Journal of Plant Biology, June 2007, 50(3) : 249-256 **REVIEW**

Historical Perspective on Breakthroughs in Flowering Field

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Plants have evolved a mechanism to synchronize flowering time in response to environments. How plants recognize specific seasons for flowering has been a long sought question, thus, more than 100 years of research has been focused on this question. Especially in the past two decades, remarkable achievements have been made in identifying the molecular mechanism for flowering. Here we summarize the breakthroughs made in this field over the past century including discoveries of photoperiodic and vernalization-induced flowering, the identification of complex genetic pathways, and the recently proposed identity of florigen. In addition, we present the currently accepted model for a molecular mechanism toward flowering.

Keywords: floral induction, florigen, flowering pathway integration, photoperiod, vernalization

People always admire and enjoy seasonal blossoming of plants in nature. How plants recognize specific seasons for flowering has been a long sought question: more than 100 years of research has been focused on this question and for the last two decades, remarkable achievements have been made especially in the molecular mechanism. Because so many reviews for flowering mechanism are available, we would like to avoid adding a similar review to the exponentially accumulating literatures. Instead, we summarize the breakthroughs made in the field of flowering over the past century, mainly focused on the molecular mechanism. In this way, we hope the general readers be more easily familiarized how the concepts of flowering mechanism have been developed.

People admire and enjoy the seasonal blossoming of plants in nature. How these organisms recognize the appropriate time for flowering has been intensively studied. More than 100 years of research has focused on this question, and over the past two decades, remarkable achievements have been made, especially in determining a molecular mechanism for this phenomenon. Rather than duplicating the numerous literature reviews already available, we instead summarize the breakthroughs made over the past century mainly focusing on how new concepts for flowering mechanism have been developed.

ERA BEFORE MOLECULAR AGE

Plants recognize specific seasons within the year by sensing the two most regularly changing environmental factors: photoperiod (day length) and temperature. Photoperiodic regulation of flowering was first reported by Garner and Allard (1920), who discovered that a mutant tobacco, 'Maryland Mammoth', bloomed only under short-day (SD) conditions. Since then, plants have been intensively analyzed and categorized, according to their photoperiodic response, as short-day plants (SDPs), long-day plants (LDPs), or day-neutral plants. The photoperiodic stimulus is perceived by the leaves. It was first demonstrated by Hamner and Bonner (1938), who showed that SD treatment of a single leaf from SDP *Xanthium* was sufficient to cause flowering even though the rest of the plant was kept in long days. That study also suggested the existence of transmissible floral signal(s) that was synthesized in the leaf and transported to the shoot apex. Afterwards the grafting experiment, a photoperiodically induced leaf onto an un-induced plant caused flowering, clearly demonstrated the presence of such a signal(s). Based on those results, Chailakhyan (1937) proposed florigen as the name of this hypothetical flowering hormone. Thereafter, biochemical purification of florigen has been tried more than half century but no significant outcome has been obtained.

A long period of winter cold is critical to the regulation of flowering and this process is called vernalization. The requirement for such a process was first reported by Gassner (1918). Later physiological analysis of henbane, a vernalization requiring LDP, demonstrated that vernalization confers competence to flower (Lang and Melchers, 1943). When those vernalized plants were kept in SD, they maintained vegetative growth, but when shifted to LD, they began to flower. Thus, vernalization was shown not to induce flowering directly, but rather to render the henbane competent to flower in response to an inductive photoperiod. That study also implied that vernalization could establish memory of prior vernalization. The site of cold perception during vernalization is the apical meristem, which was first demonstrated by localized cooling experiment using celery (Curtis and Chang, 1930). Wellensiek (1962, 1964) further refined this concept as dividing cells are required for vernalization response. He investigated whether tissues other than the shoot apex could perceive vernalization. To address those questions, Wellensiek performed *in vitro* regeneration of various tissues from vernalized *Lunaria biennis*, and found that both isolated leaves and roots could regenerate into vernalized plants if they contained actively dividing cells, if plant tissues other than shoot apex perceive vernalization. In retrospect, Wellensiek provided sharp insight into the

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molecular mechanism for vernalization, proposing that cellular memory was maintained through cell division, therefore representing the epigenetic nature of that phenomenon.

From more than 70 years of physiological analyses, researchers accumulated a tremendous amount of information about flowering, but the molecular mechanism remained vague until molecular genetics techniques were adopted. Especially due to the frustration with biochemical approach to reveal the identity of florigen, the genetic approach has been welcomed as a new tool to solve the mystery of flowering.

GENETIC CONTROL OF FLOWERING

Garden pea (*Pisum sativum*) and *Arabidopsis* have been the focus of genetic studies for flowering. In the 1970s to 1980s, the former was of great interest because lots of natural variations for flowering have been available with long history of cultivation. In fact, Mendel had much earlier attempted a genetic analysis of flowering time, although those efforts were hampered by an inability to see discrete segregation due to the complicated influence of environmental conditions on flowering (Weller et al., 1997). The genetic analyses of pea revealed many genetic factors involved in competence, transport and synthesis of floral stimuli and floral inhibitors (Weller et al., 1997). However, the cloning of corresponding genes was not easy task in pea, thus the focus of genetic analysis has been moved to Arabidopsis at 1990's.

Arabidopsis is a facultative LDP, meaning that long days accelerate flowering while short days delay, but do not prevent, it. Two different approaches have been adopted for genetic analyses analysis of flowering in *Arabidopsis*. One is to compare natural variations in flowering among different geographical ecotypes while the other uses flowering-time mutants that are induced by mutagenic treatments. Laibach (1951) pioneered such studies of summer versus winter annuals. Later, Napp-Zinn (1969) and Karlsson et al. (1993) reported that one or two major genes were responsible for those differences among ecotypes. Meanwhile, Redei (1962) first described late-flowering mutants with no other morphological phenotypes. He isolated the mutants, *constans* (*co*), *gigantea* (*gi*), and *luminidependens* (*ld*) in Columbia (Col) background. However, the most thorough examination was performed by Koornneef et al. (1991), who isolated 11 late-flowering mutants, including *co* and *gi* in the Landsberg *erecta* (Ler) background. From the physiological analyses, the mutants were assigned to one of three groups depending on their response to photoperiod and vernalization. The first grouping, *fca*, *fpa*, *fve*, and *fy*, showed strong responses to both; the second, *fe*, *ft*, *fd*, and *fwa*, responsed to photoperiod but showed little or no response to vernalization; and the third group, *co*, *gi*, and *fha* (=*cryptochrome2*) did not resdpond to either stimulus. Later experiments refined this grouping into genes associated with an autonomous pathway (i.e., members of the first group, plus *LD*) or those genes related to the photoperiod pathway (Groups 2 and 3).

Here, it would be useful to introduce briefly the currently accepted genetic model for flowering in *Arabidopsis* (Fig. 1). So far, more than 80 flowering-time genes have been isolated; these are classified into four interdependent genetic pathways: long-day, autonomous, vernalization, and gibberellin (GA)-dependent. Among these, *CONSTANS* (*CO*) and *FLOWERING LOCUS C* (*FLC*) act as central regulators. The former mediates the long-day pathway, serving as a floral activator, whereas the latter integrates the autonomous and vernalization pathways, such that *FLC* expression is negatively regulated both by an autonomous pathway and by vernalization (Fig. 1). In contrast to *CO*, *FLC* acts as a floral repressor (Henderson and Dean, 2004; Putterill et al., 2004; Lee, 2005). These two central regulators control the expression of common downstream target genes, the so-called flowering-pathway integrators, *FT*, *SUPPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1/AGL20*), and *LEAFY* (Sim-

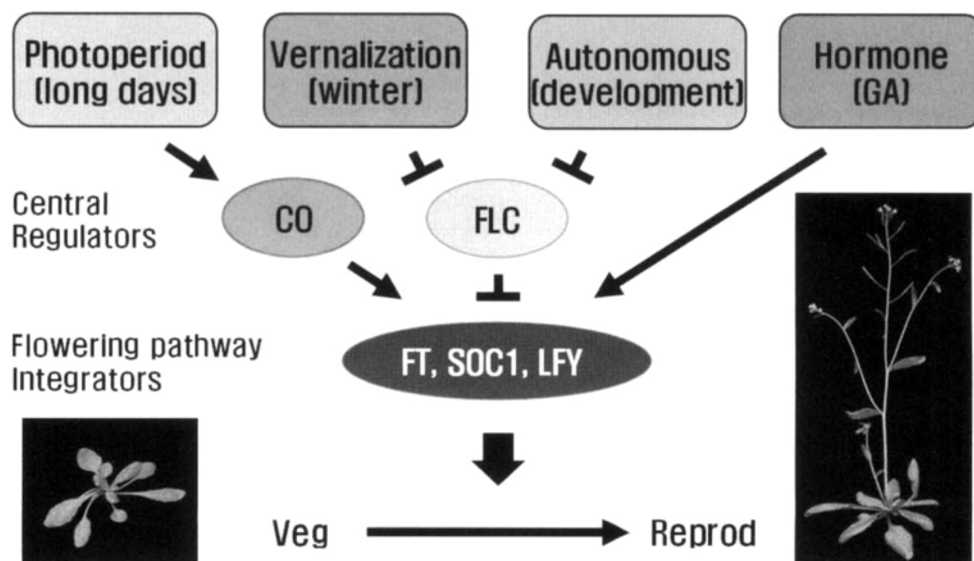

Figure 1. Current model for flowering in *Arabidopsis*. Four interdependent genetic pathways regulate two central genes, *CO* and *FLC*, and three flowering pathway integrators, *FT*, *SOC1*, and *LFY*.

pson and Dean, 2002). *CO* up-regulates whereas *FLC* down-regulates those genes (Fig. 1). The GA pathway modulates *SOC1* and *LFY* independent of *CO* and *FLC* (Blázquez and Weigel, 2000; Moon et al., 2003). An exact flowering time is eventually determined by the expression level of those integrators.

CLONING OF FLOWERING-TIME GENES

Because forward genetics (i.e., the cloning of genes based on mutant phenotypes) have proven to be relatively easy with *Arabidopsis*, flowering-time genes have been competitively obtained since the 1990s, with *LD* the first to be cloned (Lee et al., 1994a). Sequencing of *LD* did not reveal any obvious functional motif, so cloning itself was not useful for ascertaining its biochemical functioning. Later, a homeodomain was detected in the LD sequence, but its role is still unknown (Aukerman et al., 1999). Next year, Coupland group cloned *CO*, the central regulator of photoperiod pathway, by map-based gene cloning technique (Putterill et al., 1995). They reported that *CO* encodes a protein with two zinc finger motifs and the expression is higher in long days than short days. The following year Dean group cloned *FCA* also by map-based gene cloning approach (Macknight et al., 1997). This gene encodes an RNA binding protein with two RRM motifs. Interestingly, the *FCA* transcript itself showed alternative splicing, which affects flowering time (Macknight et al., 1997, 2002). Since those reports, myriad other flowering-time genes have been cloned, thereby deepening our understanding of a possible molecular mechanism for flowering.

MOLECULAR CHARACTERIZATION OF *FLC*

As mentioned already, the natural variations in flowering time among different ecotypes is regulated by two major genes, *FRIGIDA* (*FRI*) and *FLC* (Gazzani et al., 2003; Michaels et al., 2003; Werner et al., 2005). The chromosomal locus of *FRI* was first mapped during the characterization of a single dominant gene that confers a late-flowering trait in the cross between winter annual 'San Feliu-2' (SF2) and summer annual 'Col' (Lee et al., 1993). This map position was then confirmed by the cross between 'Stockholm' and 'Limburg-5', which had originally been analyzed by Napp-Zinn (Clarke and Dean, 1994). The *FLC* gene was identified by chance in the cross between SF2 and L*er* (Lee at al., 1994b). While a single dominant gene, *FRI*, was segregated in the cross of SF2 and Col, two genes were segregated in the cross of SF2 and L*er*. In this cross, one was *FRI* and the other was *FLC*. Both Col and L*er* have an *FRI* null allele while Col has a fully functional *FLC* allele and L*er* possesses a weak allele of *FLC*. This finding solved the previous mystery as to why, in certain cases, either one gene or two segregate(s) for flowering-time variations among ecotypes. The *FLC* gene was coincidentally identified by the Koornneef group, who mapped two genes segregating in the cross between *ld* (in the Col background) and the L*er* wild type (Koornneef et al., 1994). Afterward, the source for this weak activity of *FLC* in L*er* proved to be a transposon insertion in the first intron, which caused transcriptional silencing (Liu et al., 2004).

The *FLC* gene was cloned independently and almost simultaneously by two groups, with one lab using a transposon-tagged mutant and the other taking a labor-intensive map-based gene cloning approach (Michaels and Amasino, 1999; Sheldon et al., 1999). This gene encodes a MADS box transcription factor of which the expression is suppressed by both vernalization and autonomous pathway genes, while being activated by *FRI*. The genetic analyses showed that the *flc* null completely eliminates the late-flowering phenotype in *FRI* as well as all of the autonomous-pathway mutations (Michaels and Amasino, 2001), thereby demonstrating that *FLC* is the only target of *FRI* and the autonomous-pathway genes. Recently, many transcriptional activators have been reported for *FLC*, including *FRI*, *FRL1* (*FRIGIDA-LIKE1*), *FES1* (*FRIGIDA ESSENTIAL1*), and *SUF4* (*SUPPRESSOR OF FRIGIDA4*) (Michaels et al., 2004; Schmitz et al., 2005; Kim et al., 2006). Moreover, a putative protein complex that comprises *FRI*, *FRL1*, *FES1*, and *SUF4* has been suggested to act as a transcription factor for *FLC* (Kim et al., 2006).

EPIGENETIC REGULATION OF *FLC* AND VERNALIZATION

The vernalization effect is maintained through mitotic cell divisions, suggesting an epigenetic nature. Such an hypothesis was first examined by the Dennis and Peacock group (Burn et al., 1993), who showed that treatment with the DNA demethylating agent 5-azacytidine causes early flowering in vernalization requiring ecotypes and late-flowering mutants whereas it fails to cause early flowering in *ft*, a late-flowering mutant insensitive to vernalization. That same research group also reported that the decline in DNA methylation (which is induced by the antisense of *MET1* (*METHYLTRANSFERASE1*) or *ddm1* (*decreased DNA methylation1*) mutation) causes early flowering especially in a vernalization-requiring genetic background (Finnegan et al., 1998). Thus, they proposed that vernalization has a general DNA demethylating effect that releases the block to flowering. Because a DNA methylation pattern can be maintained through mitosis but reset via meiosis, that proposal was considered very attractive as an explanation for the epigenetic nature of vernalization. However, a more exciting discovery was made afterward, in which chromatin modification of the *FLC* gene through a change in the histone code proved to have a major influence on vernalization (Sung and Amasino, 2004b). It is noteworthy that vernalization effects on DNA methylation has not been directly proven yet.

The first clue for epigenetic regulation of *FLC* was reported by Amasino group (He et al., 2003). They found that *FLOWERING LOCUS D* (*FLD*), an autonomous pathway gene, encodes a putative component of the histone deacetylase (HDAC) complex (this later turned out to be a homolog of histone demethylase). The mutant *fld* exhibits a very late-flowering phenotype due to increased expression of *FLC*, which results from the hyperacetylation of histone 4 (H4) in

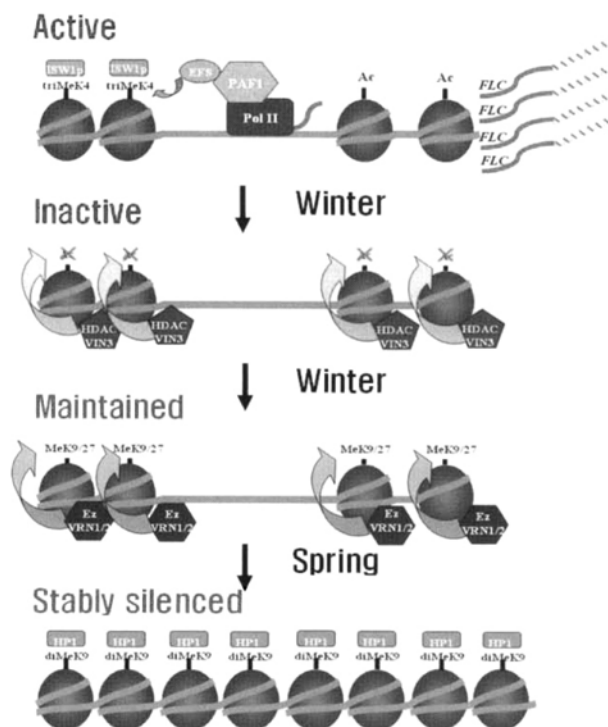

Figure 2. Model for vernalization-induced silencing of *FLC*. In winter annuals, *FLC* expression is activated by positive regulators, such as PAF1 (RNA polymerase associated factor1) complex and EFS (a homolog of SET domain methyltransferase), which causes trimethylation of H3 K4 (lysine 4 in histone 3). After a long period of winter cold, the vernalized state is established via deacetylation (caused by VIN3), and maintained by methylation (caused by VRN1/2) of *FLC* chromatin. Eventually, structural change to heterochromatin occurs for stable repression (caused by LHP1). Adapted from Sung and Amasino (2004b).

the *FLC* chromatin. Since then, *FVE*, a component of the HDAC complex, has been found to affect H3 deacetylation (Ausín et al., 2004; Kim et al., 2004) while the *Arabidopsis* homolog of the PAF1 (RNA polymerase associated factor 1) complex influences the trimethylation of H3-K4 (lysine 4) of the *FLC* chromatin (He et al., 2004). In addition, one of ATP-dependent chromatin remodeling factor, SWR1-like complex has been reported to regulate *FLC* expression, most likely through the replacement of H2A with the histone variant H2AZ (Choi et al., 2005; Deal et al., 2005, 2007; Martin-Trillo et al., 2006; Kim et al., 2007; March-Diaz et al., 2007).

The most exciting breakthrough in the area of vernalization was made in early 2004. The Amasino and Dean groups published consecutive papers showing that vernalization causes the methylation of H3K9 and H3K27 followed by the deacetylation of H3 in the *FLC* chromatin (Bastow et al., 2004; Sung and Amasino, 2004a; Fig. 2). Those genetic analyses led to the isolation of three mutants with impaired vernalization responses: *vernalization 1* (*vrn1*), *vernalization 2* (*vrn2*), and *vernalization insensitive 3* (*vin3*) (Gendall et al., 2001; Levy et al., 2002; Sung and Amasino, 2004a). *VRN1* encodes a myb-related DNA-binding protein, whereas *VRN2* encodes a polycomb group protein homologous to *SUPPRESSOR OF ZESTE-12 (Su(z)12)*, a component of the polycomb repressor complex 2 (PRC2) that possesses histone methyltransferase activity (Gendall et al, 2001; Levy et al., 2002; Chanvivattana et al., 2004).

VIN3 encodes a PHD domain protein, which is presumably a component of the chromatin modifying complexes; its expression is induced not by short-term exposure to cold but by vernalization (Sung and Amasino, 2004a). During that phase, the *FLC* chromatin undergoes a series of histone modifications - the deacetylation of H3 and the following methylation of H3-K9 and H3-K27 in the *FLC* chromatin which require VIN3, VRN1 and VRN2 respectively (Bastow et al., 2004; Sung and Amasino, 2004a). Those experiments elegantly demonstrate that histone modification is the molecular basis for vernalization.

PHOTOPERIODIC REGULATION OF FLOWERING AND EXTERNAL COINCIDENCE

The simplified genetic model for photoperiodic regulation of flowering is a hierarchical regulation of three genes, *GI*, *CO* and *FT* (Hayama and Coupland, 2004). *GI* positively regulates *CO*, and *CO* activates *FT* gene expression, then the level of *FT* expression determines flowering time. Photoperiod is composed of two physical components - light and time - both eliciting a circadian rhythm that must somehow be related to the photoperiodic regulation of flowering. As expected, many mutations that affect circadian processes also cause aberrant flowering-time phenotypes (Hayama and Coupland, 2004). One good example is the mutant *gi*, which is late-flowering and shows a defect in its circadian rhythm (Fowler et al., 1999; Park et al., 1999). The first indication that CO mediates between the circadian clock and flowering regulation was reported by the Coupland group (Suárez-López et al., 2001). In their experiments, numerous mutations in the circadian regulators, including *LHY* (*LATE AND LONG HYPOCOTYL*), *GI*, and *EARLY FLOWERING3* (*ELF3*), were associated with alterations in the circadian rhythm of *CO*. In addition, the late-flowering effect of *lhy* and *gi* was completely offset by *35S-CO*, indicating that *CO* is a downstream target of those circadian regulators. The Coupland group also reported that the *CO* rhythm is similar in LD and SD, but the difference is *CO* expression level at the end of the light period; low under SD but high under LD. It was later beautifully demonstrated by Yanovsky and Kay (2002) that the *CO* circadian rhythm is a key component in the recognition of an inductive photoperiod for flowering (Fig. 3). They showed that *35S-CO* can activate *FT* expression only under light. If transferred to dark, the *FT* expression decreased rapidly, suggesting floral induction requires both higher expression of *CO* and light. Under long days, the level of *CO* reaches a certain threshold during the day time, activating *FT* and resulting in flowering. In contrast, under short days, the *CO* level peaks only at night, thus being unable to activate *FT* and, therefore, failing to promote flowering. Later, it is reported that the role of light is to protect CO protein from degradation (Valverde et al., 2004). Such results strongly support the external coincidence model that explains the mechanism for photoperiodic flowering. This model states that flowering occurs

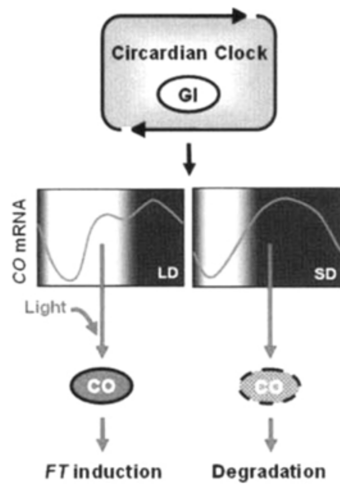

Figure 3. Model for photoperiodic regulation of flowering. Genetic hierarchy and external coincidence of CO and light are shown. In rice, genetic regulation of CO/FT is opposite that for Arabidopsis. That is, the FT homolog is negatively regulated by the CO homolog.

when the internal circadian rhythm of CO coincides with the external light (Fig. 3).

EVOLUTIONARY CONSERVATION OF THE FLOWERING MECHANISM

It was of great interest whether the molecular mechanism of flowering in *Arabidopsis* is conserved in other plant species. Orthologs of many flowering time genes such as *CO, FT, SOC1,* and *LFY* have been identified in many species, suggesting evolutionary conservation of the flowering mechanism. The most exciting discovery has been made in SDP rice, a model plant for monocots. Analysis of its quantitative trait loci (QTL) has revealed that *Heading-date1* (*Hd1*) and *Heading-date3a* (*Hd3a*), which are responsible for the variation in flowering time, are respective orthologs of *CO* and *FT* (Yano et al., 2000; Kojima et al., 2002). Interestingly, the diurnal expression pattern of *Hd1* is similar to that of *CO*; *Hd1* expression at the end of the day is high under LD but is low under SD, and enhanced expression under SD occurs only at night (Izawa et al., 2002; Kojima et al., 2002). The function of *Hd3a* is similar to that of *FT*, with overexpression of *Hd3a* causing early flowering, thereby indicating a role as a floral activator (Kojima et al., 2002). However, the opposite response to photoperiod in Arabidopsis and rice is caused by the opposite regulation of *Hd3a/FT* by *Hd1/CO* (Izawa et al., 2002; Hayama et al., 2003). Under long days, *Hd1* represses the expression of *Hd3a* with the help of phytochrome, whereas under short days, *Hd1* induces that *Hd3a*, leading to early flowering (Izawa et al., 2002). Consistent with this, the loss-of-function of Hd1 causes early flowering in LD but late flowering in SD. The genetic hierarchy of *GI-CO-FT* in *Arabidopsis* also is conserved in rice (Hayama et al., 2003). Overexpression of *OsGI*, an ortholog of *GI* in rice, causes late flowering under both SD and LD because it increases the expression of *Hd1* but decreases that of *Hd3a*. This, therefore, demonstrates that the hierarchy is conserved but regulation of the *FT* gene by *CO* is reversed in rice (Hayama et al., 2003). Recently, the Nilsson group has shown that the *CO-FT* regulatory module is also conserved in *Populus* (Böhlenius et al., 2006).

In contrast to the photoperiodic regulation of flowering, components of the vernalization pathway seem not to be conserved. The functional relevant of *FLC* in wheat is *VRN2*, because it acts as a floral repressor and its expression is decreased by vernalization (Yan et al., 2004). However, *VRN2* encodes a protein with a zinc finger domain and a CCT (*CO, CO-LIKE*, and *TOC1*) domain rather than a MADS-box protein. In addition, no *FLC* homolog has been found in the genome of rice, poplar, or *Medicago*.

INTEGRATION OF FLOWERING PATHWAYS

Although genetic analyses have been performed for several decades, a never-flowering mutant has not been reported, a phenomenon that may indicate the presence of redundant genetic pathways for flowering. Consistent with this idea, multiple flowering pathways have been described that are integrated into a few of the downstream floral activators (Simpson and Dean, 2002). The concept of integration was introduced by the Weigel group (Blázquez and Weigel, 2000), who showed that the *LFY* promoter integrates the GA signal and a long-day signal for flowering in *Arabidopsis*. Since then, it has been proven that the photoperiod and vernalization/autonomous pathways are integrated at the level of *FT* and *SOC1* (Lee et al., 2000; Samach et al., 2000). Furthermore, the expression levels of those three eventually determine flowering time. Thus, Dr. Dean grouped these three genes as flowering pathway integrators (Simpson and Dean, 2002).

FT, A COMPONENT OF FLORIGEN

The most exciting discovery in this molecular era of flowering research has been that *FT* is a component of florigen. Its overexpression causes extremely early flowering, irrespective of photoperiod (Kardailsky et al., 1999; Kobayashi et al., 1999). In addition, *FT* expression is regulated by *CO*, a mediator of circadian rhythm and flowering regulation, implying that the expression level of *FT* determines flowering time. Unexpectedly, however, neither *FT* nor *CO* expression is detected in the shoot apex where floral evocation occurs, but is, instead, detected in the leaf vasculature (Takada and Goto, 2003). Later, Coupland group demonstrated by using heterogenous promoter system that CO promotes flowering when expressed in phloem tissues but fails to promote flowering when expressed in shoot apex, suggesting CO acts cell autonomously to produce or transport systemic flowering signal (An et al., 2004). In contrast, *FT* promotes flowering when expressed in both the phloem and the shoot apex, thereby implying that *FT* itself may act as a systemic signal (An et al., 2004). In 2005, three research groups simultaneously reported that *FT* may act as a long-distance signal for flowering (Abe et al., 2005; Huang et al., 2005; Wigge et al., 2005). Araki group showed that another photoperiod

pathway gene *FD* is required in order for *FT* to promote flowering, and is expressed only in shoot apex. In contrast to *FT*, it was shown that the ectopic expression of *FD* in the leaves fail to rescue the late-flowering phenotype of *fd* mutant. They also showed that the interaction of FD and FT activates the expression of *AP1*, a floral meristem identity gene, regulating the initiation of flower development (Abe et al., 2005). Weigel group showed similar results and the direct binding of FD/FT complex to the promoter of *AP1* by chromatin immunoprecipitation technique (Wigge et al., 2005). Such results strongly suggested that the *FT* transcript or protein synthesized in the vasculature of leaves moves to the shoot apex where FD is synthesized, and that the interaction of those two proteins promotes floral transition and flower development. Finally, the Nilsson group had reported the movement of *FT* transcript by using a heat shock promoter fusion with the *FT* coding sequence (Huang et al., 2005). There, the *FT* transgene that was induced in the leaves via heat shock moved to the shoot apex at a rate similar to that previously calculated for florigen transport through physiological analysis. However, that claim was retracted in 2007. Instead, the Coupland group and Shimamoto group have now proposed that it is not *FT* transcript but FT protein that relocates from the leaves to the shoot apex in both *Arabidopsis* and rice (Corbesier et al., 2007; Tamaki et al., 2007). Such results are consistent with a previous report that *FT* transcript movement does not occur in tomato (Lifschitz et al., 2006). The hypothesis that the 'FT protein acts as a florigen' has minor problems, as explained below. First, *FD* expression, in fact, is not restricted to the shoot apex, but is also highly expressed in the leaves (personal observation). Second, it has not yet been clearly demonstrated that FT protein transport actually causes the activation of flowering or that floral induction promotes the transfer of FT protein.

CONCLUDING REMARKS

For the last two decades, the molecular mechanism of flowering has been intensively studied and, due to such endeavors, we now have a deep understanding of this phenomenon. However, we have many mysteries to solve yet. First, the FT florigen hypothesis must be confirmed in other species, especially those that show a qualitative photoperiodic response. Other possible components of florigen also should be revealed. Because the *ft* mutant itself has a relatively weak flowering phenotype, the more critical components of florigen are expected to be discovered. Second, the molecular mechanism for vernalization must be resolved in genera besides *Arabidopsis*. Because *FLC* homologs have not been detected in many other plants, researchers cannot confirm whether the same molecular mechanism is associated with vernalization in crops such as wheat and barley. Third, the molecular mechanism is still obscure for most autonomous-pathway genes. Although numerous genes have been discovered that encode RNA-binding or processing proteins, we do not know their targets. Therefore, further examination is required in this field of research.

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