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**Review**

# *FT* genes and regulation of flowering in the legume *Medicago truncatula*

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**Abstract.** Flowering time is an important contributor to plant productivity and yield. Plants integrate flowering signals from a range of different internal and external cues in order to flower and set seed under optimal conditions. Networks of genes controlling flowering time have been uncovered in the flowering models *Arabidopsis*, wheat, barley and rice. Investigations have revealed important commonalities such as *FT* genes that promote flowering in all of these plants, as well as regulators that are unique to some of them. *FT* genes also have functions beyond floral promotion, including acting as floral repressors and having a complex role in woody polycarpic plants such as vines and trees. However, much less is known overall about flowering control in other important groups of plants such as the legumes. This review discusses recent efforts to uncover flowering-time regulators using candidate gene approaches or forward screens for *spring* early flowering mutants in the legume *Medicago truncatula.* The results highlight the importance of a Medicago *FT* gene, *FTa1*, in flowering-time control. However, the mechanisms by which *FTa1* is regulated by environmental signals such as long days (photoperiod) and vernalisation (winter cold) appear to differ from *Arabidopsis*.

**Additional keywords:** *CO-like* genes, circadian, FLC, repressor, pea.

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## Introduction

Coincidence of flowering with optimal conditions is a prerequisite to successful sexual reproduction and the yield of seeds, grains and fruit. Thus, flowering time is both an important adaptive trait and an agronomic trait targeted by plant breeders (Putterill *et al*. 2004; Jung and Muller 2009). In addition, flowering time and its control remains a great source of curiosity and interest. For example, bamboo forests in India and China grow vegetatively for decades, then related plants synchronously flower in response to an unknown cue, and die. Understanding flowering in polycarpic species provides a further grand challenge. These plants include trees that often have an extended juvenile period, sometimes of many years, in which they do not flower. Once they have flowered they do not die; rather, cycles of vegetative growth and flowering can continue for many years.

Investigation of flowering time control in the best-studied flowering time models – *Arabidopsis*, rice, wheat, barley – has revealed many flowering-time genes that regulate the transition to flowering (Higgins *et al*. 2010; Andrés and Coupland 2012) (Table 1). The models have diverse responses to seasonal cues and some of the regulators are unique to each species. For example, daylength requirements vary amongst them as short days (SD, 8 h light/16 h dark) promote flowering in rice and long days (LD, 16 h L/ 8 h dark) induce flowering in *Arabidopsis*, wheat and barley. Activators of rice flowering in SD such as *Ehd1* have no counterparts in *Arabidopsis*, and activators of LD flowering in *Arabidopsis* like *CO*, have a dual role (as activators or repressors) in rice depending on daylength.

The need for extended winter cold (vernalisation) can also vary. This is not required for subtropical rice, but flowering is

**Table 1. Genetic regulation of flowering time**

| Flowering time genes | Candidate *Medicago* genes | Pathway/function |
|---|---|---|
| *Arabidopsis* | | |
| *APETALA 1* (*AP1*) | PROLIFERATING INFLORESCENCE MERISTEM (*PIM*) | Floral meristem identity gene |
| *CONSTANS* (*CO*) | *MtCO*, *COLa to d* | Long day pathway (LD) |
| *EARLY FLOWERING 3*(*ELF3*) | *ELF3* | LD |
| *ELF4* | *ELF4* | LD |
| *FLAVIN-BINDING KELCH REPEAT F BOX PROTEIN 1* (*FKF1*) | *FKF1* | LD |
| *FLOWERING LOCUS C* (*FLC*) | Not found | Floral repressor |
| *FLOWERING LOCUS D* (*FD*) | *FD* | Floral promoter |
| *FLOWERING LOCUS T* (*FT*) | *FTa1*, *FTa2*, *FTb1*, *FTb2*, *FTc* | Floral integrator |
| *GIGANTEA* (*GI*) | *GI* | LD |
| *LATE ELONGATED HYPOCOTYL* (*LHY*) | *LHY* | LD |
| *LEAFY* (*LFY*) | *SINGLE LEAFLET1* (*SGL1*) | Floral integrator and floral meristem identity |
| *PHYTOCHROME A*) | *PHYA* | Light signalling |
| *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1* | *SOC1a to c* | Floral integrator |
| *TIMING OF CAB EXPRESSION 1* (*TOC1*) | *TOC1a/b* | LD |
| *TERMINAL FLOWER 1* (*TFL1*) | *TFL1* | Floral repressor and inflorescence identity |
| *TWIN SISTER OF FT* (*TSF*) | *FTa1*, *FTa2*, *FTb1*, *FTb2*, *FTc* | Floral integrator |
| *Temperate cereals* | | |
| *VERNALISATION 2* (*VRN2*) | Not found | Floral repressor |
| *VRN3* (*FT* orthologue) | *FTa1*, *FTa2*, *FTb1*, *FTb2*, *FTc* | Floral integrator |

promoted by vernalisation in winter varieties of *Arabidopsis* and the temperate cereals wheat and barley. In these vernalisation-responsive plants there is a common theme of repression of a repressor of flowering, directly or indirectly by winter cold, but the identity of the repressor differs (Kim *et al*. 2009). FLC, a MADS domain transcription factor, represses flowering in *Arabidopsis* whereas VRN2 – a CCT domain protein, represses flowering in wheat and barley until after vernalisation. This ensures that these plants grow vegetatively over the winter and that flowering and sexual reproduction occurs rapidly in the milder conditions of spring, rather than in autumn and winter.

In addition to environmental cues, the role of endogenous signals such as developmental stage, hormones and carbohydrate level are well established and the mechanisms of the age-dependent pathway (Wang *et al*. 2009; Yang *et al*. 2011) and sugar signalling (Wahl *et al*. 2013) are now described in *Arabidopsis*. The role of gibberellins (GAs) in flowering is becoming better understood for some species, although it is not universal (Wilkie *et al*. 2008; Mutasa-Göttgens and Hedden 2009; Turnbull 2011), and there is little evidence for GA involvement in regulation of flowering time in legumes such as pea (Murfet and Reid 1987). Cytokinins (CKs) can also promote flowering by regulation of flowering time genes in the leaf, and potentially by systemic transport and direct action in the shoot apical meristem (D'Aloia *et al*. 2011).

There are also important commonalities such as the key role of *FT* genes in promoting flowering. FT is a small ~23 kDa PEBP family protein which functions as an important mobile promoter of flowering (the long sought florigen) that is produced in leaves in response to inductive daylengths (Turck *et al*. 2008; Turnbull 2011). In *Arabidopsis*, FT and a related protein TSF, are expressed in the leaf veins, mobilised into the phloem and then in the shoot apical meristem. They are proposed to partner with a transcription factor FD to activate the transcription of the floral integrator gene *SOC1* and the floral meristem identity gene *AP1*. *Arabidopsis FT* is an important floral integrator gene that receives signals from the long day pathway and the vernalisation pathway and also is regulated by light quality and ambient temperature cues (Srikanth and Schmid 2011).

Many plants possess *FT* and *FT*-like genes that promote flowering (Andrés and Coupland 2012). In *Arabidopsis* and the other models, once the repressors (described above) have been downregulated by winter cold, inhibition of *FT* genes (*VRN3* in wheat and barley) and of the floral integrator gene *SOC1* in *Arabidopsis*, is alleviated (Michaels *et al*. 2005; Trevaskis *et al*. 2007). In spring, the LD pathway then is activated and *FT* expression is upregulated. The LD pathway (Andrés and Coupland 2012) involves interplay among the circadian clock, light perception and signalling and clock-regulated flowering time genes including *GI*, *FKF1* and *CO*. *CO* expression is cyclical, and its protein levels are stabilised by coincidence with light. This coincidence occurs only in the afternoon in LD enabling CO to directly upregulate *FT* expression in the vascular tissue. In rice, the circadian clock is also involved in daylength sensing and flowering time control. Two *FT* genes are involved in triggering flowering in short day inductive daylengths – *Hd3a* and *RFT1*. The role of *FT* genes stretches beyond florigen and promotion of flowering (Pin and Nilsson 2012). Other functions include control of

stomatal opening (Kinoshita *et al*. 2011) and lateral shoot outgrowth in *Arabidopsis* (Hiraoka *et al*. 2012), regulation of growth and heterosis for yield in tomato (Lifschitz *et al*. 2006; Shalit *et al*. 2009; Krieger *et al*. 2010) and control of tuberisation in potato (Navarro *et al*. 2011). Some *FT*-like genes have assumed the role of floral repressors. In sugar beet, *BvFT2* is essential for floral induction, but *BvFT1* delays flowering (Pin *et al*. 2010). Vernalisation represses *BvFT1* and LD exposure activates *BvFT2*, leading to initiation of flowering. Vernalisation-independent annual beets flower in response to LDs; *BvFT1* transcription is reduced whereas *BvFT2* is activated in direct response to LDs. Annual growth habit is conferred by dominant allele *B*, a recently characterised pseudo-response regulator protein BvBTC1 (Pin *et al*. 2012), which is the upstream regulator of *BvFT1* and *BvFT2*. Another example of *FT*-mediated repression of flowering and role of *FT* in domestication is presented in sunflower (*Helianthus annus*) (Blackman *et al*. 2010) and antagonistic *FT*-like paralogs were also described in tobacco (*Nicotiana tabacum*) (Harig *et al*. 2012). Therefore, some *FT* genes acquired a function usually associated with the TFL1/CEN subclade of PEBP family proteins that can act antagonistically to FT (Shalit *et al*. 2009).

In woody polycarpic species, *FT* genes do not participate only in regulation of flowering, but may also regulate other aspects of development. In poplar (*Populus* spp.), *FT1* is expressed predominantly in winter, and is responsible for floral induction, whereas *FT2* is expressed predominantly in spring, and is responsible for regulation of vegetative growth (Hsu *et al*. 2011).Two *FT* paralogs in apple, *MdFT1* and *MdFT2*, share the floral promoting role but have distinct expression patterns (Kotoda *et al*. 2010). In *Citrus* species, three *FT*-like genes were identified, but only one appeared to be associated with transition to flowering and floral induction by low temperature (Nishikawa *et al*. 2007) and promoted flowering upon ectopic expression in trifoliate orange (Endo *et al*. 2005). Grape *FT* (*VvFT*) is relatively highly expressed irrespective of the flowering process, suggesting a role other than flowering control, although it promoted flowering upon constitutive expression in *Arabidopsis* (Sreekantan and Thomas 2006; Carmona *et al*. 2007) and responded to oxidative stress required to elevate bud dormancy (Vergara *et al*. 2012). Upregulation of kiwifruit *FT* in response to cold correlated with winter chilling requirement and bloom time of kiwifruit cultivars (Varkonyi-Gasic *et al*. 2013). Ectopic expression of kiwifruit *FT* promoted flowering in *Arabidopsis*, but in kiwifruit resulted in reduced plant growth and survival without precocious flowering. This suggests that kiwifruit *FT* may confer meristem termination, but is not sufficient to promote floral fate, a finding that is in line with the proposal that *FT* and *TFL1* homologues regulate the balance of growth but are not directly involved in cell and organ fate (Shalit *et al*. 2009).

There are several comprehensive recent reviews on *FT* genes and the control of flowering time (see Srikanth and Schmid 2011; Turnbull 2011; Andrés and Coupland 2012; Pin and Nilsson 2012). Our aim here is to complement these by describing how the use of 'Medicago' as a genetic model for flowering time has led to identification of *spring* early flowering mutants and highlighted the importance of a *FT* gene named *FTa1*.

## Medicago as a model plant for studying flowering time

*Medicago truncatula* ('Medicago' or 'barrel medic', named for its barrel-shaped seed pod) is a temperate forage legume most closely related to alfalfa and clover, to important food crops like pea, chickpea and lentil, and more distantly to soybean and common bean. Networks of flowering-time genes that regulate the transition to flowering have been uncovered in flowering model plants such as rice and *Arabidopsis*. However, although flowering time is an important regulator of yield and productivity, flowering time control in many other plants including the legumes is much less well understood. Medicago has several advantages for use as a legume model (Box 1) (Hecht *et al*. 2005; Julier *et al*. 2007; Pierre *et al*. 2008; Rose 2008; Tadege *et al*. 2008, 2009; Weller *et al*. 2009, 2012; Young *et al*. 2011).

Fig. 1 and Table 1 show selected candidate Medicago floral regulators. These were identified in Medicago and soybean genome and EST sequencing projects on the basis of predicted

### Box 1

Medicago is an in-breeding diploid plant with a medium-sized genome of ˜550 Mb that has been largely sequenced and analysed for candidate flowering time genes. The promotion of Medicago flowering by the seasonal cues of vernalisation and long-day photoperiods bears similarities to flowering control in the well studied winter varieties of *Arabidopsis*, barley and wheat. Medicago has a relatively rapid life cycle and small stature allowing it to be grown in pots under artificial lights in glasshouses or controlled environment rooms. Medicago can be transformed using *Agrobacterium* and large mutant populations were developed by introducing a tobacco retroelement *Tnt1* tagging cassette and regenerating plantlets with multiple stable *Tnt1* insertions. Together with the *Tnt1* lines, fast neutron and ethane methanesulfonate mutagenesis lines are also available for screening using forward and reverse genetics. Collections of natural accessions are maintained. There is natural variation for flowering time and flowering time quantitative trail loci (QTL) have been mapped in crosses of natural accessions. Some accessions are being re-sequenced in the Medicago Hapmap (http://www.medicagohapmap.org/, accessed 13 June 2013) project to identify DNA polymorphisms that can be used for linkage studies to map important traits using QTL analysis or genome wide association studies. Medicago is also being used successfully in comparative mapping and genomics to identify the genes affected in flowering-time mutants in garden pea and corresponding to loci conferring natural variation in flowering time in temperate legumes.

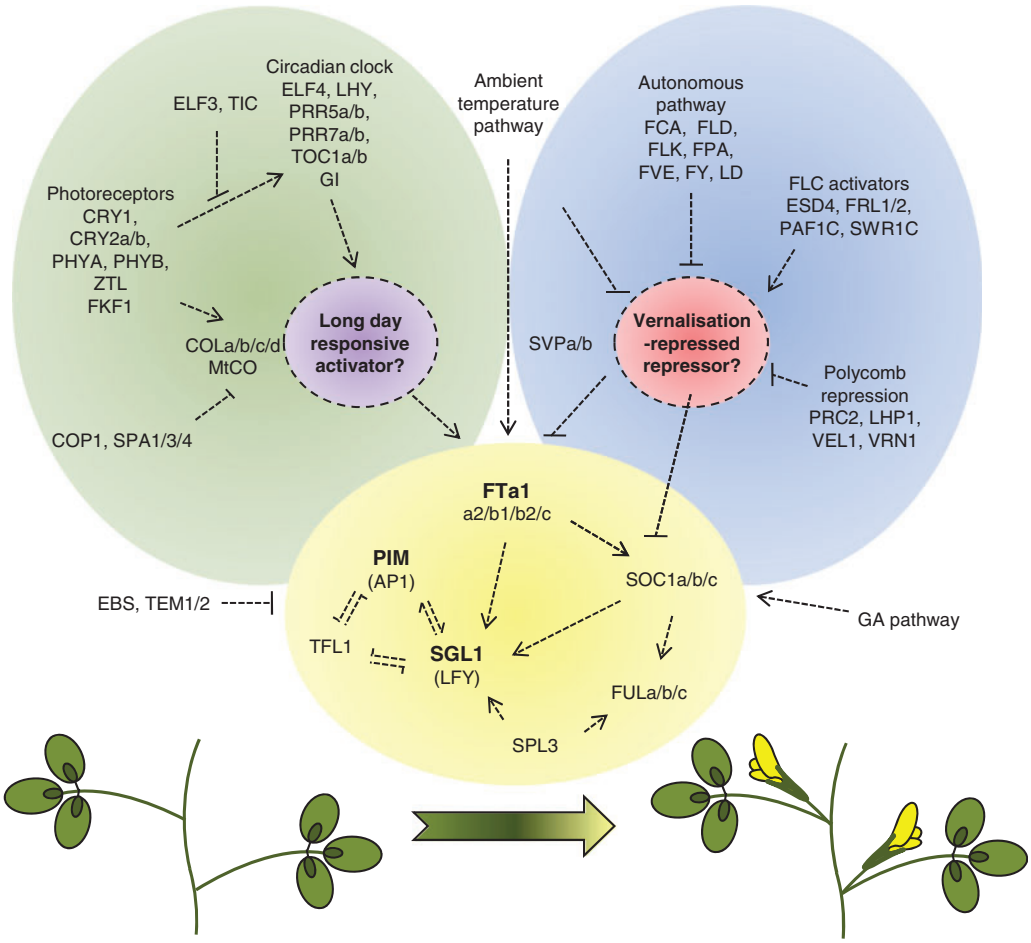


**Fig. 1.** Schematic showing selected candidate Medicago flowering regulators. Candidate Medicago floral regulators have been identified on the basis of their predicted protein similarity to regulators of *Arabidopsis* flowering. Using reverse genetics, *FTa1* has been demonstrated to regulate flowering time and Medicago *LFY* (*SGL1*), and Medicago *AP1* (*PIM*) to have roles in floral meristem identity (gene names in bold). Expression of the *FTa1* gene is upregulated after a period of extended cold (vernalisation) followed by warm long day photoperiods. However, Medicago lacks a *FLC* gene and candidate Medicago *CO*-like genes do not appear to function like *Arabidopsis CO*. Therefore, the identities of the predicted upstream vernalisation – repressed repressor and long-day responsive activator of *FTa1* remain to be discovered (signified by ?). None of the other candidate genes shown have yet been demonstrated to influence Medicago flowering. Hypothetical positive and negative interactions are based on the *Arabidopsis* network and are shown by a dotted line with an arrow or a blocked line respectively.

protein similarity to *Arabidopsis* regulators (Hecht *et al*. 2005; Weller *et al*. 2009; Jung *et al*. 2012) (J. Putterill and M. Balcerowicz, unpubl. data). Of these, three Medicago genes have a null mutant phenotype using reverse genetics with the *Tnt1* insertion mutants. These are *FTa1* that regulates flowering time (Laurie *et al*. 2011) and Medicago *LFY* (*SGL1*) and Medicago *AP1* (*PIM*), which have roles in Medicago floral meristem identity (Benlloch *et al*. 2006; Wang *et al*. 2008). The function of the other Medicago candidate genes in Fig. 1 remains to be proven. As described below, expression of the *FTa1* gene is upregulated after a period of extended cold (vernalisation) followed by warm LD photoperiods. However, Medicago lacks a *FLC* gene and candidate Medicago *CO*-like genes do not appear to function like *Arabidopsis CO* (described below). Therefore, the identities of the predicted upstream vernalisation-repressed repressor and LD responsive activator of *FTa1* remain a mystery.

## The Medicago *FTa1* gene

The Medicago *FTa1* gene is the first candidate flowering-time gene that has been shown by genetic analysis to have an important role in Medicago flowering time control (Laurie *et al*. 2011; Yeoh *et al*. 2013). Medicago has 5 *FT* genes (*FTa1*, *a2*, *b1*, *b2* and *c*). Phylogenetic analyses of the predicted Medicago FT proteins indicate that they fall into three FT subclades (FTa1/a2, b1/b2 and c) that are found only in legumes (Hecht *et al*. 2011). These subclades contain proteins encoded by five pea *FT* genes and 10 soybean *FT* genes. In Medicago, all five of the genes are on chromosome 7; *FTa1*, *a2* and *c* are clustered within ~30 kb of

each other and *FTb1* and *b2* are next to each other towards the top of the chromosome.

In *Arabidopsis*, the opposite activity of FT and TFL1 is conferred by differences in two key motifs common to all PEBP proteins – a putative ligand-binding pocket and an external loop. Specific amino acid residues within these motifs determine the regulatory activity of these proteins and a single amino acid change is sufficient to convert TFL1 to an activator of flowering (Hanzawa *et al*. 2005; Ahn *et al*. 2006). All of the Medicago *FT* genes are predicted to encode the FT-like Tyr85 and all except FTc have Gln140 (Laurie *et al*. 2011; Yeoh *et al*. 2011). In FTc it is replaced by a histidine that differs from both FT and TFL1. Medicago also possesses a *TFL1-like* gene.

*FTa1* gene expression correlates well with conditions that promote flowering (Laurie *et al*. 2011). It is expressed predominantly in leaves, the site of *Arabidopsis FT*/*TSF* expression, before the transition to flowering. It is the only Medicago *FT* gene to be strongly upregulated by the combination of vernalisation (~3 weeks at 4°C) followed by warm LDs (16 h light/ 8 h dark). These gene expression analyses thus strongly indicate that *FTa1* integrates vernalisation and photoperiod signals. However, it is not yet known whether *FTa1* is expressed in the veins of leaves, nor if *FTa1* acts as a florigen. However, this seems likely as the pea *FTa1* orthologue *GIGAS* is involved in regulation of a graft transmissible floral promotive signal (Hecht *et al*. 2011).

Molecular-genetic analyses confirm an important role for *FTa1* in Medicago flowering time control (Laurie *et al*. 2011; Yeoh *et al*. 2011). *FTa1* overexpression strongly promotes flowering in transgenic *Arabidopsis* and rescues the *ft-1* mutant. In Medicago, overexpression of *FTa1* strongly accelerates flowering independently of photoperiod and vernalisation. Reverse genetics in the *Tnt1* retroelement insertion lines, identified two *fta1* null mutants that are late flowering in inductive conditions.

However, the molecular identity of the postulated repressor that inhibits Medicago flowering until after winter remains unknown. As in many plants other than the Brassicaceae, Medicago has no clear MAF-FLC clade and VRN2, the repressor in cereals, is not present either (Hecht *et al*. 2005; Jung *et al*. 2012).

## Candidate genes in the long day pathway and regulation of *FTa1*

How is *FTa1* controlled by the Medicago long day flowering pathway? Many candidate components of the LD pathway including circadian clock genes, LD flowering pathway genes and light signalling pathways are present in Medicago (Hecht *et al*. 2005; Weller *et al*. 2009; Jung *et al*. 2012) (J. Putterill and M. Balcerowicz, unpubl. data) (Fig. 1). Yet *FTa1* appears to be regulated very differently from *Arabidopsis FT* by LD. Medicago *FTa1* expression is constitutive through the day/night cycle in LD. This contrasts with *FT*, which peaks only at the end of LD following direct upregulation by the CO protein. This raises interesting questions about how Medicago discriminates between days of different length and the nature of the upstream daylength-responsive regulators of *FTa1*.

Candidate upstream members of the Medicago LD flowering pathway, Medicago *LHY*, *TOC1a*, *GI* and *FKF1*, are expressed in a similar way to orthologs in *Arabidopsis* (Andrés and Coupland 2012). The Medicago genes are all expressed with a diurnal cycle and are circadian -clock and photoperiodically controlled (J. Putterill, L. Zhang and M. Balcerowicz, unpubl. data). In *Arabidopsis*, GI acts with a small class of F box proteins, including FKF1, to remove repressors of CO transcription, allowing it to upregulate *FT* (Andrés and Coupland 2012). GI can also act directly to upregulate *FT* (Sawa and Kay 2011). Roles for *GI* or *FKF1* in Medicago flowering time control have not yet been demonstrated. However, the pea *GI* orthologue (*LATE BLOOMER 1*) upregulates pea *FTa1* and promotes flowering in LD (Hecht *et al*. 2007). *GI* variants have also been linked to flowering time variation in soybean (Watanabe *et al*. 2011). Genetic studies in pea have also identified roles for other candidate genes in the light signalling/clock/long day pathway including pea *PHYA*, pea *ELF4* (*DNE*) and pea *ELF3* (*HR*) (Weller *et al*. 2004, 2012; Liew *et al*. 2009). We note that variation in pea *ELF3* plays an important role in the reduced photoperiod response characteristic of the spring habit in pea and lentils (Weller *et al*. 2012).

## Medicago *CO-like* (*COL*) genes

The role of *COL* genes in the LD pathway and flowering control in Medicago is an open question. A *CO-like* gene, *MtCO*, was implicated in Medicago flowering time control by QTL mapping (Pierre *et al*. 2008, 2011). *MtCO* is a Class 3 *COL* gene most closely related to *Arabidopsis* COL14/15, which are not known to control flowering in *Arabidopsis*. *MtCO* transcript levels weakly cycle with an afternoon peak of expression in LD. Its expression is unaffected by vernalisation (J. Putterill and L. Zhang, unpubl. data). *MtCO* along with other flowering time genes including three *FTs* (*FTa1*, *FTa2* and *FTc*) falls within a flowering time QTL interval on chromosome 7. We identified an *MtCO* mutant with a *Tnt1* insertion in the first exon that disrupts gene expression (J. Putterill and L. Zhang, unpubl. data). The mutant flowered at the same time as wild type in LD, with or without vernalisation. Therefore, in the R108 genotype, *MtCO* is not involved in promotion of flowering in response to vernalisation and LD. It remains possible that allelic variants may have a role in other accessions of Medicago.

The four most closely-related Medicago *COLs* to *Arabidopsis CO* are the Class 1 *COLs COLa* to *d* (Hecht *et al*. 2005). These encode proteins with the two characteristic B Boxes and a CCT domain. MtCOLa is the most closely related to *Arabidopsis* CO, but falls into a sister clade to three *Arabidopsis* COLs (CO, COL1 and COL2). *COL1* and *COL2* do not appear to regulate flowering in *Arabidopsis* (Ledger *et al*. 2001). The expression of Medicago *COLa-d* differs strongly from *Arabidopsis CO*. *COLa* and *COLb* peak at dawn, or show weak (*COLd*) or no cycling (*COLc*) (J. Putterill, L. Zhang and M. Balcerowicz, unpubl. data). This lack of similarity to *Arabidopsis CO* was observed previously in pea – pea *COLa – c* expression are unaffected in pea *gi* or pea *elf4* mutants, unlike *Arabidopsis CO* (Weller *et al*. 2009). Medicago *COLa* to *c* show little response to daylength. Thus, their expression does not correlate with the expression of *FTa1*.

*COLd* appears to be expressed at slightly higher levels in LD than in SD. Nevertheless, a strict *Arabidopsis* CO mechanism does not appear to be operating in Medicago as coincidence of expression of the *COLs* with light does not vary much between LD and SD.

### Other Medicago *FT* genes

What role might the other Medicago *FT* genes play in flowering control? Some of their features suggest possible functions in flowering (Laurie *et al*. 2011). Four of the *FT* genes (*FTa1* and *FTa2*, *b1* and *b2*) are expressed predominantly in leaves. Unlike *Arabidopsis FT*/*TSF*, the fifth gene *FTc* is expressed mainly in the shoot apex. Expression of all five *FT* genes precedes expression of the floral meristem identity gene *PIM* (*AP1*). Three *FT* genes are induced by LD photoperiods (*FTa1* and *FTb1*/*b2*) with *FTb1*/*b2* showing a bimodal diurnal pattern. In contrast, *FTa2* is expressed at higher levels in SD than in LD. *FTa1* and *a2* respond to vernalisation – *FTa1* only after growth in warm LD, whereas *FTa2* is upregulated during exposure to cold. *FTc* strongly promotes flowering when overexpressed in *Arabidopsis* and complements the *ft-1* mutant. However, Medicago *FTc* mutants with *Tnt1* retroelement insertions have no flowering phenotypes in LD. *FTb1* partly complements the *ft-1* mutant, but *FTa2* and *b2* do not. It is possible that one of the five Medicago *FT* genes may function as a repressor of flowering. However, none of the five *FT* genes is downregulated by vernalisation (Laurie *et al*. 2011) indicating that they are not regulated in the same way as *BvFT1*(Pin *et al*. 2010).

### Pea and soybean *FT* genes

Overall, these results concur quite well with discoveries in garden pea where an orthologous pea *FTa1* gene, *GIGAS*, has an important role in floral promotion by a mobile signal (Hecht *et al*. 2011; Laurie *et al*. 2011). However, detailed comparisons reveal differences in the role of pea and Medicago *FT* genes (Laurie *et al*. 2011). For example, in pea, all five *FT* genes complement *Arabidopsis ft-1* to some extent. Pea *FTa1* and *FTb2* are each proposed to be involved with mobile flowering signals, with *FTb2* likely to be an early photoperiod responsive gene. *FTb2* is up- regulated in LD, but not in the pea *gigantea* mutant, which has lost its LD photoperiod response. *FTb2* is also unaffected in the pea *FTa1* mutant *gigas*. Pea *gigas* mutants may not produce flowers in LD, whereas Medicago *FTa1* mutants do produce flowers. In the SD plant soybean, *FT* expression differs as might be expected (Kong *et al*. 2010). Here two of the 10 *FT* genes, *GmFT2a* and *GmFT5a*, FTa and FTc class genes, respectively, are induced by SD and expressed in a cyclical way. Both genes can promote flowering in *Arabidopsis*.

### Medicago *spring* mutants overexpress *FTa1*

We have been screening the *Tnt1* mutant populations (in collaboration with the Noble Foundation, Ardmore, OK, USA) and other mutant populations for flowering time mutants using forward and reverse genetic approaches (Tadege *et al*. 2009). We obtained three *spring* mutants (*spring1–3*) with dominant early flowering that is largely independent of vernalisation (Yeoh *et al*. 2013) (J. Putterill, L. Zhang, C. Yeoh and M. Jaudal, unpubl. data). Rapid flowering in *spring1–3* and upregulation of *FTa1* is dependent on LD photoperiods (J. Putterill, L. Zhang, C. Yeoh and M. Jaudal, unpubl. data).

We used linkage analysis to map *spring1* (Yeoh *et al*. 2013). It co-located with markers within an interval on chromosome 7 containing the three *FT* genes, *FTa1*, *a2* and *c*, amongst other genes. Microarray analysis of gene expression indicated that the only gene in the interval whose expression was altered in *spring1* was *FTa1*. DNA sequence analysis of the *FTa1* genomic region did not reveal promoter, coding or intron sequence mutations that might cause misregulation of the gene. Recently, we discovered an endogenous retroelement insertion just downstream of the *FTa1* gene in *spring1* that is not present in wild type (J. Putterill and L. Zhang, unpubl. data). We have also identified *Tnt1* retroelement insertions in *spring2* and *spring3* that are located in (*spring3*) or downstream (*spring2*) of the *FTa1* gene and show 100% co-segregation with the early flowering phenotype (unpubl. data). The *spring* retroelement insertions may thus function by interrupting negative regulatory sequence(s) of *FTa1*, as was proposed for a dominant wheat *FT* allele with similar properties and a retroelement insertion in its promoter (Yan *et al*. 2006). In *Arabidopsis*, the negative regulator SMZ has been shown to bind downstream of the *FT* gene using chromatin immunoprecipitation, but no *SMZ* genes have been identified in Medicago or soybean (Mathieu *et al*. 2009; Jung *et al*. 2012).

A fourth flowering time mutant, *spring4*, falls into a different class (J. Putterill and M. Jaudal, unpubl. data). It confers recessive early flowering in LD conditions. *FTa1* is upregulated. Early flowering occurs independently of vernalisation, but depends on the LD pathway, as it flowers late in SD like wild-type plants. This type of mutation might be expected as a consequence of interference with a repressor of flowering. Heterozygous plants have a wild-type phenotype consistent with the idea that one functional copy of the repressor could still repress flowering. Work is underway to characterise the molecular basis of the *spring4* phenotype, with the aim to determine the nature of the interaction of the putative repressor with *FTa1*.

## Perspectives

Numerous studies, including recent work in Medicago, have shown that *FT*-like genes promote flowering in many plants in response to external and internal cues. The Medicago *FTa1* gene integrates vernalisation and LD cues and has a central role in regulation of flowering time. However, it is striking how much the finer details of *FT* regulation and function can differ from *Arabidopsis* (Andrés and Coupland 2012). For example, *Arabidopsis FT* expression is induced in LD conditions and is expressed cyclically with a peak in the late afternoon. Medicago *FTa1* is also upregulated by LD, but is expressed throughout the day/night cycle. This indicates that Medicago and *Arabidopsis* are likely to measure and respond to daylength in different ways. Indeed, differences exist even amongst quite closely related plants as highlighted for Medicago and pea (Laurie *et al*. 2011). Significant progress is being made in Medicago, with identification and characterisation of the *spring* flowering time mutants and reverse genetic analysis of candidate regulators. However, many unanswered questions about the environmental regulation of genes such as *FTa1*

remain. Forward and reverse genetics, analysis of natural variation and genome-wide gene expression studies should uncover many more of the components of the LD and vernalisation pathways that upregulate Medicago *FTa1* and promote flowering.

*FT* genes can also act as floral repressors or contribute to regulation of other developmental events. The highly diverse nature of additional roles raises questions about the underlying mechanisms and potential diversity of necessary co-factors. As an example, the florigenic role of FT and its homologues is dependent on interaction with FD homologues (Pnueli *et al*. 2001; Abe *et al*. 2005; Wigge *et al*. 2005), but Mimida *et al*. (2011) identified transcription factors within the TCP and VOZ families as apple FT protein partners, which may be crucial for FT-mediated regulation during leaf and fruit development.

The other area expected to attract attention is the interaction of *FT* with classical plant hormones. Tomato *SFT* impacts on apical dominance, radial stem expansion, generation of abscission zones and leaf architecture, which are all auxin-regulated traits (Shalit *et al*. 2009). In *Arabidopsis*, cytokinin treatment activates *TSF* but not *FT* to affect flowering (D'Aloia *et al*. 2011) and gibberellin directly promotes the expression of *FT* and *TSF* in leaves independently of *CO* and *GI* (Galvão *et al*. 2012). Another focus is the role of sugar signalling in flowering via trehalose 6-phospate and *FT* regulation (Wahl *et al*. 2013).

Flowering time is an important component of yield in legumes and other plants (Jung and Muller 2009; Yeoh *et al*. 2011). Changes in the *cis*-regulatory DNA sequences that regulate *FT* expression contribute to natural variation in flowering time (Yan *et al*. 2006; Schwartz *et al*. 2009; Strange *et al*. 2011). In addition, the ability of FT proteins to promote flowering in many plants has significant practical implications for crop improvement. A range of potential biotechnological applications were identified for use in crop breeding and improvement where ectopic or conditional expression of *FT* might be used to customise flowering (Jung and Muller 2009; Yeoh *et al*. 2011). For example, in forages, delayed flowering may increase pasture productivity and improve nutritional characteristics. In other plants, early flowering may be desirable to shorten the time between planting and harvest in particular geographic locations. Reducing the length of the juvenile phase in long-lived trees is useful for breeding. Obtaining synchronous flowering may be an advantage in fruiting vines like kiwifruit. Alternatively, the ability to time flowering so as to avoid peak stress conditions such as high temperatures or high salt stress might be critical. Further applications and limitations will likely be revealed with further understanding of multiple *FT* roles in plant development.

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