perspective on *FT* family evolution within legumes, we performed BLAST searches of the soybean genome at Phytozome (www.phytozome.net). Although legume genomes share an ancient whole-genome duplication event, soybean has experienced an additional, more recent whole-genome duplication event and as a result contains larger gene families with readily identifiable pairs of homoeologs (Schmutz et al., 2010). Our searches identified 10 putative full-length *FT* genes in soybean (see Supplemental Table 1 online), eight other full-length PEPB genes more similar to *Arabidopsis TFL1*, *BFT*, or *MFT* (see Supplemental Table 1 online), and several other apparent pseudogenes.

Phylogenetic analysis (Figure 1B) shows that legume PEBP genes fall into previously described *FT*, *TFL1*, and *MFT* clades. Figure 1B also shows that the *FT* genes fall into three distinct subclades, and microsynteny around the soybean and *Medicago* genes further demonstrates the affinities of genes within each clade (see Supplemental Figure 1 and Supplemental Table 2 online). These results indicate that expansion of the *FT* family occurred relatively early in legume evolution and suggest that the three clades are likely to be represented in other crop and model legumes. To provide consistency in nomenclature across the three species, and a framework for naming *FT* genes from other legumes, we designated these subclades as *FTa*, *FTb*, and *FTc* and propose to rename the *Medicago* and pea genes accordingly (see Supplemental Table 1 online).

The *FTa* subclade is represented in *Medicago* and pea by two genes, *FTa1* (formerly *FTLa*) and *FTa2* (formerly *FTLb*), and in soybean by four genes, a pair of homoeologs (*FTa1* and *FTa2*) and two other linked genes (*FTa3* and *FTa4*) without clear homoeologs. The *FTb* subclade is also represented by two genes in both pea and *Medicago*: *FTb1* (formerly *FTLd* in *Medicago*) and *FTb2* (formerly *FTLe* in *Medicago* and *FTL* in pea). In *Medicago*, this pair of genes is located in tandem at the top of chromosome 7, and we mapped the pea *FTb1* gene to the corresponding region of linkage group V. Soybean also has four *FTb* genes in two homoeologous blocks on chromosomes 18 (*FTb1* and *FTb2*) and 8 (*FTb3* and *FTb4*) (see Supplemental Figure 1 online). The *FTc* subclade is the least complex, consisting of a single gene in pea and *Medicago* (formerly *FTLc*) and a single pair of homoeologs (*FTc1* and *FTc2*) in soybean (Figure 1). In both *Medicago* and soybean, these genes are located immediately adjacent to *FTa* genes (see Supplemental Figure 1 online), supporting their orthologous nature and likely origin from the same duplication event.

Previous efforts to define structural features that distinguish flower-promoting FT-like function from flower-inhibiting TFL1-like function identified two critical pairs of residues:Tyr-85/Gln-140 in FT and His-88/Asn-144 in TFL1 (Hanzawa et al., 2005; Ahn et al., 2006). All legume FT proteins carry the conserved Tyr residue, and FTa and FTb proteins also contain the conserved Gln characteristic of other FT proteins (Ahn et al., 2006). However, FTc proteins share a His at this position and thus differ both from other FT sequences and from TFL1. FTc proteins also carry several other conserved substitutions in the adjacent region (segment B in Ahn et al., 2006) which may have the potential to alter protein function (see Supplemental Figure 2 online).

Pea *FT* Genes Show Distinct Patterns of Regulation

We used photoperiod transfer experiments to define a temporal window from 11 to 16 d after sowing during which pea plants of

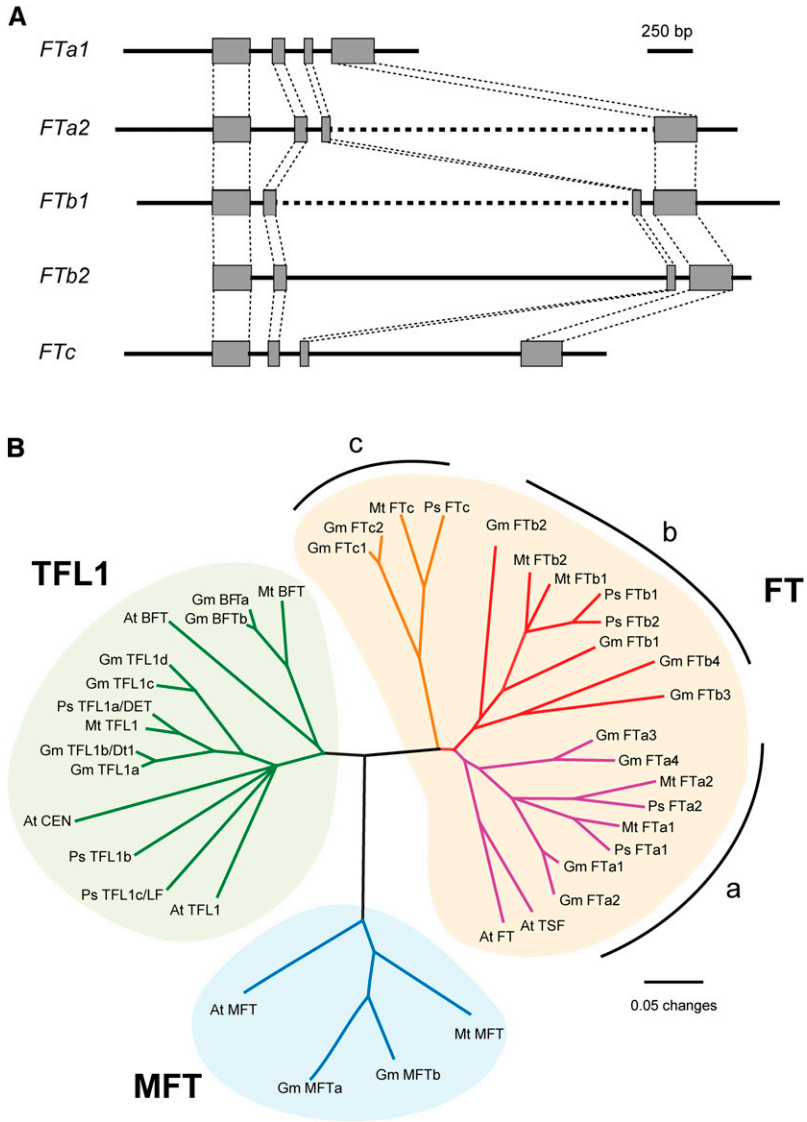

Figure 1. Legumes Have Three Distinct Groups of *FT* Genes.

(A) Genomic organization of pea *FT* genes. Exons are represented by shaded boxes. Bold dashed lines indicate introns of unknown size.
(B) Phylogram of legume PEBP protein sequences. Branches with bootstrap values <60% have been collapsed. The analysis is based on the sequence alignment shown in Supplemental Figure 2 online. Sequence details are available in Supplemental Table 1 online.
[See online article for color version of this figure.]

line NGB5839 become irreversibly committed to flower in long days (LD) conditions at 20°C (Figure 2A). Expression of the pea *AP1* ortholog *PROLIFERATING INFLORESCENCE MERISTEM* (*PIM*) (Taylor et al., 2002) in apical buds (dissected to 2 to 3 mm in size) showed clear induction from day 21 in LD or day 42 in short days (SD), corresponding to the appearance of visible flower buds ~1 week later. Significant induction of the pea *LFY* ortholog *UNIFOLIATA* (*UNI*) (Hofer et al., 1997), *SEPALLATA1* (*SEP1*) (Hecht et al., 2005), and the *TFL1* paralog *DETERMINATE* (*DET*/*TFL1a*) (Foucher et al., 2003) also occurred from day 21 in LD or day 42 in SD (Figure 2B). Expression of the pea *FD* ortholog showed weak induction by day 21 in LD relative to the SD level (Figure 2B).

Interestingly, *FT* genes showed distinct patterns of regulation with respect to photoperiod, timing, and tissue specificity. In the uppermost fully expanded leaf of LD-grown seedlings, expression of *FTb2* was strongly induced by day 14, whereas induction of *FTa1* and *FTa2* expression occurred more gradually from days 14 to 35 (Figure 2C). In the corresponding tissue from SD-grown seedlings, *FTb2* was not expressed, whereas *FTa1* showed weak induction by day 21 similar to LD, and *FTa2* was also induced to a similar extent as in LD but with a delay of ~1 week.

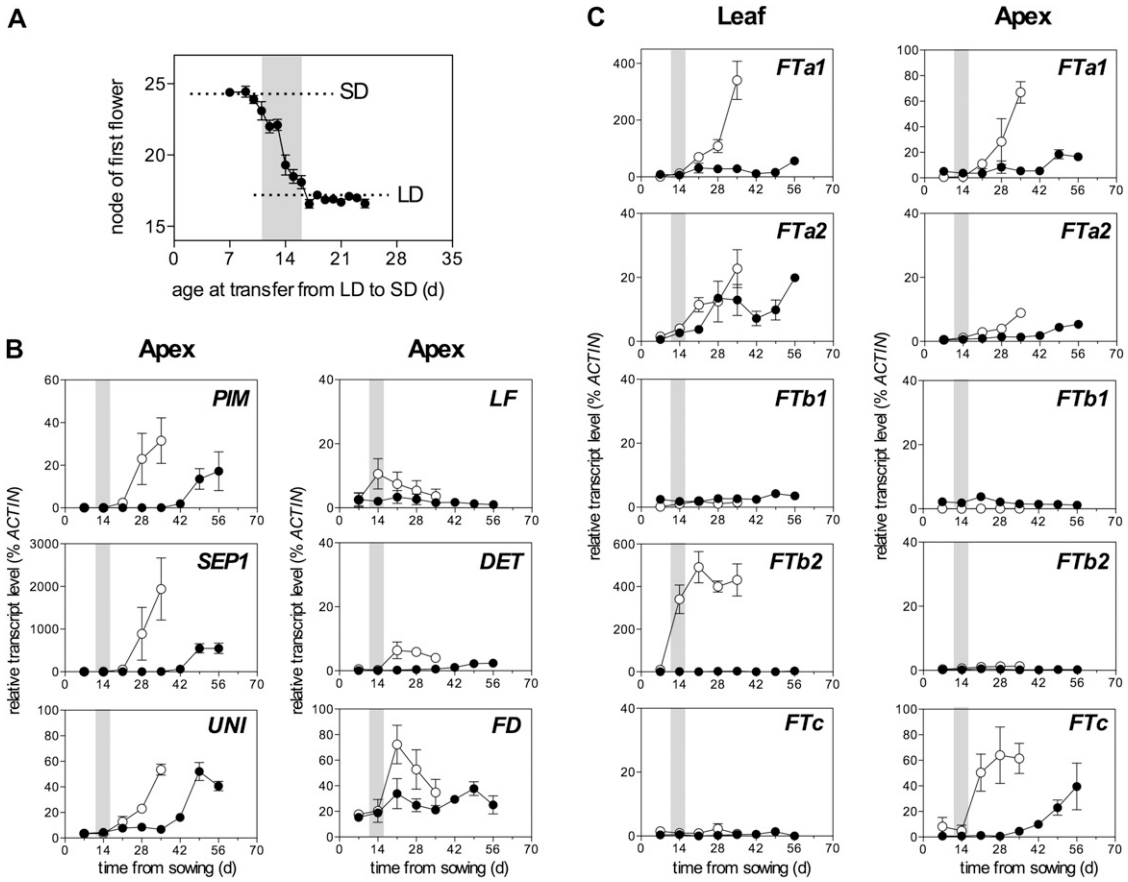

Figure 2. Pea *FT* Genes Show Different Expression Patterns.

(A) Photoperiod transfer defines a window for commitment to flower in wild-type (NGB5839) plants grown in LD. Plants were grown from sowing under LD conditions and transferred to SD conditions at the times indicated. There was no significant effect of LD exposure up until day 12, and by day 17, there was no significant effect of the SD transfer. The window during which commitment to flower is established is indicated by gray shading. Values represent mean ± SE for $n = 6$

(B) and **(C)** Expression of inflorescence identity genes **(B)** and *FT* genes **(C)** during development. Relative transcript levels were determined in dissected shoot apices or the uppermost fully expanded leaf during development in LD (16 light/8 dark; open circles) or SD (8 light/16 dark; closed circles). Gray shading represents the commitment window determined in **(A)**. Values have been normalized to the transcript level of the *ACTIN* gene and represent mean ± SE for $n = 2$ to 3 biological replicates, each consisting of pooled material from two plants.

FTc transcript was not detected above background in leaf tissue under LD or SD.

We also detected significant expression of *FT* genes in apical buds. In LD, *FTa1* and *FTc* expression was both clearly induced and *FTa2* weakly induced by day 21 in this tissue (Figure 2C). In SD, *FTc* showed the strongest induction, increasing gradually from day 35 to a level similar to the wild type in LD, whereas expression of *FTa1* and *FTa2* was more weakly induced from day 42, similar to that of *PIM* and other inflorescence identity genes (Figures 2B and 2C). Neither *FTb1* nor *FTb2* transcript was expressed above background in shoot apices under either photoperiod. The interpretation of expression in these apical bud samples must be qualified by the fact that they contained other tissues in addition to the shoot apical meristem itself, including leaf, petiole, and vascular primordia. It will be interesting in the future to examine in more detail the spatial expression patterns of *FT* genes at the shoot apex. Nevertheless, even with the most conservative interpretation, the specific expression of *FTc* in these samples and the absence of *FTb2* expression does indicate a strong, contrasting tissue specificity for expression of these *FT* genes.

Pea *FT* Genes Promote Flowering in Transgenic *Arabidopsis*

We next tested the ability of the pea *FT* genes to complement the *Arabidopsis ft-1* mutant. Figure 3 shows that a representative highly expressing *35S:*Ps-*FTa1* line grown in LD flowered significantly earlier than the untransformed *ft-1* control. *FTc* overexpression resulted in a striking early-flowering phenotype in which the majority of transformants produced only two distorted cauline leaf–like structures before termination of the shoot apex in a single terminal flower (see Supplemental Figure 3 online).

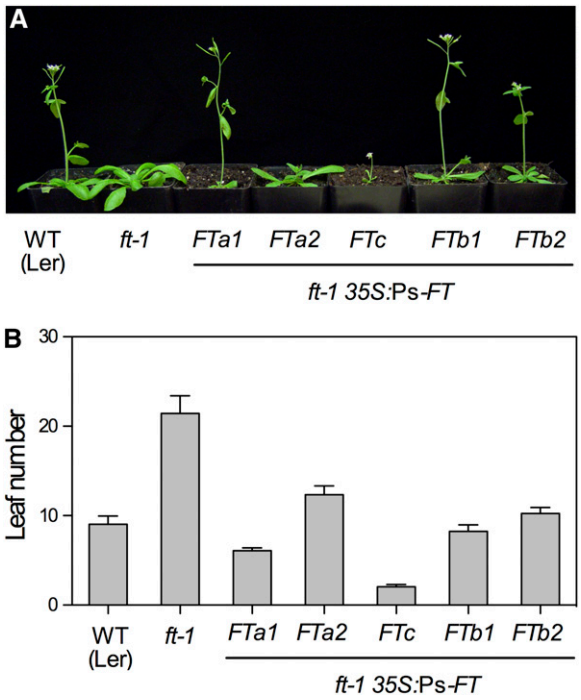

Figure 3. Pea *FT* Genes Show Differing Activities in Transgenic *Arabidopsis*.

Complementation of the *Arabidopsis ft-1* mutant with pea *FT* genes
(A) Representative plants grown in LD. Flowering has occurred in all lines but not in the untransformed *ft-1* control. WT, wild type.
(B) Total leaf number at flowering for representative *Arabidopsis* lines. Data are mean ± SE for a minimum of 10 plants.
[See online article for color version of this figure.]

This phenotype is stronger than reported for *35S:FT* expression in *Arabidopsis* and more similar to the combined effects of *35S:AP1* and *35S:FT* (Kardailsky et al., 1999). The remaining pea genes (*FTa2*, *FTb1*, and *FTb2*) showed weaker activity, with transformants flowering earlier than the untransformed *ft* mutant but not as early as the wild type. This shows that all five pea *FT*-like genes possess *FT* function to some extent and suggests that different FT proteins may differ in their inherent activity.

Aberrant Regulation of *FT* Genes in Photoperiod Pathway Mutants

We next examined whether *FT* genes might be misregulated in two mutants with impaired responses to photoperiod. The *late1* mutant delays flowering and blocks the LD response, whereas the *dne* mutant promotes flowering and has a LD phenotype in SD (Hecht et al., 2007; Liew et al., 2009).

Under a 16-h LD in growth cabinets, the *late1* mutation delayed flowering by approximately six nodes and the induction of *PIM* in dissected shoot apical buds by 2 weeks (Figure 4A). The expression patterns for the three leaf-expressed *FT* genes in the *late1* mutant were similar to those seen in SD-grown wild-type plants (Figure 2C), consistent with the SD-like phenotype of LD-grown *late1* plants (Hecht et al., 2007). Induction of *FTb2* in the leaf was completely blocked by *late1* (Figure 4A), consistent with a previous report showing lower expression of this gene in *late1* (Hecht et al., 2007). In *late1* shoot apices, the patterns of *FTa1*, *FTa2*, and *FTc* expression were also similar to that in SD-grown wild-type plants, with reduced *FTa1* expression and delayed *FTc* induction relative to LD-grown wild type and no major difference in *FTa2* expression (Figure 4A).

Under an 8-h SD in growth cabinets, the *dne* mutation promoted flowering by approximately eight nodes and the induction of *PIM* by ~3 weeks (Figure 4B). The *dne* mutant also showed significantly higher expression of *FTa1*, *FTa2*, and *FTb2* in leaves and of *FTa1*, *FTa2*, and *FTc* in shoot apices. The *dne* mutant flowers earlier in SD than the wild type in LD (Liew et al., 2009), and this was also reflected in certain *FT* expression patterns. Specifically, leaf expression of *FTa1* and *FTa2* was much stronger in SD-grown *dne* than in LD-grown wild type, as was expression of *FTa2* in apical buds. However, *FTb2* expression, although elevated in *dne* relative to the wild type in SD (Figure 4B), did not show the strong early induction (i.e., by day 14) that is typical of the wild type in LD (Figure 2C).

This misregulation of *FT* genes in leaves of *late1* and *dne* mutants is consistent with previous findings that both mutants alter production of graft-transmissible flowering signal(s) (Hecht et al., 2007; Liew et al., 2009). However, it does not identify which among the *FT* genes may contribute to this signaling.

The *GIGAS* Locus Corresponds to *FTa1*

The previously described *gigas* mutant (*gigas-1*) is late flowering under SD and flowers late or not at all under LD, depending on the spectral quality of the photoperiod extension (Beveridge and Murfet, 1996). A second mutant allele (*gigas-2*) has a slightly more severe phenotype, failing to flower under any LD conditions (Reid et al., 1996). Whereas the SD phenotype of *gigas* mutants is essentially similar to the wild type, apart from a delay in flowering (Beveridge and Murfet, 1996), the LD phenotype is unusual. Despite their failure to flower, the mutants show a number of vegetative changes in response to LD, including reduced internode length, reduced leaf size, and axillary bud outgrowth (Beveridge and Murfet, 1996; Figure 5A). This phenotype is very different from that of photoperiod response mutants *late1* and *phyA*, which essentially phenocopy SD-grown wild-type plants (Weller et al., 2004; Hecht et al., 2007), and is more similar to the phenotype of other pea mutants that impair flowering and/or inflorescence development, such as the nonflowering *veg1* and *veg2* mutants (Reid et al., 1996; Benlloch et al., 2007). Nevertheless, normal flowering can be restored in *gigas* by grafting to the wild type, implicating *GIGAS* in the production of a graft-transmissible flowering stimulus (Beveridge and Murfet, 1996) and suggesting *FT*-like genes as candidates.

The pea and *Medicago* genomes are closely syntenic (Aubert et al., 2006), and the location of *GIGAS* corresponds approximately to that of the *FTa*/*FTc* cluster in *Medicago* (Hecht et al., 2005). We confirmed a conserved location for these genes in pea and then examined whether any might be disrupted in *gigas* mutants. We found no differences within the coding regions of

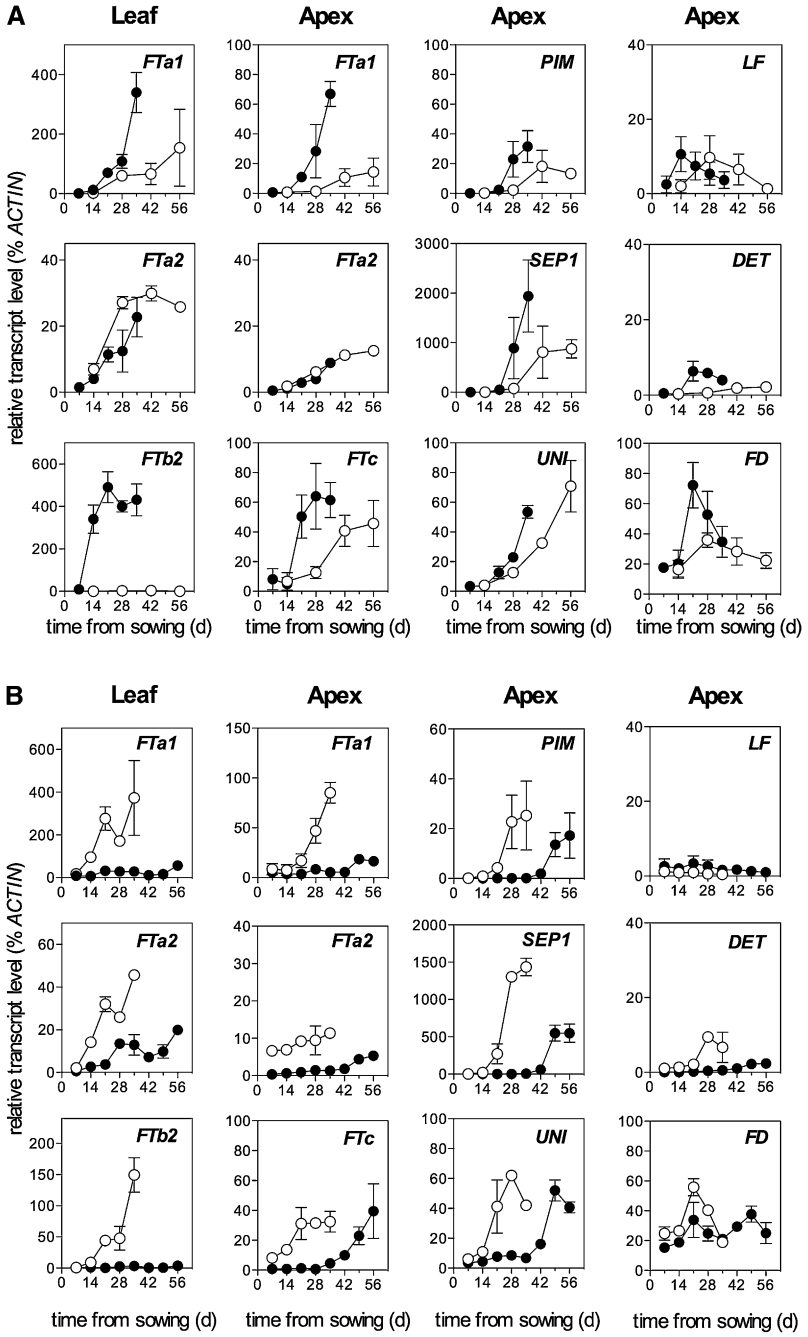

Figure 4. Pea *FT* Genes Are Misregulated in Photoperiod Response Mutants.

(A) Gene expression in the wild type (closed circles) and the *late1-2* mutant (open circles) during development under LD conditions (16 light/8 dark).
(B) Gene expression in the wild type (closed circles) and the *dne-1* mutant (open circles) during development under SD conditions (8 light/16 dark).
Relative transcript levels were determined in dissected shoot apices or the uppermost fully expanded leaf. Values have been normalized to the transcript level of the *ACTIN* gene and represent mean ± SE for $n = 2$ to 3 biological replicates, each consisting of pooled material from two plants.

FTa2 and *FTc* in *gigas-1* or *gigas-2* but identified significant changes in the *FTa1* gene in both mutants relative to their progenitor lines cv Virtus (*gigas-1*) and cv Porta (*gigas-2*). The *gigas-1* mutant contained a single nucleotide substitution in the 3′ splice site of intron 2 (Figure 5C), predicted to result in skipping of exon 3 and an immediate termination of translation after Trp-88. PCR with *FTa1* primers on *gigas-1* cDNA resulted in amplification of only the expected mutant product, a cDNA molecule missing the 41 bp of exon 3, and the wild-type *FTa1* cDNA could not be detected. In the case of the *gigas-2* mutant, failure to

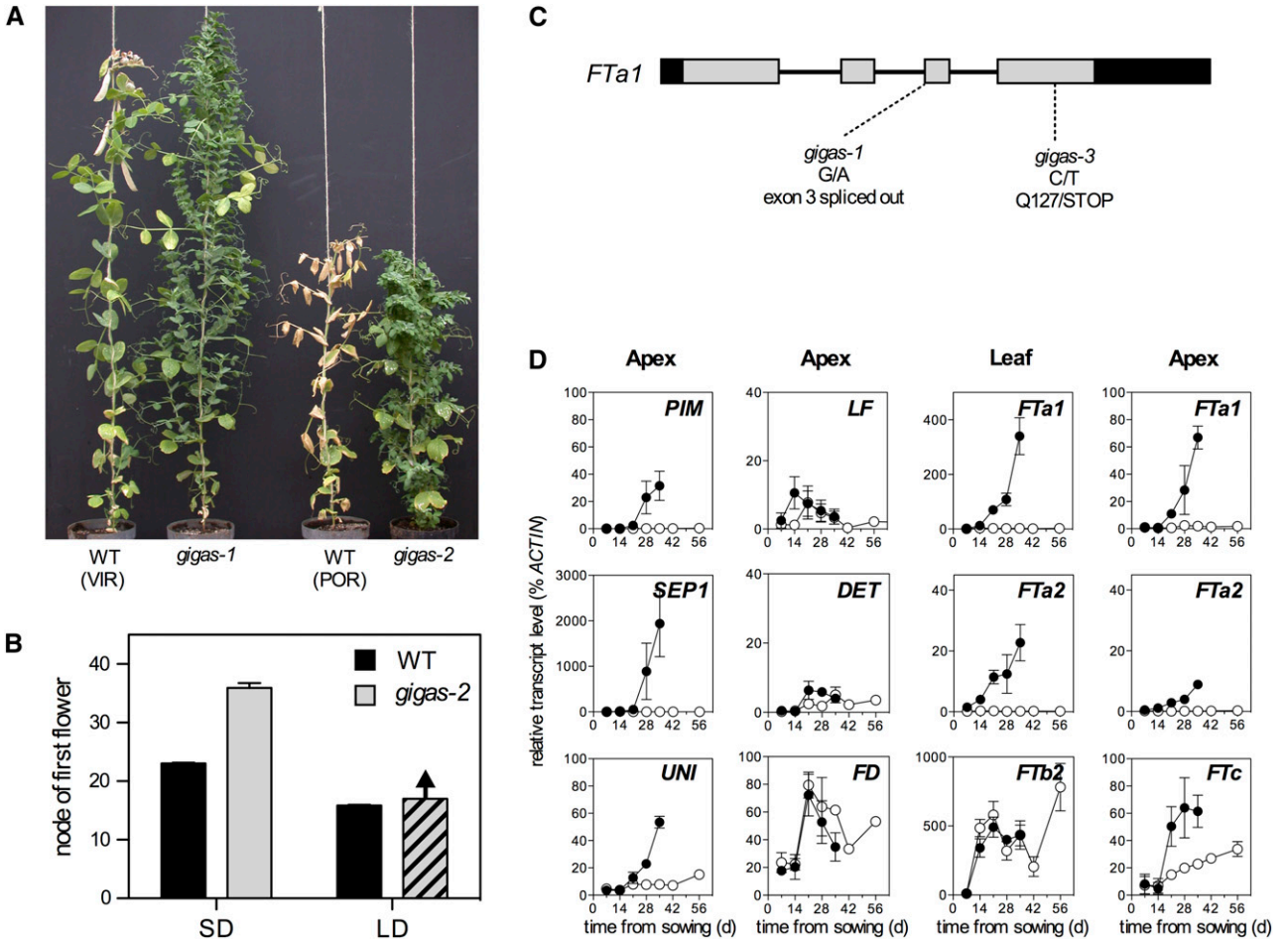

Figure 5. The *GIGAS* Locus Corresponds to *FTa1.*

(A) Representative plants of two independent *gigas* mutants and their original wild-type (WT) progenitor lines Virtus (VIR) and Porta (POR). Plants were grown for ~12 weeks under LD conditions (18 light/6 dark) in the glasshouse.

(B) Effect of the *gigas-2* mutation on flowering node in the NGB5839 genetic background. Plants were grown under SD (8 light/16 dark) and LD (16 light/8 dark) conditions in the phytotron. Values represent mean ± SE for $n = 8$ to 12. The nonflowering *gigas* LD phenotype shown in **(A)** is represented by diagonal hatching and an arrow. These plants are shown with a nominal value that corresponds approximately to the node at which the short-internode, highly branched phenotype commenced.

(C) Diagram of the *FTa1* gene showing the nature and location of mutations in *gigas* mutants. Exons are shown as boxes, with coding sequence shaded in gray and untranslated regions in black. In the *gigas-2* mutant, *FTa1* is completely deleted.

(D) Gene expression in the wild type (closed circles) and the *gigas-2* mutant (open circles) during development under LD conditions (16 light/8 dark). Relative transcript levels were determined in dissected shoot apices or the uppermost fully expanded leaf. Values have been normalized to the transcript level of the *ACTIN* gene and represent mean ± SE for $n = 2$ to 3 biological replicates, each consisting of pooled material from two plants.

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amplify *FTa1* from genomic DNA suggested the presence of a substantial deletion or rearrangement. A third *gigas* mutant (*gigas-3*) was identified among late-flowering mutants from an ethyl methanesulfonate–mutagenized population (Hecht et al., 2007) and found to carry a C-to-T substitution in exon 4 of *FTa1* that converted Gln-127 to a stop codon (Figure 5C). *FTa2* sequence was identical to the wild type in *gigas-3*, and as recombinants with *FTc* were identified during mapping, the *FTc* sequence was not examined in *gigas-3*. Transformation of the *Arabidopsis ft-1* mutant with the *FTa1* cDNA from *gigas-1* failed to rescue the late-flowering phenotype (see Supplemental Figure 4 online), confirming that *FTa1* function is significantly impaired by the *gigas-1* mutation, as expected.

Molecular Consequences of *gigas* Mutants

We next examined how loss of *FTa1* function affected the developmental regulation of genes related to inflorescence identity and the floral transition. Figure 5D shows that expression of *PIM* and *SEP1* is completely absent in *gigas*, consistent with the general failure of *gigas* mutants to produce flowers under LD conditions. Expression of *UNI* was much lower than in the

wild type, but a residual weak induction was still suggested. By contrast, induction of *DET* and *FD*, although relatively weak, was similar in the wild type and *gigas* (Figure 5D). The timing and apex-specific expression of *FTc* in wild-type plants under LD (Figure 2B) suggested that *FTc* might be a transcriptional target of other leaf-expressed *FT* genes, so we also examined how loss of *FTa1* affected expression of *FTc* and other *FT* genes. Figure 5D shows that the weak induction of *FTa2* expression in wild-type leaf tissue was completely absent in *gigas*, whereas the strong induction of *FTb2* expression was unaffected. The *gigas* mutation also prevented expression of *FTa2* in apical tissue, whereas *FTc* was induced with similar timing but to a lower level in *gigas* than in the wild type (Figure 5D). Thus, *FTa1/GIGAS* is essential for expression of inflorescence identity genes *PIM* and *SEP1* in LD and also makes a major contribution to induction of *UNI*. In addition, *FTa1/GIGAS* is also required for the normal expression of *FTa2* in the leaf and apex and *FTc* in the apex. This suggests that cross-regulation among *FT* genes may be an important feature during the induction of flowering in pea.

Genetic Interactions of *gigas* with Other Flowering Mutants

In view of the distinct flowering and molecular phenotypes shown by *gigas* and photoperiod response mutants, we next examined their genetic interactions. Surprisingly, the introduction of the *late1* mutation to the *gigas* background restored flowering and reverted the overall phenotype to one more similar to that of *late1* (see Supplemental Figure 5 online). However, the *late1 gigas* mutant flowered later than the *late1* single mutant in LD (Figure 6A), and the difference between *late1* and *late1 gigas* in LD was similar to the difference between the wild type and *gigas* in SD. This suggests that the effect of *late1* is merely to confer a SD phenotype on the *gigas* mutant in LD. It also provides further illustration that the *gigas* mutant, despite not flowering in LD, retains a strong response to LD that can be blocked by the *late1* mutation. Double mutants between *gigas* and the early-flowering photoperiod unresponsive mutant *dne* were readily distinguished in SD by a nonflowering phenotype very similar to the *gigas* single mutant grown under LD (Figure 6A). Thus, the effect of *dne* on *gigas* is the converse of *late1*, conferring a LD phenotype on the *gigas* mutant when grown in SD. Both of these interactions can be seen as being essentially additive in the sense that *DNE* and *LATE1* exert their influence on photoperiod responsiveness independently of *GIGAS*. We also examined how the interaction of *late1* and *gigas* mutations and the restoration of flowering in the *gigas* background by *late1* might be reflected in gene expression. Figure 6B and Supplemental Figure 6 online show that the late flowering of the *late1 gigas* double mutant is associated with clear induction of *PIM*, *SEP1*, and *UNI* expression at the apex despite the lack of detectable expression of any *FT* gene in leaf tissue.

Finally, we examined the genetic interaction between *GIGAS/FTa1* and the *LATE FLOWERING* (*LF*) gene, a paralog of *Arabidopsis TFL1* (Figure 1) that delays flowering in a photoperiod-independent manner (Murfet, 1975; Foucher et al., 2003). We found that the early-flowering phenotype of a putative null *lf* mutant (*lf-22*) was completely epistatic to the nonflowering *gigas* phenotype under LD, with the double *lf gigas* mutant indistinguishable from the single *lf* mutant (Figure 6A; see Supplemental Figure 5 online). This indicates that *GIGAS* promotes flowering through a negative influence on *LF* function.

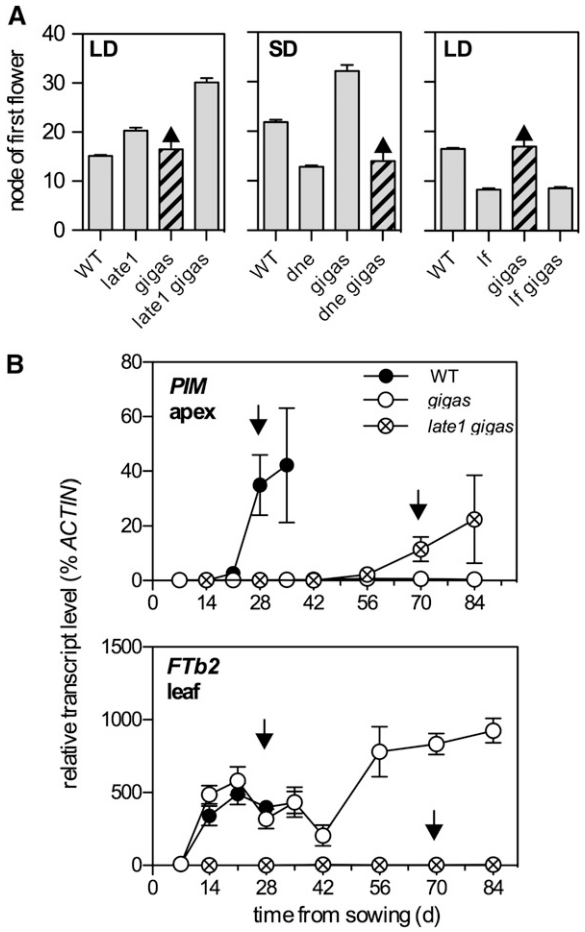

Figure 6. Genetic Interactions of *GIGAS*.

(A) Comparison of flowering node in double mutants *late1-2 gigas-2*, *dne-1 gigas-2*, and *lf-22 gigas-2* in wild-type (WT) NGB5839, and corresponding monogenic mutants. Plants were grown in the phytotron in either LD (16 light/8 dark) or SD (8 light/16 dark). As in Figure 5B, the nonflowering *gigas* LD phenotype is represented by diagonal hatching and an arrow. Plants displaying this phenotype are shown with a nominal value that corresponds approximately to the node at which the short-internode, highly branched phenotype commenced.

(B) Gene expression in NGB5839 (wild type), *gigas-2*, and the *late1-2 gigas-2* double mutant during development under LD conditions (16 light/8 dark). Relative transcript levels were determined in dissected shoot apices or the uppermost fully expanded leaf. Values have been normalized to the transcript level of the *ACTIN* gene and represent mean ± SE for $n = 2$ to 3 biological replicates, each consisting of pooled material from two plants. Arrows correspond to visible flower bud appearance in the dissected apices in the wild type and *late1-2 gigas-2* double mutants.

Effects of *gigas* on Graft-Transmissible Flowering Signals

Previous grafting experiments showed that both the late-flowering SD phenotype and the nonflowering LD phenotype of

the *gigas-1* mutant could be rescued by grafting to wild-type graft stocks bearing leaves (Beveridge and Murfet, 1996). Figure 7A shows that this is also true for the *gigas-2* mutant and confirms that *GIGAS*/*FTa1* is necessary for the production of a graft-transmissible stimulus of flowering. In previous experiments, the late-flowering phenotype of the *late1* mutant could be similarly rescued by grafting to leafy wild-type graft stocks (Hecht et al., 2007). One possible interpretation of these results is that *LATE1* acts through *GIGAS*/*FTa1* to control the same mobile stimulus, which might either be *GIGAS*/*FTa1* itself or some other signal downstream of *FTa1*. Such an interpretation would predict that *gigas* mutant graft stocks should be less effective than the wild type for rescue of the *late1* mutant. Figure 7B shows that *late1* scions grafted onto *gigas* stocks did flower significantly later (14.4 $\pm$ 0.1 nodes) than when grafted to wild-type stocks (13.2 $\pm$ 0.1 nodes, $P < 0.01$) but still flowered much earlier than *late1* self-grafted plants (19.7 $\pm$ 0.5 nodes, $P < 0.01$). Also, *late1* stocks were only slightly less effective than the wild type in promoting flowering in *gigas* scions (Figure 7B; 15.1 $\pm$ 0.2 versus 13.2 $\pm$ 0.1 nodes, $P < 0.01$), indicating that the small effect of *late1* on *GIGAS*/*FTa1* expression in leaves (Figure 4A) has only a minor effect on the mobile flowering signal. These complementary effects of *late1* and *gigas* indicate that *GIGAS*/*FTa1* does contribute to the flowering signal but also suggest a role for a component of the signal that is independent of *GIGAS*.

We tested this conclusion further by examining how *gigas* might affect the ability of *dne* mutant graft stocks to promote flowering across a graft junction under SD conditions. The *dne* mutant has elevated expression of *FTa1*, *FTa2*, and *FTb2* in leaf tissue (Figure 4B), and we reasoned that the elimination of *FTa1* activity from graft stocks by the *gigas-2* mutation would show whether any of the remaining leaf-expressed *FT* genes could influence flowering in scions. Figure 7D confirms that 3-week-old SD-grown *dne gigas* seedlings lack *FTa1* transcript as expected and also fail to express *FTa2* or *FTb1* but do express substantially higher levels of *FTb2* than the wild type or the *gigas* single mutant. This is accompanied by an increased ability to promote flowering in wild-type scions under SD conditions (Figure 7C; 17.0 $\pm$ 1.2 versus 21.7 $\pm$ 0.6 nodes, $P < 0.01$), suggesting that *FTb2* is associated with the production of a second mobile flowering signal that is independent of *GIGAS*/*FTa1*.

DISCUSSION

It is increasingly clear that members of the *FT* family play important roles in the floral transition. Compared with *Arabidopsis*, where the most detailed studies have been performed, *FT* families in many other species are considerably more complex and functional analyses are much less advanced. In this study, we characterized the *FT* family in three model legumes, soybean, *Medicago*, and pea, and identified three main branches that are likely common to all legumes. We then examined the contribution of the pea genes to control of flowering, photoperiod responsiveness, and mobile signaling.

Expression of Pea *FT* Genes

Compared with the simple *Arabidopsis FT* family, in which *FT* and *TSF* share similar expression patterns, we observed distinctly different patterns of expression for pea *FT* genes. In leaf tissue from plants in LD, only *FTb2* was clearly induced during the period in which the plant becomes physiologically committed to flower (Figure 2). This induction is photoperiod specific and together with the apparent lack of expression in apical buds suggests that *FTb2* might have a role as, or in the production of, a mobile signal for flower induction in LD. Like *FTb2*, *FTa1* and *FTa2* are also induced in leaves, but their expression is not differentially regulated by daylength over the period prior to floral commitment in LD, suggesting that they are less likely to participate in initial signaling for LD flower induction. However, in apical buds, *FTa1* and *FTc* showed clear, LD-accelerated induction that occurred after floral commitment with similar timing to induction of inflorescence identity genes *PIM*, *UNI*, and *SEP1*. This raises the possibility that both of these *FT* genes might play a role in the apex that is intermediate between a mobile *FT* protein and genes specifying inflorescence identity or act in parallel with these genes as florigen targets. It is of particular interest that *FTc* expression is reduced in both *gigas* and *late1* mutants (Figures 4 and 5), and the effect of *late1* is at least partly independent of *GIGAS* (see Supplemental Figure 6 online). This provides a clear example of transcriptional cross-regulation among members of an *FT* gene family and further supports the possibility that *FTc* may integrate long-range signaling from other *FT* genes.

The existence of multiple, differently regulated *FT* family members that we observe here in pea has also been described for several other species. For example, in barley (*Hordeum vulgare*), two *FT*-like genes are preferentially expressed under LD but differ in developmental and diurnal timing, while a third gene is preferentially expressed under SD (Faure et al., 2007; Kikuchi et al., 2009). In sunflower (*Helianthus annuus*), one of three functional *FT*-like genes is specifically expressed in the shoot apex but not in leaves (Blackman et al., 2010), while in maize, multiple *FT*-like genes are expressed in a variety of tissues, including roots, leaves, developing inflorescences, and seeds (Danilevskaya et al., 2008). The next broad challenge will be to assess the functional relevance of these differences. *Arabidopsis* complementation experiments show that all five pea FT proteins have the capacity to function like *Arabidopsis* FT to some extent. They also suggested distinct differences in activity, but proof of this will clearly require functional analysis in the pea system.

GIGAS/*FTa1* Function

Identification of both *gigas-1* and *gigas-2* mutants as RNA-null alleles of *FTa1* enabled a first step in understanding the functions of pea *FT* genes. These mutants clearly show that *FTa1* has an important role in control of flowering not only under LD but also under SD. Studies in rice and *Arabidopsis* also report minor effects of *FT* genes on flowering in noninductive conditions, so this is not unexpected (Yamaguchi et al., 2005; Komiya et al., 2008). However, the *gigas* LD phenotype, which combines a failure to flower with the retention of LD responses for other traits,

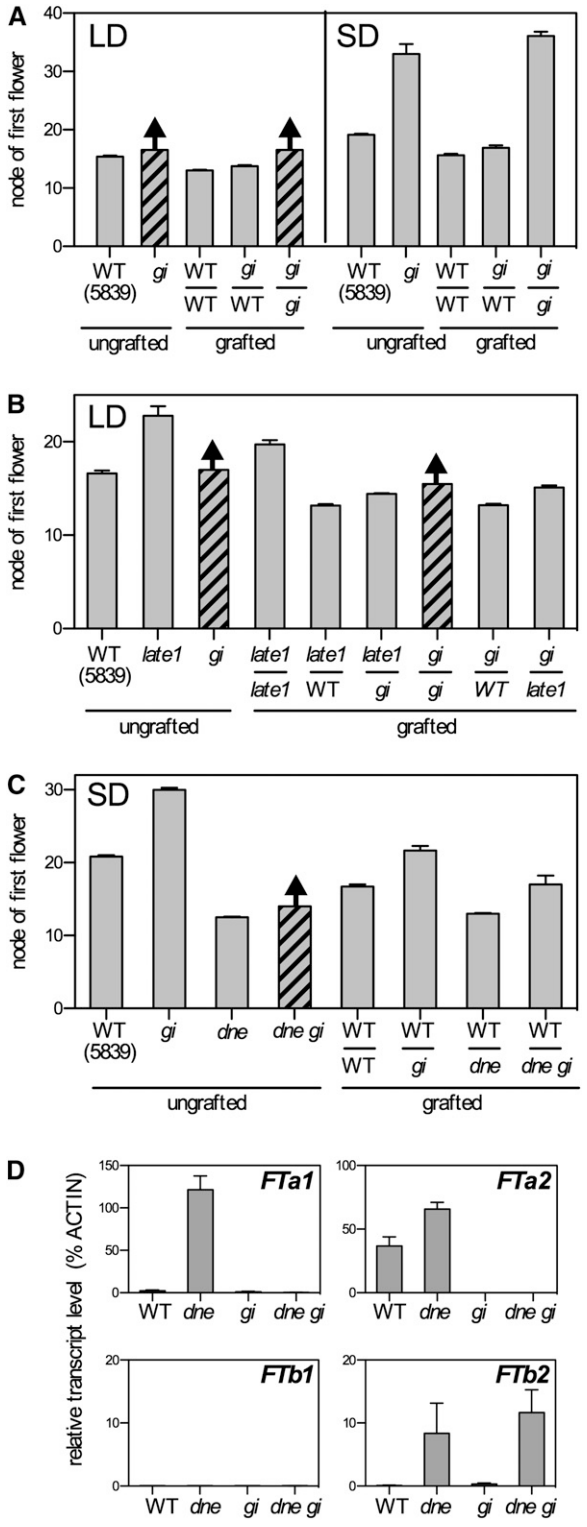

Figure 7. Effects of *GIGAS* on Graft-Transmissible Floral Signaling.

The effects of *gigas* mutations on mobile floral signals were examined by grafting 6-d-old shoots excised at the epicotyl (graft scion) onto the main stem of 3-week-old plants above the uppermost fully expanded leaf (graft stock). For each graft combination, the genotypes of scion (top) and stock (bottom) are shown, separated by a horizontal line.

(A) Grafting to the wild type (WT) rescues the *gigas-2* phenotype under LD and SD. Self-grafted *gigas* displayed a phenotype typical of ungrafted *gigas* control plants, whereas grafting of *gigas* onto the wild type restored a near- wild-type phenotype.

(B) Complementary graft-transmissible promotion of flowering by *gigas-2* and *late1-2* in LD. As both *late1* and *gigas* mutants were previously observed to affect a graft transmissible stimulus, their ability to complement each other across a graft union was examined, in comparison with self-grafts and grafts of mutants onto the wild type.

(C) *GIGAS*-independent graft-transmissible promotion of flowering by the *dne-1* mutant in SD. The importance of *GIGAS*/*FTa1* for the graft-transmissible effect of *dne* was examined by comparing the ability of *dne* single mutant and *dne gigas* double mutant stocks to promote flowering of wild-type scions held in SD.

(D) Expression of *FT* genes in the uppermost expanded leaf of graft stocks used in **(C)**. Values have been normalized to the transcript level of the *ACTIN* gene and represent mean ± SE for $n = 4$ to 6 biological replicates.

All plants were grown in the phytotron in either LD (16 light/8 dark) or SD (8 light/16 dark). Data in **(A)** to **(C)** are mean ± SE for $n = 6$ to 15. As in Figure 5B, the nonflowering *gigas-2* LD phenotype is represented in **(A)** to **(C)** by diagonal hatching and an arrow. Plants displaying this phenotype are shown with a nominal value that corresponds approximately to the node at which the short-internode, highly branched phenotype commenced.

differs from *FT* mutants in other species and shows that *FTa1* does not mediate the general response to photoperiod. The greater similarity of *gigas* to pea inflorescence identity mutants, such as *veg1* and *veg2*, instead indicates that the role of GIGAS/FTa1 is restricted to the induction of flowering (Reid and Murfet, 1984; Benlloch et al., 2007). It is also consistent with the observation that *FTa1* expression in wild-type leaves is not induced by LD in the physiological commitment window but occurs slightly later, in parallel with induction of the inflorescence identity genes and its own induction in apical buds (Figure 2). Further support is provided by the *late1* mutant, which despite a lack of photoperiod responsiveness shows only minor effects on *FTa1* expression in leaves (Figure 4A; Hecht et al., 2007) during the physiological commitment window.

In addition to supporting a role for *FTa1* in the acquisition of inflorescence identity and excluding a major role in photoperiod responsiveness, the comparison between *late1* and *gigas* suggests an association between another leaf-expressed *FT* gene, *FTb2*, and the response to photoperiod. Similar to the wild-type in SD, *late1* mutants in LD show delayed induction of *FTa1* and *FTc*, and no clear effect on *FTa2*, but fail completely to induce *FTb2* expression, while in *gigas*, *FTb2* is induced normally (Figures 4A and 5D). Several lines of evidence thus identify *FTb2* as a strong candidate for the primary *FT* gene controlling photoperiod response: it is not expressed in SD and is strongly upregulated during floral induction in LD, and this upregulation is blocked in a photoperiod response mutant and unaffected in *gigas* mutants.

In *Arabidopsis* and temperate cereals, *FT* genes also integrate signaling from vernalization pathways (Kim et al., 2009). We have not examined vernalization in this study, but a previous report

showed that vernalization could induce flowering of *gigas-1* in LD and was no less effective in *gigas-1* than in the wild type for promotion of flowering in SD (Beveridge and Murfet, 1996). This suggests that *FTa1* is not a major target for regulation by vernalization in pea and that vernalization may oppose whatever factor is responsible for the LD vegetative phenotype of *gigas*. However, vernalization in pea is reported to act through distinct mechanisms in leaves and at the apex (Reid and Murfet, 1975), and the leaf-based response in *gigas* is apparently missing (Beveridge and Murfet, 1996), suggesting that *FTa1* may nevertheless mediate a component of the vernalization response. It will be interesting in the future to examine how vernalization may regulate pea *FT* genes and the broader molecular phenotypes of *gigas* and photoperiod response mutants.

Two Distinct Mobile Flowering Signals in Pea

Grafting experiments show that the lack of *FTa1* in the shoot can clearly be compensated for by some factor moving from leaves or other stock tissues (Figure 7A). In view of the growing number of examples where FT-like proteins act as mobile signals (Turck et al., 2008), and the clear induction of *FTa1* in leaves under LD, the most likely explanation for graft rescue of *gigas* mutants is the movement of the FTa1 protein itself. An alternative explanation is that the signal is some other molecule that is regulated by *FTa1*. For example, as expression of *FTa2* in leaves is dependent on *GIGAS*/*FTa1* (Figure 5D), it is possible that *FTa2* could play this role.

As in the case of *gigas*, the late-flowering phenotype of the *late1* mutant can also be rescued by grafting to the wild type (Hecht et al., 2007), even though *gigas* and *late1* mutants have distinctly different phenotypes and *FT* expression profiles (Figures 4A and 5D). The simplest interpretation of this would be that the same *FTa1*-dependent signal is deficient in scions of both mutants and can be supplied by wild-type stocks, but several lines of evidence suggest that this is not the case. First, *late1* mutants show only a small reduction in *FTa1* expression and have similar *FTa2* expression compared with the wild-type. Second, *gigas* scions grafted onto *late1* stocks are induced to flower and flower much earlier than *late1* self-grafts (Figure 7B; Hecht et al., 2007). This would not be the case if the sole reason for the late flowering of *late1* and the nonflowering of *gigas* were a deficiency in the same mobile signal. Indeed, the fact that among the five *FT* genes only *FTa1* and *FTa2* are expressed in leaf tissue of the *late1* mutant provides additional support for the idea that *FTa1* is responsible for generation of a mobile signal in *late1* stocks capable of rescuing the *gigas* mutant. Third, significant promotion of flowering occurs in *late1* scions when grafted to *gigas* stocks under LD. The fact that *gigas-2* stocks lack the *FTa1* gene clearly excludes FTa1 protein as the active molecule in this case, and in view of the negligible *FTa2* expression level, the participation of FTa2 protein is also unlikely. Furthermore, out of the three leaf-expressed *FT* genes, only *FTb2* is significantly expressed in *gigas*, implying that if the graft rescue of *late1* scions by *gigas* stocks reflects *FT*-dependent signal generation in stock leaves, then *FTb2* is the only likely candidate. This conclusion is supported by results from grafts with the *dne gigas* double mutant under SD (Figure 7C). Like the *gigas* single mutant, *dne gigas* does not express *FTa1* or *FTa2* but is more effective than *gigas* at promoting flowering of wild-type scions and expresses much higher levels of *FTb2* transcript in leaf tissue (Figure 7D). The common feature in both these two latter grafting experiments is thus an association between flower-promoting ability and the presence of significant *FTb2* expression in leaves: in *gigas* stocks under LD and in *dne gigas* stocks under SD.

Overall, these results point to the coexistence of two distinct graft-transmissible flower-promoting signals in pea that are correlated with expression of *FTa1* and *FTb2* genes in leaf tissue. In addition, the different expression profiles of these genes during development of the wild type in LD and their association with different flowering phenotypes in the *late1* and *gigas* mutants both suggest a hypothesis in which *FTa1* and *FTb2* influence the flowering process via mechanisms that are at least partially distinct. One plausible interpretation is that *GIGAS*/*FTa1* may confer photoperiod-independent monitoring of plant size or other environmental variables and acts specifically on the induction of flowering, whereas *FTb2* may act as the primary leaf-derived signal for acceleration of flowering by LD and is also responsible for other aspects of the LD response (Figure 8). Several recent

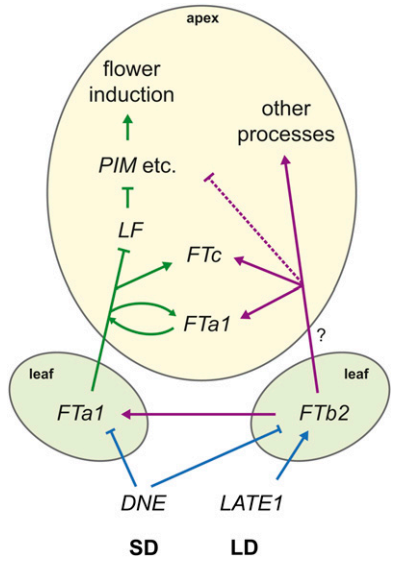

Figure 8. A Model for the Role and Interactions of Pea *FT* Genes in Flowering and Photoperiod Responsiveness.

This model summarizes the major results from the study and the main hypotheses derived from them. Photoperiod response genes *DNE* and *LATE1* regulate the expression of two key *FT* genes in leaves: *FTa1* and *FTb2*. We propose that both genes generate distinct mobile signals that influence flowering at the apex. The mobile *FTa1* signal acts specifically on flowering in both LD and SD, potentially through antagonizing the repression by *LF* of *PIM* and other inflorescence identity genes. The *FTa1*-dependent signal also contributes to induction of *FTc* and potentially to the expression of *FTa1* itself at the apex. LD induce expression of *FTb2* in leaves and the generation of a second, potentially *FTb2*-dependent, signal that influences flowering through induction of *FTa1* in leaves and (directly or indirectly) at the apex. Independently of *FTa1*, this second signal also induces *FTc* at the apex, regulates a number of other photoperiod-responsive processes, and may inhibit flower induction.
[See online article for color version of this figure.]

studies indicate that *FT* genes may have roles beyond the simple initiation of flowering. For example, in tomato, where flowering is not responsive to photoperiod, *FT* and *TFL1* homologs interact to regulate growth and determinacy across several developmental processes (Shalit et al., 2009), and in poplar (*Populus* spp), *FT* and *TFL1* homologs also have a role in the induction of dormancy (Böhlenius et al., 2006; Ruonala et al., 2008; Mohamed et al., 2010). Future isolation of *FTb2* mutants will clearly be an important test of this possibility in pea and will clarify other aspects of *FTb2* function, including its regulatory relationship with *FTa1* and *FTc* and its interaction with *TFL1* homologs.

Control of Flowering without *FTa1*

Flowering of the *gigas* single mutant in SD or in the *late1 gigas* mutant in LD shows that in pea, the floral transition can still proceeds in the absence of significant expression of any *FT* gene in leaves. This implies the existence of an underlying default pathway ensuring that flowering will eventually occur regardless of daylength and suggests that the *FT* module may be superimposed on this to provide a means of accelerating flowering under favorable conditions. In *Arabidopsis*, plants lacking both *FT* and *TSF* still flower, and mechanisms are known that bypass *FT* to induce expression of floral meristem identity genes, including regulation of *SOC1* by gibberellin (Moon et al., 2003; Jang et al., 2009). It will be interesting to examine whether a similar mechanism also operates in pea.

Grafting experiments indicate a positive role for *FTa1* and *FTb2* in the initiation of flowering. However, the nonflowering of the *gigas* mutant in LD and the fact that restoration of flowering by the *late1* mutation is coupled with a loss of *FTb2* expression clearly imply a more complex regulation. One possibility could be that LD conditions induce or activate some factor that inhibits flower formation in the absence of *GIGAS*/*FTa1*, and one potential candidate for this factor could be the *TFL1* paralog *LF* (Foucher et al., 2003). In both *Arabidopsis* and tomato, FT and TFL1 proteins interact antagonistically to regulate floral induction, leading to the suggestion that activation of target genes depends on balance of these two gene activities (Kardailsky et al., 1999; Kobayashi et al., 1999; Shalit et al., 2009). In pea, loss of *LF* function confers extremely early flowering, and the complete epistasis of *lf* over *gigas* (Figure 6; see Supplemental Figure 5 online) is consistent with a mechanism in which *GIGAS* opposes *LF*-mediated inhibition of flowering. Therefore, it is tempting to speculate that the vegetative phenotype of *gigas* in LD could result from a LD-specific increase in *LF* activity that would normally be opposed in the wild type by *GIGAS*/*FTa1* activity. In *Arabidopsis*, *TFL1* is transcriptionally upregulated by *CO* (Simon et al., 1996), and in pea, both *LF* and *DET* are expressed at a higher level in LD than in SD (Figure 2), and their expression is delayed (*LF*) or reduced (*DET*) in *late1* relative to the wild type under LD (Figure 4A). However, the fact that expression of *LF* is lower in the nonflowering *gigas* mutant than in the *late1 gigas* double mutant (see Supplemental Figure 6 online) rules out transcriptional regulation of *LF* as a straightforward explanation for the LD-specific vegetative phenotype. Other explanations could include spatially localized, posttranscriptional regulation of LF activity or the action of some other factor. Detailed molecular comparisons of *gigas* with photoperiod response mutants may give more insight into this matter.

In conclusion, this study highlights the important role of pea *FT*-like genes in both regulating the timing of flowering and specifying floral identify. Our results suggest a more complex mechanism for induction of flowering in pea compared with *Arabidopsis* that involves cross-regulation among different *FT* genes with distinct patterns of expression and different inherent activities and the action of at least two mobile signals, of which one is GIGAS/FTa1. Given that expanded *FT* families with diverse patterns of regulation have now been reported in a range of species, it is likely that such complexity will prove to be relatively common in higher plants. Our results also pave the way for in-depth functional analyses of *FT* genes in other legumes and should accelerate molecular analysis of the genetic variation for flowering time and photoperiod responsiveness that exists in many crop legume species.

METHODS

Plant Material, Growth Conditions, and Grafting

The origins of the *gigas-1*, *le-3*, *dne-1*, and *late1-2* mutants have been described previously (King and Murfet, 1985; Beveridge and Murfet, 1996; Lester et al., 1999; Hecht et al., 2007). The *gigas-2* mutant was generated by W.K. Swiecicki following fast-neutron mutagenesis of cultivar Porta, and the *gigas-3* mutant was generated from line NGB5839 by ethyl methanesulfonate mutagenesis (Hecht et al., 2007). Plants for all gene expression studies (Figures 2B, 2C, 4, 5D, and 6B), *Arabidopsis thaliana* flowering experiments (Figure 3) and photoperiod transfer experiments (Figure 2A) were conducted in growth cabinets at 20°C, whereas flowering time and grafting experiments (Figures 5A, 5B, 6A, and 7) were conducted in the Hobart phytotron using previously described growth media, light sources, phytotron conditions, and grafting protocols (Hecht et al., 2007). Standard phytotron SD conditions consisted of an 8-h photoperiod of natural light, which was extended for 8 h with white light from compact fluorescent tubes at an irradiance of 10 μmol m^{-2} s^{-1} to give a 16-h LD.

Gene Isolation and Phylogenetic Analysis

All pea (*Pisum sativum*) *FT* genes except *FTc* were isolated from leaf cDNA and genomic DNA of the wild type (NGB5839) using PCR techniques, rapid amplification of cDNA ends (SMART RACE cDNA amplification kit; Clontech), and genome walking (GenomeWalker Universal kit; Clontech), using specific primers designed on an initial DNA fragment obtained with degenerate primers (Hecht et al., 2005). *FTc* full genomic sequence was obtained by screening a commercially made pea genomic library (Clontech). All PCR fragments were cloned in pGEM-T easy (Promega) and sequenced at the Australian Genome Research Facility. Primer details are given in Supplemental Table 3 online. For the phylogenetic tree shown in Figure 1B, amino acid sequences of legume PEBP proteins were aligned using ClustalX (Thompson et al., 1997). Distance and parsimony-based methods were used for phylogenetic analyses in PAUP*4.0b10 (http://paup.csit.fsu.edu/) using the alignment shown in Supplemental Figure 2 online.

Arabidopsis Complementation

The *Arabidopsis ft-1* mutation in the Landsberg *erecta* background was previously described (Koornneef et al., 1991; Kardailsky et al., 1999;

Kobayashi et al., 1999) Full-length cDNA fragments for pea *FT* genes were generated by PCR from pea wild-type line NGB5839 and additional full-length *FTa1* cDNAs from the original *gigas-1* mutant and its progenitor cultivar Virtus using primers listed in Supplemental Table 3 online. The cDNA fragments were were first cloned in pCR8/GW/TOPO vector (Invitrogen) and then recombined into the binary vector pB2GW7 using Gateway cloning (Karimi et al., 2002) and confirmed by sequencing. *Arabidopsis* transformation was conducted by floral dipping (Bechtold et al., 1993), and the flowering phenotypes of several independent transformants per construct were characterized through several generations.

Gene Expression Studies

Harvested tissue consisted of both leaflets from the uppermost fully expanded leaf or apical buds dissected to a size ~2-mm wide and 3-mm long. Samples were frozen in liquid nitrogen and total RNA extracted using the SV Total RNA isolation system (Promega). RNA concentrations were determined by spectrophotomoter analysis using a NanoDrop 8000 (Thermo Scientific). Reverse transcription was conducted in 20 μL with 1 μg of total RNA using MMLV high performance reverse transcriptase (Epicenter) according to the manufacturer's instructions. RT-negative (no enzyme) controls were performed to monitor for contamination with genomic DNA. First-strand cDNA was diluted five times, and 2 μL was used in each real-time PCR reaction. Real-time PCR reactions using SYBR green chemistry (Sensimix, Quantace, Bioline) were set up with a CAS-1200N robotic liquid handling system (Corbett Research) and run for 50 cycles in a Rotor-Gene RG3000 (Corbett Research). Two technical replicates and two to three biological replicates were performed for each sample. Transcript levels of several potential reference genes, including *EF1a* (Johnson et al., 2006), *UBI* (Platten et al., 2005), and *ACTIN* (Weller et al., 2009a), were examined in the different tissue series. Of these genes, *ACTIN* was found to be the most stably expressed, and this gene was therefore used to evaluate transcript levels of flowering genes, as previously described (Weller et al., 2009a). Primer sequences are given in Supplemental Table 3 online.

Accession Numbers

Please refer to Supplemental Table 1 online for accession numbers of pea, *Medicago*, and soybean FT genes. Accession numbers for other genes are as follows: *DET* (AY340579), *LF* (AY343326), *PIM* (AJ291298), *SEP1* (AY884290), and *UNI* (AF010190).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. Microsynteny around *FT* Genes in *Medicago* and Soybean.

Supplemental Figure 2. Alignment of Legume PEBP Amino Acid Sequences.

Supplemental Figure 3. Overexpression of *PsFTc* in Transgenic *Arabidopsis*.

Supplemental Figure 4. The *gigas-1* Mutation Completely Impairs the Activity of Pea *FTa1* in Transgenic *Arabidopsis*.

Supplemental Figure 5. Photographs of Representative Plants of *late1 gigas* and *lf gigas* Double Mutants.

Supplemental Figure 6. Gene Expression in NGB5839 (WT), *gigas-2*, and the *late1-2 gigas-2* Double Mutant during Development under LD.

Supplemental Table 1. Details of *FT*-like sequences in pea, *Medicago*, and soybean.

Supplemental Table 2. Microsynteny in the *FTa*/*FTc* region in soybean and *Medicago*.

Supplemental Table 3. Primers.

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