Pea \textit{LATE BLOOMER1} Is a \textit{GIGANTEA} Ortholog with Roles in Photoperiodic Flowering, Deetiolation, and Transcriptional Regulation of Circadian Clock Gene Homologs\textsuperscript{1[W][OA]}

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Genes controlling the transition to flowering have been studied in several species, including Arabidopsis (\textit{Arabidopsis thaliana}) and rice (\textit{Oryza sativa}), but have not yet received much attention in legumes. Here, we describe a new allelic series of late-flowering, photoperiod-insensitive mutants in the pea (\textit{Pisum sativum}) \textit{LATE BLOOMER1 (LATE1)} gene and show that \textit{LATE1} is an ortholog of Arabidopsis \textit{GIGANTEA}. Mutants display defects in phytochrome B-dependent deetiolation under red light and in the diurnal regulation of pea homologs of several Arabidopsis circadian clock genes, including TIMING OF \textit{CAB1}, \textit{EARLY FLOWERING4}, and \textit{CIRCADIAN CLOCK ASSOCIATED1/LATE ELONGATED HYPOCOTYL}. \textit{LATE1} itself shows strongly rhythmic expression with a small but distinct acute peak following dark-to-light transfer. Mutations in \textit{LATE1} prevent the induction of a \textit{FLOWERING LOCUS T (FT)} homolog \textit{FTL} in long days but cause only minor alteration to the rhythmic expression pattern of the only known group 1a \textit{CONSTANS} homolog \textit{COLa}. The late-flowering phenotype of \textit{lalte1} mutants can be completely rescued by grafting to the wild type, but this rescue is not associated with a significant increase in \textit{FTL} transcript level in shoot apices. Genetic interactions of \textit{lalte1} with the photoperiod-insensitive, early-flowering sterile node (\textit{sn}) mutant and impairment of the \textit{LATE1} diurnal expression rhythm in \textit{sn} plants suggest that \textit{SN} may also affect the circadian clock. These results show that several functions of Arabidopsis \textit{GIGANTEA} are conserved in its pea ortholog and demonstrate that genetic pathways for photoperiodic flowering are likely to be conserved between these two species. They also suggest that in addition to its role in the floral transition, \textit{LATE1} also acts throughout reproductive development.

Plant development is highly attuned to the environment, and light is one of the most important environmental factors shaping plant form. Plants can respond not only to changes in light quality, quantity, and direction, but also to changes in the daily photoperiod. The most prominent process regulated by photoperiod is the induction of flowering, but photoperiod has also been reported to control a wide variety of other developmental processes, including stem elongation, bud dormancy, axillary branching, leaf growth, and the formation of storage organs (Thomas and Vince-Prue, 1997).

Over recent years, the genetic network controlling photoperiod perception and response has been extensively studied in the long-day (LD) plant Arabidopsis (\textit{Arabidopsis thaliana}). As currently understood, the response to photoperiod depends on accumulation of the transcription factor \textit{CONSTANS} (CO), which in Arabidopsis occurs specifically under LD (Valverde et al., 2004). The main role of CO appears to be the transcriptional activation of \textit{FLOWERING LOCUS T (FT)}, a gene of unknown biochemical function that also mediates the effects on flowering of other environmental variables such as light quality and temperature (Blázquez et al., 2003; Cerdán and Chory, 2003; Wigge et al., 2005). LD-specific CO accumulation is achieved through several distinct mechanisms, including control of \textit{CO} transcription by light (Imaizumi et al., 2003, 2005) and the circadian clock (Suárez-López et al., 2001; Yanovsky and Kay, 2002), and light regulation of CO protein stability (Valverde et al., 2004).

Light perception and circadian rhythms are therefore central to the generation of photoperiodic responses. This is evident in the fact that mutations in genes necessary for these processes generally confer altered flowering time and an impaired ability to respond to differences in photoperiod. For example, loss-of-function mutations in the circadian clock-related genes \textit{EARLY FLOWERING3 (ELF3)}, \textit{LATE ELONGATED HYPOCOTYL (LHY)}, \textit{CIRCADIAN CLOCK ASSOCIATED1 (CCA1)}, \textit{ELF4}, \textit{LUX ARRHYTHMO}, and TIMING OF \textit{CAB1 (TOC1)} all confer early flowering (Strayer et al., 2000; Hicks et al., 2001; Doyle et al., 2002; Mizoguchi et al., 2002; Hazen et al., 2005). In contrast, mutations in
the GIGANTEA (GI) gene, the blue-light (B) photoreceptor CRYPTOCHROME2, or the putative B photoreceptor FLAVIN-BINDING KELCH-REPEAT F-BOX1 confer a late-flowering phenotype (Guo et al., 1998; Fowler et al., 1999; Park et al., 1999; Nelson et al., 2000; Imaizumi et al., 2003). In many of these cases, the flowering defect results from aberrant diurnal expression of CO that in turn causes inappropriate regulation of FT.

As the genetic control of photoperiodism has become increasingly well understood in Arabidopsis, other studies have begun to explore the function of corresponding genes in other species (Hayama and Coupland, 2004; Turner et al., 2005; Böhlenius et al., 2006; Lifschitz et al., 2006). The legume family is a large plant group that includes many important crop species and several genetic model species, including garden pea (Pisum sativum), Medicago truncatula, Lotus japonicus, and soybean (Glycine max). Pea has been a prominent model for genetics and physiology of flowering and photoperiodism for some time (Murfet, 1977; Reid et al., 1996), and several different pea flowering mutants have been described. These include the early-flowering, photoperiod-insensitive sterile nodes (sn), die neutralis (dne), and photoperiod (ppd) mutants; the late-flowering, photoperiod-insensitive phytochrome A (phyA) mutant; the early-flowering, photoperiod-sensitive late flowering (lf) and phyB mutants; and the late-flowering, photoperiod-sensitive gigas and vegetative2 (veg2) mutants (Murfet, 1977; Reid et al., 1996). Grafting experiments have suggested that gigas mutants impair the production or transport of a mobile flowering stimulus, whereas the sn, dne, and ppd mutants impair the production or transmission of a mobile flowering stimulus (Murfet, 1977; Reid et al., 1996). The phenotype of gigas mutants suggests that the GIGAS-controlled stimulus is specific for flower initiation (Beveridge and Murfet, 1996), whereas the photoperiod-specific pleiotropic phenotypes of sn, dne, and ppd mutants suggest that the inhibitor may have a wider role in the control of range of photoperiod-responsive traits (Murfet, 1985).

Despite the accumulation of considerable physiological data on these mutants, meaningful comparison with Arabidopsis has been difficult because of the relatively small number of flowering loci known in pea and the lack of molecular information about these loci. We recently reported the isolation of many different Arabidopsis flowering gene homologs in pea (Hecht et al., 2005), and have now begun to analyze their functions using forward and reverse genetics and expression analysis. In this study, we describe the isolation and transcriptional regulation of the pea ortholog of Arabidopsis GI and use newly isolated loss-of-function mutants to investigate its function in flowering, phanomorphogenesis, and the regulation of circadian clock gene homologs. The results from our experiments provide a useful point of comparison for photoperiod response pathways in LD plants and suggest that previous models for flowering control in the pea system (Murfet, 1977; Reid et al., 1996) may need revision.

RESULTS

Phenology of Flowering in Pea

Pea is a quantitative LD plant with a caulescent growth habit. Following floral induction, simple axillary racemes are initiated at successive nodes along a main shoot axis. These lateral (or secondary) inflorescences typically possess one to three flowers, although some genotypes may produce as many as seven (Murfet, 1985; Reid et al., 1996). The transition to flowering is quantified either in terms of the node of first flower initiation or the time to first open flower. These two traits are equivalent when the first formed flower is the first to open, but, under certain conditions or in certain genotypes, one to several of the first-formed flowers may abort at various stages prior to flower opening (Haupt, 1969; Murfet, 1985). Following the transition to flowering, all subsequent nodes also bear inflorescences, except in some conditions where vegetative reversion has been observed (Murfet, 1985). The reproductive phase is concluded by a rapid growth reduction and senescence of the shoot apical meristem, which remains indeterminate. Growth under non-inductive short days (SD) results not only in delayed flower initiation, but also less vigorous reproductive development and an extended reproductive phase. This is seen as a tendency for abortion of inflorescence and flower initials, a slower rate of development of inflorescences, flowers and pods relative to the rate of leaf opening, increased number of flowers per inflorescence, reduced number of mature seeds formed per pod, and an increased number of reproductive nodes (Haupt, 1969; Murfet, 1985). Other vegetative traits are also affected by photoperiod, most notably the tendency for outgrowth of axillary meristems. In LD, lateral branching is normally absent, whereas in SD strong lateral branches are often formed from basal nodes (Murfet, 1985; Reid et al., 1996). Some of these features are illustrated for a commonly-used, wild-type tall 'Torsdag' and its isogenic dwarf derivative NGB5839 in Supplemental Table S1 and Figure 1A. Among known late-flowering pea mutants, two phenotypic classes can be distinguished: mutants that exhibit these additional SD-like phenotypes in LD (e.g. the phyA mutant; Weller et al., 1997) and those that do not (e.g. the gigas and veg2 mutants; Murfet and Reid, 1993; Beveridge and Murfet, 1996; Fig. 1B).

Isolation of Pea Mutants Impaired in LD-Specific Promotion of Flowering

We chose to conduct flowering studies in the NGB5839 dwarf genetic background (Ross and Reid, 1991) in view of its small size and greater convenience for handling. This line will be referred to hereafter as the wild type. The NGB5839 line carries a mutation (le-3) in the GA 3β-hydroxylase gene LE and is deficient in the active GA, GA1 (Lester et al., 1999). Comparison with the isogenic LE ‘Torsdag’ shows that the GA deficiency imposed by the le-3 mutation has no
substantial effect on node of flower initiation or a range of other flowering-related traits under either LD or SD (Supplemental Table S1; Fig. 1E). This is consistent with previous reports that GAs have minimal influence on the transition to flowering or on photoperiodism in pea (Murfet and Reid, 1987).

To identify new photoperiod response loci, we screened an ethylmethane sulfonate-mutagenized M2 population for mutants that were late flowering in LD. We identified several different phenotypic classes of late-flowering mutants that defined several genetic loci, which we have designated LATE BLOOMER (LATE) loci. We initially focused on a group of mutants that we considered to be putative photoperiod-response mutants because they were not only late flowering but were also similar to SD-grown wild-type plants in other respects, including increased basal branching and an increased number of reproductive nodes. This group included new phyA mutants and several new allelic groups of late mutants (Fig. 1B).

The LATE1 locus is represented by six mutant alleles (late1-1 to late1-6) that all behave in a normal Mendelian recessive manner. When grown under LD conditions, these mutants are all significantly later flowering than the wild type and show a range of other pleiotropic characteristics in addition to late flowering, including increased basal branching and an extended reproductive phase (Fig. 1, B–D). We observed significant differences among the late1 mutants in flowering under LD and SD and in their degree of photoperiod responsiveness. In addition, in plants grown under seasonally variable conditions in the phytotron, the severity of the phenotypes of some mutant alleles also varied with season.

Under LD conditions in the phytotron, where plants were grown under a photoperiod of 8 h of natural daylight extended with weak white fluorescent light, three of the mutants, late1-1, late1-2, and late1-4, consistently flowered at a similar node to wild-type NGB5839 plants grown under SD, regardless of season (Fig. 1, D and E). In autumn sowings (e.g. Fig. 1D), the late1-3 and late1-5 mutants showed a less severe phenotype, flowering earlier in LD than the wild type in SD. However, in spring sowings (e.g. Fig. 1E), only late1-5 showed...
a weaker LD phenotype, and late1-3 mutants flowered as late in LD as the late1-1 and late1-2 mutants. The late1-6 mutant was consistently much later flowering than the wild type or any other late1 mutant under LD. Also, although this mutant showed an extended reproductive phase compared with the wild type, it had a disproportionately small number of reproductive nodes compared to the other mutants (Fig. 1D). Under SD conditions in the phytotron (8 h of natural daylight), three of the mutants (late1-2, late1-4, and late1-5) showed no clear phenotype and flowered at the same time as the wild-type plants (Fig. 1E). The other three mutants (late1-1, late1-3, and late1-6) were always observed to flower later than SD-grown wild-type plants, although this difference was greater in spring sowings than in autumn sowings (e.g. late1-1 mutant in Figs. 1E and 2F).

These differences in flowering under SD and LD meant that the late1 mutants also differed in their response to photoperiod. The late1-2 and late1-4 alleles consistently showed no response to photoperiod, whereas the remaining mutants did show a significant response (Fig. 1E).

**LATE1 Participates in PhyB-Dependent Seedling Photomorphogenesis**

The similarity of the late1 flowering phenotype to that of previously described phyA mutants (Weller et al., 1997) suggested the possibility that LATE1 could act in a phyA signaling pathway. As phyA is necessary both for LD-specific promotion of flowering and for seedling deetiolation, an early block to phyA signaling might be expected to cause both flowering time and deetiolation defects. In addition, several Arabidopsis photoperiod pathway genes also affect seedling deetiolation, with mutants showing either increased (e.g. ztl, fkh1, cca1/phy) or decreased (e.g. cry2, gi, elf3, elf4, and prr5/7/9) responsiveness to light (Lin et al., 1998; Huq et al., 2000; Nelson et al., 2000; Somers et al., 2000; Liu et al., 2001; Doyle et al., 2002; Mizoguchi et al., 2002; Nakamichi et al., 2005). Figure 2A shows that the late1-1 mutation does not have a significant effect on deetiolation under continuous high-irradiance far-red (FR) or B light. In different experiments, we did occasionally observe small differences between wild-type and late1 mutants under both conditions, but late1 mutants were never more than 10% longer than the wild type. In contrast, late1 mutations had very clear effects on deetiolation under red light (R), where mutant seedlings had internodes approximately twice as long as the wild type and also showed reduced leaf expansion (Fig. 2, A and B). These defects in response to R are similar to those seen in a phyA null mutant but less severe than in a phyB null mutant (Fig. 2, A and B). Overall, the severity of deetiolation and flowering phenotypes of the different late1 mutants showed no consistent correlation, with the weaker late1-3 allele showing a greater loss of response for elongation under R and the stronger late1-1 allele having weaker effects on deetiolation.

Because both phyA and phyB participate in R sensing in pea seedlings, late1 mutants could be defective in R-specific signaling from one or both of these phytochromes. We addressed this question genetically by constructing late1 phyA and late1 phyB double mutants and examining their responsiveness to R. Compared with the phyA single mutant, the late1 phyA double mutant had longer internodes under R (Fig. 2C), showing that the photomorphogenic effects of LATE1 under R do not depend only on phyA. In fact, given that the effects of the late1 mutations on internode length were similar in magnitude on both a phyA and PHYA background, it seems most likely that at least under high-irradiance R, late1 influences photomorphogenesis independently of phyA. In contrast, late1 mutations had no additional effect on seedling deetiolation on a phyB mutant background (Fig. 2D). This is in clear contrast to the synergistic effects of phyA and phyB mutations (Weller et al., 2001) and shows that the photomorphogenic effects of LATE1 depend on the presence of functional phyB.

**LATE1 Shows Distinct Interactions with PhyA and PhyB in the Control of Flowering**

We also used late1 phyA and late1 phyB double mutants to examine how the late-flowering phenotype of late1 mutants was dependent on the phyA and phyB photoreceptors. Figure 2E shows that the late1-2 phyA double mutant flowered later than the wild type but at a similar node to both single mutants, formally suggesting that phyA and late1 act in the same genetic pathway for the promotion of flowering by LD. Under both SD and LD, the loss of phyB significantly promoted flowering of late1 plants, such that the flowering node of the late1-1 phyB double mutant did not differ significantly from that of the phyB single mutant under either condition (Fig. 2F). This epistatic relationship suggests that as in the case of photomorphogenesis, the flower-promoting effects of LATE1 also depend on active phyB.

Although late1 phyA double mutants were indistinguishable from either single mutant on the basis of the node of flower initiation, several differences were apparent for other reproductive characteristics. Whereas both late1 and phyA single mutants grown in LD were similar in most respects to a wild-type plant grown in SD (Fig. 1; Weller et al., 1997), the late1 phyA double mutant showed more severe defects in development of floral initials and in fruit setting than either single mutant, resulting in a significant increase in the number of reproductive nodes produced before apical arrest (Fig. 2E). This result suggests that PHYA and LATE1 act additively to promote several aspects of reproductive development.

**late1 Mutants Show Altered Expression Rhythms of Circadian Clock Gene Homologs**

In Arabidopsis, the photoperiod response depends critically on rhythms of gene expression generated by
the circadian clock, and mutations in several different clock-related genes confer photoperiod-specific flowering defects. We next examined whether clock gene homologs showed rhythmic expression in pea seedlings grown under light/dark cycles and whether mutations in \textit{LATE1} had any effect on these rhythms. Figure 3 shows that in 2-week-old wild-type pea seedlings grown under LD (16 h light [L]:8 h dark [D]), the transcript level of the \textit{LHY}/\textit{CCA1} homolog \textit{MYB1} cycles with peak expression in the morning at around 2 h after dawn, whereas \textit{TOC1} and \textit{ELF4} show evening peaks around 14 h after dawn. In the \textit{late1} mutant, a phase advance of between 2 and 4 h was seen for all three genes. In addition, the \textit{ELF4} and \textit{MYB1} rhythms were reduced in amplitude in \textit{late1} relative to the wild type (Fig. 3). These effects imply that \textit{LATE1} may have a role in regulation of the circadian clock mechanism, either as part of the clock itself or in input of light signals to the clock.

\textbf{LATE1 Is the Pea Ortholog of Arabidopsis GI}

The co-occurrence of photoperiod-insensitive, late-flowering, phyB-dependent photomorphogenic defects and impaired expression rhythms of clock-related genes in \textit{late1} mutants resembles most closely the phenotypic syndrome described for mutants in the Arabidopsis gene \textit{GI} (Koornneef et al., 1991; Fowler et al., 1999;
Park et al., 1999; Huq et al., 2000). We previously reported the identification of GI orthologs in both pea and Medicago and showed that these genes map to syntenic regions on pea chromosome 6 (linkage group II) and Medicago chromosome 1 (Hecht et al., 2005). During the construction of the late1 phyA mutant, we observed linkage between the LATE1 and PHYA loci, placing LATE1 in linkage group II. We then determined the position of LATE1 more precisely in a cross between the late1-2 mutant (in its original ‘Torsdag’ le background) and ‘Térèse,’ using random-amplified polymorphic DNA and cleaved-amplified polymorphic sequence markers (Laucou et al., 1998; Hecht et al., 2005). This analysis located LATE1 close to marker C1_580 and completely linked to PsGI, consistent with the potential identity of these two genes.

To confirm this possibility, the partial pea GI sequence previously reported (GenBank accession no. AY826733) was extended by degenerate-primer PCR and RACE-PCR, and a cDNA clone encompassing the full coding sequence was isolated (GenBank accession no. EF185297). A full amino acid sequence alignment of LATE1 with GI homologs from other angiosperm species is provided in Supplemental Figure S1 and shows that the GI protein is highly conserved with sequence identity of 74% to 80% in the three pairwise comparisons among legume, poplar (Populus trichocarpa), and Arabidopsis sequences. Figure 4A shows a diagrammatic representation of the pea GI gene and cDNA. Reverse transcription (RT)-PCR amplification of PsGI from the wild type and the six late1 mutant alleles showed that late1-1 to late1-5 all produced PsGI transcript, but no transcript could be detected in cDNA from the late1-6 mutant. Sequencing of the PsGI cDNA in late1-1 to late1-4 revealed single G-to-A nucleotide substitutions that all resulted in significant changes at the protein level (Fig. 4A), and molecular markers designed to detect these mutations cosegregated perfectly with mutant phenotypes. The late1-5 and late1-6 mutants were not analyzed further. The late1-2 and late1-4 mutations both introduce premature stop codons, whereas the late1-1 and late1-3 mutations both result in the substitution of amino acids perfectly conserved in all available angiosperm GI orthologs (Fig. 4A; Supplemental Fig. S1). Interestingly, the two nonsense mutants (late1-2 and late1-4) were also the two mutants that we consistently found to behave as true photoperiod-response mutants (Fig. 1E). The presence of molecular defects in PsGI in all late1 mutants examined and the phenotypic similarities of pea late1 and Arabidopsis gi mutant phenotypes together demonstrate that LATE1 is the pea ortholog of Arabidopsis GI. Like the pea late1 mutants, Arabidopsis gi mutants also show considerable allelic variability for flowering in SD and LD, photoperiod responsiveness, and other traits (e.g. Martin-Tryon et al., 2007).

The Accumulation of LATE1 Transcript Is Regulated by Light and Photoperiod

Arabidopsis GI is regulated by the circadian clock and shows evening-phased rhythmic expression under several different light regimes. Figure 4B shows that the LATE1 transcript shows rhythmic accumulation under light/dark cycles, peaking at dusk under 8-h SD conditions and 4 h before dusk under 16-h LD. The phase of the LATE1 rhythm under LD and SD is also consistent with that observed for Arabidopsis GI and orthologs in a number of other species (Fowler...
et al., 1999; Park et al., 1999; Hayama et al., 2003; Dunford et al., 2005; Zhao et al., 2005).

In addition to the clock-regulated evening peak, *Arabidopsis* GI expression also shows an acute morning peak (Locke et al., 2005; Paltiel et al., 2006). Figure 4C shows that pea seedlings grown under LD cycles (16 h L:8 h D) also show a small shoulder in *LATE1* expression at approximately 2 h after dawn, distinct from the major expression peak at 12 h. An induction of *LATE1* transcript accumulation by light is also seen when etiolated seedlings are transferred from darkness to continuous light. Figure 4D shows that R, B, and FR light are all effective for this induction, implying that several different photoreceptors may interact to regulate *LATE1* expression.

**Effects of *LATE1* on Expression of *CO* and *FT* Homologs in LD**

In both Arabidopsis and rice (*Oryza sativa*), GI appears to regulate flowering at least in part by influencing the expression of *CO*, which in turn regulates the floral integrator *FT* (Suárez-López et al., 2001; Yanovsky and Kay, 2002). The expression rhythm of *CO* is more similar to those of Arabidopsis *COL1* and *COL2*, which also peak at dawn and lack the evening shoulder (Ledger et al., 2001). In the *late1* mutant, the rhythm and peak amplitude of *COLa* expression was similar to that in wild-type plants, with statistically significant differences occurring at only a few time points across a 48-h harvest period (Fig. 5A). The level of *FTL* transcript in wild-type plants also shows a clear peak at dusk or early in the night with a shoulder in the evening (Suárez-López et al., 2001; Yanovsky and Kay, 2002). However, *FTL* expression was strongly reduced in *late1* mutants throughout the daily cycle (Fig. 5B).
Multiple Roles for Pea GIGANTEA Ortholog

**LATE1 Controls the Production of a Mobile Floral Stimulus**

It is well established in many species that the response to photoperiod involves the communication between leaf and shoot apex. Grafting experiments have traditionally been used as one way to examine the nature of this communication, and, in Arabidopsis, grafting of *co* mutant scions onto leafy wild-type stocks results in a substantial rescue of the late-flowering phenotypes of this mutant (An et al., 2004). We tested the effect of *LATE1* on mobile flowering signals in pea by performing reciprocal grafts between the wild type and *late1* under LD conditions, using a grafting system in which the epicotyl of a 7-d-old seedling was grafted onto a 3-week-old stock possessing several expanded leaves (Fig. 6, A and B). Figure 6C shows that self-grafted *late1* scions remained as late flowering as ungrafted *late1* control plants, whereas grafting of *late1* mutant scions onto wild-type stocks resulted in the promotion of flowering to a level not significantly different from self-grafted wild-type plants. In contrast, wild-type scions flowered equivalently early regardless of whether they were grafted onto wild-type or *late1* stocks. These results support a role for *LATE1* in the production of a mobile flowering stimulus and argue against the alternative possibility that *late1* mutants might have elevated levels of a mobile inhibitor of flowering.

Recent studies in Arabidopsis suggest that *FT* mRNA and/or protein may act as a mobile flowering stimulus (An et al., 2004; Huang et al., 2005) and that this stimulus is primarily responsible for mediating the effects of inductive photoperiods on flowering. Because *LATE1* is necessary for the induction of the *FTL* expression in LD conditions (Fig. 5B), we tested whether graft rescue of *late1* mutant scions was associated with elevated levels of *FTL* mRNA in the shoot apex. This analysis was performed using manually excised shoot apices of 2 to 3 mm in length, which possessed four to five leaf primordia in addition to the apical meristem. A comparison of leaf number and timing of flower initiation in ungrafted wild-type plants (C. Knowles and J. Weller, unpublished data) allowed us to estimate the timing of flower initiation in graft-rescued *late1* scions at around day 20 after grafting. Figure 6D shows that although wild-type and *late1* stock leaves clearly differed substantially in their level of *FTL* mRNA, apices from *late1*/wild type and *late1*/late1 grafts contained similar amounts of *FTL* mRNA at three different time points spanning the estimated time of floral initiation.

**Genetic Interactions with LATE1 Suggest a Clock-Related Role for the SN Locus**

Previous studies of the photoperiod response in pea have largely centered on the roles of genes necessary for delay of flowering. Mutations at the *SN*, *DNE*, and *PPD* loci all confer an early-flowering, photoperiod-insensitive phenotype and influence graft-transmissible floral signaling (Murfet, 1971a; King and Murfet, 1985; Taylor and Murfet, 1996), but the corresponding genes have not been identified. The isolation of *LATE1* as a conserved component of the photoperiod response pathway in pea provided the opportunity to examine the position of these genes in the pathway by construction of double mutants. Figure 7A shows that an *sn* late1 double mutant has a novel phenotype that combines the early initiation of flowering typical of *sn* single mutants with the extended reproductive phase typical of *late1* single mutants. This suggests that *late1* and *sn* may interact in different ways to control different aspects of the photoperiod response, with *sn* epistatic to *late1* in the control of flower initiation but *late1* epistatic to *sn* in controlling the length of the reproductive phase.

We also examined how these genes might interact to control seedling deetiolation. Figure 7B shows that under R and B, internode elongation in the *sn* mutant is reduced relative to the wild type. Under B, this short-internode phenotype is dependent on *LATE1* because it is completely reverted by *late1*, even though by itself the *late1* mutation has no effect on elongation under B. Under R, the double mutant shows a phenotype intermediate between the two single mutants, although more similar to *late1* than to *sn*. 

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**Figure 5. Effects of LATE1 on rhythmic expression of CO and FT homologs under LD.** Wild-type (black symbols) and *late1-2* (white symbols) seedlings were grown for 28 d under LD (16 h L:8 h D) and leaflets from the sixth leaf were harvested at 4-h intervals over the next 48 h. Relative transcript levels of *COLa* (A) and *FTL* (B) were analyzed by RT-qPCR and quantified based on nonequal efficiencies (Pfaffl, 2001). For each data point, n = 3 and bars represent SE.
Figure 6. *LATE1* regulates a graft-transmissible flowering stimulus. A, Representative grafted plant 2 weeks after grafting. Arrow indicates site of graft. Bar = 1 cm. B, Representative examples of self-grafted plants and reciprocal graft combinations between the wild type and the *late1-1* mutant at approximately 7 weeks after grafting. The *late1/late1* self-graft has not yet flowered, whereas all other graft combinations have flowered and terminated apical growth. Arrow indicates site of graft. Bar = 10 cm. C, Node of flower initiation in ungrafted controls, self-grafts, and reciprocal grafts between the wild type and *late1-1*. For each data point, *n* = 12 to 16 and bars represent se. D, Relative transcript levels of *FTL* in the uppermost stock leaf at 21 and in dissected apices of self-grafted *late1-1* scions and in *late1-1* scions grafted to the wild type. Harvests took place at 17, 21, and 28 d after grafting to span the estimated time of flower initiation at around day 20 after grafting. Relative transcript levels were analyzed by RT-qPCR and quantified based on nonequal efficiencies (Pfaffl, 2001). For each data point, *n* = 3 and bars represent se. All plants were grown in the phytotron under LD conditions (8-h photoperiod of natural daylight extended for 16 h with white fluorescent light at 10 μmol m⁻² s⁻¹).

Another possibility raised by this interaction is that *SN* might be involved in the transcriptional regulation of *LATE1*. Figure 7C shows that the rhythm of *LATE1* expression is clearly altered in the *sn* mutant. Peak expression levels in the wild type and *sn* were not significantly different, but peaks occurred earlier in the day and trough levels were substantially higher in *sn* than in the wild type. This suggests that *SN* is needed for normal cycling of *LATE1* expression, which places it either upstream of *LATE1* or at a similar level in the genetic hierarchy governing circadian rhythms and flowering.

**DISCUSSION**

The molecular physiology of flowering time control has been intensively investigated in Arabidopsis for more than a decade but has only more recently come under systematic investigation in other species (Carmel-Goren et al., 2003; Hayama et al., 2003; Brunner and Nilsson, 2004; Hecht et al., 2005). In pea, classical genetic and physiological analyses have identified several flowering genes and established a model for their interactions (Murfet, 1977; Reid et al., 1996), but apart from the demonstration that the *LF* gene encodes a *TFL1* homolog (Foucher et al., 2003), the molecular nature of these genes is not known. We have recently begun the molecular genetic analysis of photoperiod responsiveness in pea through isolation of new mutants and isolation of photoperiod pathway gene homologs (Weller et al., 2003; Hecht et al., 2005). In this study, we have used a range of approaches to define the physiological and molecular roles of *LATE1*, the pea ortholog of Arabidopsis *GI*, in photoperiodic flowering, photomorphogenesis, and the generation of rhythmic gene expression. We have also characterized rhythmic expression of pea homologs for a number of Arabidopsis clock genes, providing a basis for exploring the molecular nature of a range of other pea flowering loci. Finally, we have explored the role of *LATE1* in mobile signaling and examined its interaction with the well-known pea flowering locus *SN*. Results from these experiments provide the first points of contact between previous physiological models for flowering in pea and molecular models derived mainly from Arabidopsis.

**Roles of *LATE1* in Regulation of Photomorphogenesis and Expression of Circadian Clock Gene Homologs**

Diurnal expression rhythms for pea circadian clock gene homologs *TOC1*, *MYB1* (*LHY/CCA1* homolog), *ELF4*, and *LATE1* conform closely to those reported for the corresponding Arabidopsis genes (Matsushika et al., 2000; Doyle et al., 2002; Mizoguchi et al., 2002), suggesting that the regulatory patterns that form the basis of the Arabidopsis circadian clock may also be present in pea. Loss of *LATE1* function results in a clear disruption to these patterns, with reduced amplitude and a phase advance seen for the rhythms of *MYB1*, *TOC1*, and *ELF4* (Fig. 3). These clear effects of *LATE1* on phasing of expression rhythms of core clock gene homologs suggest that one of the roles of *LATE1* may be to function as part of the central clock mechanism. An important role for Arabidopsis *GI* in the clock has emerged...
Interaction of LATE1 and SN. A, Interaction of late1 (late1-1) and sn (sn-4) mutations in the control of initiation of flowering and the duration of the reproductive phase under LD conditions. Left, Node of flowering initiation; right, number of reproductive nodes. Plants were grown in the phytotron where they received an 8-h photoperiod of natural daylight extended for 16 h with white fluorescent light (10 μmol m\(^{-2}\) s\(^{-1}\)). For each data point, \(n = 8\) to 12 and bars represent se. B, Interaction of late1-1 and sn-4 mutations in the control of internode elongation under monochromatic R (left) and B (right). For each data point, \(n = 8\) to 12 and bars represent se. C, Diurnal rhythms of LATE1 transcript accumulation in the wild type (black symbols) and sn-4 (white symbols) mutant seedlings under LD. Growth conditions and tissue harvests were the same as in Figure 3A, and data for the wild type are replotted from Figure 4B. Relative transcript levels were analyzed by RT-qPCR and quantified based on nonequal efficiencies (Pfaffl, 2001). For each data point, \(n = 3\) and bars represent se.

Figure 7. Interaction of LATE1 and SN. A, Interaction of late1 (late1-1) and sn (sn-4) mutations in the control of initiation of flowering and the duration of the reproductive phase under LD conditions. Left, Node of flowering initiation; right, number of reproductive nodes. Plants were grown in the phytotron where they received an 8-h photoperiod of natural daylight extended for 16 h with white fluorescent light (10 μmol m\(^{-2}\) s\(^{-1}\)). For each data point, \(n = 8\) to 12 and bars represent se. B, Interaction of late1-1 and sn-4 mutations in the control of internode elongation under monochromatic R (left) and B (right). For each data point, \(n = 8\) to 12 and bars represent se. C, Diurnal rhythms of LATE1 transcript accumulation in the wild type (black symbols) and sn-4 (white symbols) mutant seedlings under LD. Growth conditions and tissue harvests were the same as in Figure 3A, and data for the wild type are replotted from Figure 4B. Relative transcript levels were analyzed by RT-qPCR and quantified based on nonequal efficiencies (Pfaffl, 2001). For each data point, \(n = 3\) and bars represent se.

from several studies (Locke et al., 2005; Gould et al., 2006), although its effects on other clock components have not been thoroughly characterized. Arabidopsis gi mutants show lower amplitude of LHY and CCA1 expression rhythms in LD and SD but no discernable phase shift (Fowler et al., 1999; Mizoguchi et al., 2005). However, several different gi alleles show a phase advance in LHY and CCA1 expression rhythms under constant light conditions (Park et al., 1999; Mizoguchi et al., 2002; Martin-Tryon et al., 2007). Effects of a putative null gi mutation on rhythmic TOC1 expression have only been examined under constant light conditions, where a phase advance is also observed (Martin-Tryon et al., 2007). Effects of GI on rhythmic ELF4 expression have not been reported. In the future, it will be of interest to determine whether LATE1 also affects clock gene expression rhythms under constant conditions and to examine in more detail the significance of the acute induction of LATE1 by light.

Like Arabidopsis gi mutants, the pea late1 mutants also show photomorphogenic defects under continuous high-irradiance R, suggesting a conserved role for GI orthologs in light signaling during deetiolation. Double mutant analyses confirm that the reduction in response to R of late1 mutants is predominantly due to a defect in phyB signaling and is largely independent of phyA. As in the case of circadian period, there also seem to be allele-specific differences in relative responsiveness to light, with two independent T-DNA insertion mutants for Arabidopsis GI showing distinctly different response to high-irradiance R (Huq et al., 2000; Martin-Tryon et al., 2007). The elongated hypocotyl phenotype of gi mutants was recently shown to depend on LHY and CCA1 (Mizoguchi et al., 2005) and can thus also be viewed as a defect in rhythmic control of elongation. Small differences in hypocotyl elongation of different Arabidopsis gi mutants under B have also been recently described (Martin-Tryon et al., 2007). We found no clear effects of late1 mutations on B responses in a wild-type background. However, in a sn mutant background, late1 clearly impairs responsiveness to both B and R (Fig. 7B), suggesting that the role of LATE1 in light signaling is not completely phyB specific.

Roles of LATE1 in Regulation of Photoperiod Pathway Gene Homologs

In Arabidopsis and other species, light and photoperiod signals are integrated at the level of FT expression. Late-flowering photoperiod pathway mutants in Arabidopsis such as gi, fkh1, and cry2 have reduced FT expression under LD conditions, whereas early-flowering mutants have increased FT expression. In pea, LATE1 is clearly necessary for induction of the FT homolog FTL (Fig. 5B), demonstrating that both the GI-FT regulatory interaction and the association between photoperiod-insensitive late flowering and low expression of FT homologs are conserved in pea. Our preliminary studies of other photoperiod response mutants, such as phyA and SD-grown wild-type plants, suggest that they also show altered FTL expression (V. Hecht and C. Knowles, unpublished data), in support of a role for FTL as a target of the photoperiod pathway.

In Arabidopsis and rice, effects of GI on FT expression are mediated at least in part through changes in the expression of CO genes (Suárez-López et al., 2001; Hayama et al., 2003). In Arabidopsis, the clade of group 1a COL genes has two other members (COL1 and COL2) in addition to CO, but these genes do not share the regulation or function of CO (Ledger et al., 2001).
Under LD, Arabidopsis CO mRNA peaks in the night with a shoulder in evening and regulates flowering but not circadian rhythmicity (Suárez-López et al., 2001; Yanovsky and Kay, 2002). In contrast, COL1 and COL2 show expression peaks at dawn, lack the evening shoulder, and affect circadian rhythms but not flowering (Ledger et al., 2001).

We previously identified a single group Ia COL gene (COLa) in pea and Medicago (Hecht et al., 2005) that, like group Ia COL genes from other eudicot families, clearly falls outside the Arabidopsis CO/COL1/COL2 clade (Supplemental Fig. S2). None of these genes can therefore be considered as a CO ortholog, and it is not clear how the functions and regulatory patterns of Arabidopsis CO/COL1/COL2 may be represented in these genes. While group Ia COL genes from some species show rhythmic expression patterns similar to Arabidopsis CO (Turner et al., 2005; Böhlenius et al., 2006), others differ markedly from CO (Ben-Naim et al., 2006), suggesting that the coincidence of light with high CO expression in both the morning and evening may not be a universal phenomenon.

We found that pea COLa showed two significant differences in comparison with Arabidopsis CO, raising the question of whether COLa is the functional equivalent of CO. First, the expression pattern of pea COLa is more similar to Arabidopsis COL1/COL2 than to CO. Second, the altered regulation of FTL in late1 mutants is not reflected in any substantial difference in transcriptional regulation of COLa, in contrast to Arabidopsis, where CO expression in gi mutants is dramatically reduced throughout the daily cycle (Suárez-López et al., 2001; Mizoguchi et al., 2005). One obvious interpretation of these differences is that COLa is not the functional equivalent of Arabidopsis CO and that this role is played by another as yet unidentified group Ia COL gene. Although we have not yet exhaustively characterized the COL gene family in pea, COLa was the only group Ia COL gene isolated using several different isolation approaches in three different labs and is the only one represented in legume EST and genomic databases. However, given that sequencing of the Medicago and Lotus genomes is not yet complete and that CO is expressed at only a very low level in Arabidopsis (Putterill et al., 1995), it remains a possibility that another CO/COL1/COL2-like gene could still be present in pea and other model legumes.

However, another possibility is that LATE1 regulation of FTL does not depend on COLa or on any CO-like gene. In addition to the CO-dependent role of Arabidopsis GI in regulation of FT, a CO-independent role in regulation of FT has also been proposed (Mizoguchi et al., 2005), and it may be that a comparable mechanism predominates in pea. A third possibility is that LATE1 may act through COLa to regulate FTL, but this is achieved either through relatively subtle changes in the transcriptional rhythm of COLa or predominantly through posttranscriptional control. In Arabidopsis, both transcriptional and posttranscriptional control of CO protein abundance have been demonstrated (Valverde et al., 2004; Imaizumi et al., 2005), and, once again, it may be that a similar photoreceptor-dependent posttranscriptional control predominates in pea.

Discrimination among these possibilities and direct testing of a role for COLa in the transduction of LATE1 signals to FTL clearly awaits the thorough characterization of the subclade of group Ia COL genes in pea and/or other related model legumes, an investigation of COLa protein regulation, and the isolation of appropriate mutants.

**Roles of LATE1 in Regulation of Mobile Flowering Signals**

The clear promotion of flowering in late1 mutants grafted onto leafy wild-type stocks and the absence of significant inhibition of flowering in the reciprocal (wild type on late1) graft combination (Fig. 6C) provides strong evidence that the induction of flowering in pea by LD is primarily achieved through the action of a mobile flowering stimulus. The effects of photoperiod on flowering in pea have previously been interpreted in terms of a mobile inhibitor of flowering (Murfet, 1971a; Murfet, 1985; Reid et al., 1996). However, these earlier studies mostly used epicotyl-epicotyl grafts in which the stock (donor) material possessed no true leaves or other photosynthetic tissue, a grafting system not ideal for studying the interaction between processes in the leaves and apex. It is also of note that other early studies in pea proposed a floral stimulus as the main mobile signal mediating the photoperiod response (Haupt, 1969). Our result does not exclude the existence of a separate inhibitor of flowering but does indicate that, if present, this inhibitor plays only a very minor role in regulating flowering or has targets that are upstream of LATE1.

Even though most discussions of mobile floral signals in pea have centered on a putative inhibitor and its role in mediating both vegetative and reproductive responses to photoperiod, the existence of a distinct mobile flowering stimulus with much more specific role in flower initiation has also been proposed based on the flowering-specific graft-transmissible effects of the GIGAS locus (Beveridge and Murfet, 1996). It may be that one of the effects of LATE1 is to induce the GIGAS signal in leaves under LD, a possibility that could be examined genetically by testing whether late1 scions can be rescued by grafting to leafy gigas stocks. However, the phenotypic differences between late1 and gigas mutant phenotypes (Fig. 1; Beveridge and Murfet, 1996) imply that, even if LATE1 and other photoperiod response genes such as PHYA act through GIGAS to affect flowering, they also have additional roles independent of GIGAS. Separation of flower initiation from other aspects of the photoperiod response was also observed in the sn late1 double mutant, but in an opposite manner, with promotion of flowering occurring in the absence of effects on other photoperiod-regulated traits (Fig. 7).

One possible explanation for these different effects of photoperiod is that the pea photoperiod detection...
mechanism converges on a single gene with multiple targets, one of which could be GIGAS. In Arabidopsis, the photoperiod response gene CO does not appear to have multiple targets (Wigge et al., 2005), but recent evidence has shown that FT homologs in tomato (Lycopersicon esculentum), poplar, and Norway spruce (Picea abies) negatively regulate various aspects of vegetative growth in addition to positively regulating flowering (Böhlenius et al., 2006; Lifschitz et al., 2006; Gyllenstrand et al., 2007), raising the possibility that a broader role for FT-like genes might be ancestral and widespread (Lifschitz and Eshed, 2006). Another possibility is that different aspects of the pleiotropic photoperiod response are generated through the interaction of light with multiple clock outputs (Blasing et al., 2005). Isolation of COLa and FTLa mutants in pea and molecular characterization of the GIGAS locus will help distinguish between these interpretations.

Analysis of gene expression in dissected shoot apices of rescued late1 graft scions found no major difference in FTLa transcript levels (Fig. 6D). Thus, if FTLa mRNA, like Arabidopsis FT, does constitute part of the mobile flowering signal, then the changes required to elicit flowering are below the detection limit and/or spatial resolution of our preliminary assay. However, in a similar experiment investigating the function of the tomato FT homolog SINGLE FLOWER TRUSS (SFT) in which sft mutants were rescued by grafting to 35S::SFT stocks, transgene-derived SFT transcript was undetectable in shoot apices or any other sft scion tissues (Lifschitz et al., 2006). This implies that the SFT regulates a mobile signal but its mRNA is not mobile, and suggests that a role for FT transcripts in mobile signaling may not be universal. Future attempts to address the involvement of pea FTLa in mobile signaling will benefit from a system able to distinguish scion- and stock-derived FTLa transcripts.

The SN Locus May Also Have a Clock-Related Role

Finally, our results have shed the first light on the molecular roles of SN, one of the first major flowering loci in pea to be physiologically characterized and one that may have agronomically significant orthologs in other legume crops (Murfet, 1971b; Sarker et al., 1999). The interactions between SN and LATE1 described here provide support for the suggestion that SN may have primary defects in the circadian clock, a suggestion initially based on flowering time comparisons with Arabidopsis mutants (Hecht et al., 2005; Weller, 2005). In fact, the clear reduction and earlier phase of morning repression of LATE1 expression in the sn mutant identifies a role for SN in LATE1 regulation distinct from those of known regulators of GI in Arabidopsis. Loss-of-function mutants for genes suggested to play an important role in regulation of Arabidopsis GI have been reported to cause phase advance (itty; Mizoguchi et al., 2005), phase advance combined with reduced peak induction (toc1; Martin-Tryon et al., 2007), or higher expression overall without effect on phase (elf3; Fowler et al., 1999), but these are all distinct from the effects of sn. The greatest similarity is seen in the prr5 prr7 prr9 triple mutant in Arabidopsis (Nakamichi et al., 2005), but unlike sn, this mutant is late flowering. Regardless of the specific molecular identity of SN, a role in the clock mechanism would imply that SN and LATE1 regulate flowering via the same downstream integrator(s) and mobile signaling pathway. The characterization of flowering- and circadian clock-related genes presented here can now be extended to test this possibility.

MATERIALS AND METHODS

Plant Material, Mutagenesis, and General Growth Conditions

All pea (Pisum sativum) lines were derived from NGBS839, a derivative of ‘Torsdag’ carrying the le-3 mutation (Lester et al., 1999). Growth media, plant husbandry, EMS mutagenesis protocols, and the phyA and phyB-3 mutants have been described previously (Weller et al., 1997, 2001, 2004). The sn-4 mutant is a novel allele identified in the same screen as the late1 mutants. All late1 mutants were subjected to at least two backcrosses to wild-type NGBS839 before use for the experiments shown. The sn-4 late1-1 double mutants were clearly distinguished among the F1 progeny of a cross between late1-1 and sn-4 on the basis of their novel phenotype, and their identity was confirmed in subsequent generations.

Devolatilization experiments and all gene expression studies were carried out in growth cabinets at a temperature of 20°C unless otherwise specified. Chromatic R, B, or FR light was obtained from light-emitting diode arrays (Nichia NSP510F W3 Super Blue diodes, Nichia; Shinkoh Electronics KL450-730GDDH [FR] and KL450-660GDDH [R] diodes, Shinkoh Electronics). Spectral output from these sources can be viewed at http://www.utas.edu.au/glasshouse/jh_fac_led.html. Light obtained from the FR diodes was filtered through one layer of FR perspex (FRF-700; Westlake Plastics) to remove low-level emission below 700 nm. White light for cabinet experiments was obtained from cool-white fluorescent tubes (L40 W/205 cool white; Osram Germany) at an irradiance of 120 to 140 μmol m⁻² s⁻¹ unless otherwise specified. Photoperiod experiments in Figures 1, 2, and 7 and grafting experiments in Figure 6 were conducted in the custom-built Hobart phytotron. Plants received an 8-h photoperiod of natural daylight before automated transfer to night compartments either with (LD) or without (SD) a 16-h extension with light from cool-white fluorescent tubes at an irradiance of 10 μmol m⁻² s⁻¹.

Sequencing, Mapping, and Molecular Markers

The partial sequence of the pea GI ortholog previously reported by Hecht et al. (2005) was extended using additional degenerate primers and RACE-PCR. Degenerate primers were designed using the CODEHOP strategy (Rose et al., 1998) based on EST sequences of GI orthologs obtained from The Institute for Genomic Research database (http://www.tigr.org; Medicago truncatula TC106069; Lotus japonicus TC0894; soybean [Glycine max] TC204605, TC204604, TC204602; tomato [Lycopersicon esculentum] TC158735; and rice [Oryza sativa] TC261930). Degenerate primers were used in combination with specific PisGI primers to amplify cDNA sequences (GI-1F and GI-GSP2 for the 5‘ region and GI-GSP4 and GI-4R for the 3‘ region). The full-length cDNA was obtained by RACE-PCR using the BD-SMART RACE cDNA amplification kit (CLONTECH) and gene-specific primers (GI-GSP2 and GI-7R for the 5‘ region and GI-2F and GI-GSP4 for the 3‘ region). All PCR fragments were cloned in pGEM-T easy (Promega) and sequenced on a Beckman CEQ8000. Intron sequences were obtained by PCR on genomic DNA using a range of different primer pairs. Details of all primers used are given in Supplemental Table S3. Mutations in late1-1, late1-2, and late1-4 were confirmed by scoring of cleaved-amplified polymorphic sequence markers (Supplemental Table S3) in segregating populations (late1-1, late1-2) and pure lines. Markers for phyA-1 and phyB-5 mutations and for mapping of PisGI have been described previously (Hecht et al., 2005; Platten et al., 2005).
Analysis of Gene Expression

Harvested tissues were immediately frozen in liquid nitrogen and total RNA extracted using the Qiagen RNeasy Plant mini kit, including an on-column DNase treatment (Qiagen). RNA concentrations were determined using Ribogreen RNA quantification reagent (Molecular Probes) in a Picofluor fluorometer (Turner Biosystems). RT was carried out in 20 μL with 2 μg of total RNA using the Omniscript Reverse Transcriptase kit (Qiagen) according to manufacturer’s instructions. RT negative (no enzyme) controls were routinely 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) were set up with a CAS-1200N robotic liquid handling system (Corbett Research). Details of primers are presented in Supplemental Table S4. Relative transcript search was carried out in a Rotor-Gene RG3000 (Corbett Research) and run for 50 cycles in a Rotor-Gene RG3000 (Corbett Research).

Relative transcript abundance was calculated using ACTIN (Hepworth S, Mouradov A, Justin S, Turnbull C, et al. (2004) CONSTANS acts in the phloem to regulate a systemic signal that induces photoperiodic flowering of Arabidopsis. Development 131: 3615–3626) and reference ACTIN as a control (Benzing OE, Gibon Y, Gunther M, Hohne M, Morcuende R, Osuna D, Thimm O, Usadel B, Scheible WR, Stitt M (2005) Sugars and carbonic anhydrase regulation make major contributions to the global regulation of diurnal gene expression in Arabidopsis. Plant Cell 17: 3257–3281). Blázquez MA, Agh JE, Weiigel D (2003) A thermosensory pathway controlling flowering time in Arabidopsis thaliana. Nat Genet 33: 168–171. Böhlenius H, Huang T, Charbonnel-Campaa L, Brunner AM, Jansson S, Strauss SH, Nilsson O (2006) CO-F1 regulatory module controls timing of flowering and seasonal growth cessation in trees. Science 312: 1040–1043. Brunner A, Nilsson O (2004) Revisiting tree maturation and floral initiation in the poplar functional genomics era. New Phytol 164: 43–51. Caramelo-Goren L, Liu YS, Lifschitz E, Zamir D (2003) The SELF-PRUNING gene family in tomato. Plant Mol Biol 52: 1215–1222. Cerdán PD, Chory J (2003) Regulation of flowering time by light quality. Nature 423: 881–885. Doyle MR, Davis SJ, Bastow RM, McWatters HG, Kozma-Boglar N, Nagy F, Millar AJ, Amasino RM (2002) The ELF4 gene controls circadian rhythms and flowering time in Arabidopsis thaliana. Nature 419: 74–77. Dunford RP, Griffiths S, Christodoulou V, Laurie DA (2005) Characterisation of a barley (Hordeum vulgare L.) homologue of the Arabidopsis flowering time regulator GIGANTEA. Theor Appl Genet 110: 925–931. Foo E, Bullier E, Goussot M, Foucher F, Rameau C, Beveridge C (2005) The branching gene RAMOSUS mediates interactions among two novel signals and auxin in pea. Plant Cell 17: 464–474. Foucher F, Morin J, Courtiade J, Cadiou S, Ellis N, Banfield M, Rameau C (2003) DETERMINATE and LATE FLOWERING are two TERMINAL FLOWER1/CENTRORADIALIS homologs that control distinct phases of flowering initiation and development in pea. Plant Cell 15: 2742–2754. Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, Puttner J (1999) GIGANTEA: a circadian clock-controlled gene that regulates photoperiodic flowering in Arabidopsis and encodes a protein with several possible membrane-spanning domains. EMBO J 18: 4679–4688. Gould PD, Locke JC, Larue C, Southern MM, Davis SJ, Hanno S, Moyle R, Milich R, Puttner J, Millar AJ, et al. (2006) The molecular basis of temperature compensation in the Arabidopsis circadian clock. Plant Cell 18: 3177–3187. Griffiths S, Dunford RP, Coupland G, Laurie DA (2003) The evolution of CONSTANS-like gene families in barley, rice, and Arabidopsis. Plant Physiol 131: 1855–1867. Guo H, Yang H, Mockler TC, Lin C (1998) Regulation of flowering time by Arabidopsis photoreceptors. Science 279: 1360–1363. Gyllenstrand N, Clapham D, Killman T, Lagercrantz U (2007) A Norway spruce FLOWERING LOCUS T homolog is implicated in control of growth rhythm in conifers. Plant Physiol 144: 248–257. Haupt W (1969) Paeurn sativum L. In: LT Evans, ed, The Induction of Flowering. Macmillan, Melbourne, pp 393–408. Hayama R, Coupland G (2004) The molecular basis of diversity in the photoperiodic flowering responses of Arabidopsis and rice. Plant Physiol 135: 667–684. Hayama R, Yoko S, Tamaki S, Yano M, Shimamoto K (2003) Adaptation of photoperiodic control pathways produces short-day flowering in rice. Nature 422: 719–722. Hazen SP, Schultz TF, Pruneda-Paz JL, Borevitz JO, Ecker JR, Kay SA (2005) LUX ARRHYTHMO encodes a Myb domain protein essential for circadian rhythms. Proc Natl Acad Sci USA 102: 10387–10392. Hecht V, Foucher F, Fernandez C, Macknight R, Navarro C, Morin J, Vardy ME, Ellis N, Beltran JP, Rameau C, et al. (2005) Conservation of Arabidopsis flowering genes in model legumes. Plant Physiol 137: 1420–1434. Hicks KA, Albertson TM, Wagner DR (2001) EARLY FLOWERING3 encodes a novel protein that regulates circadian clock function and flowering in Arabidopsis. Plant Cell 13: 1281–1292.
Multiple Roles for Pea GIGANTEA Ortholog

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