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Environmental regulation of flowering

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ABSTRACT The timing of flower initiation is a highly plastic developmental process. To achieve reproductive success, plants must select the most favourable season to initiate reproductive development; this in turn requires continuous monitoring of environmental factors and a properly response. Environmental factors which change in a predictable fashion along the year, such as light and temperature, are the most relevant in terms of selection of the flowering season. In *Arabidopsis* and more recently in a few other species, molecular genetic analyses are providing a way to identify the genes involved in the regulation of flowering time. From gene sequences it is possible to develop hypotheses regarding molecular function and to infer some of the molecular mechanisms involved in the environmental regulation of flowering time. In this paper, we summarize recent discoveries concerning the mechanisms which plants use to perceive and respond to major environmental factors (light and temperature) and their different components. We focus mainly on annual plants and especially on *Arabidopsis* because most of the available molecular and functional data come from this species. However, additional information arising from other plant systems is also considered.

KEY WORDS: *flowering time, photoperiod, light quality, vernalization, natural variation*

Plants are sessile organisms that grow and reproduce at the site of seed germination. In contrast to animals, most plant development takes place post-embryonically and is very sensitive to environmental conditions. This interaction determines that plant development is not fixed but shows a wide plasticity based on a constant adjustment of developmental regulation to changing environmental conditions. One of the most plastic developmental decisions in the life cycle of plants is the timing of the floral transition. To achieve reproductive success, plants must select the most favourable season to initiate reproductive development. This selection requires the existence of molecular mechanisms to continuously monitor environmental factors and to properly respond to the adequate conditions. Many environmental factors influence flowering time (Bernier and Perilleux, 2005). Those changing in a predictable fashion along the year, such as light and temperature, are the most relevant in terms of the selection of the flowering season. These predictable factors show complex patterns of variation and interaction in different temporal ranges (i.e. diurnal versus annual variation in light and temperature). However, even less predictable factors such as nutrient or wind can also modulate flowering time, depending on the species. Environmental factors display patterns of variation in the short (i.e. diurnal variation) and long ranges (i.e. seasonal annual fluctuation). Plants are able to perceive all this environmental variation and modulate their growth and development with responses that can be in the short term such as growth response to ambient temperature or in long terms like the flowering response to vernalization. This complexity determines the need for different molecular mechanisms in the perception of environmental variation and the generation of different temporal responses.

Diversity is also broad from the side of the plant species. Generally, we can distinguish between plants that complete development within a year, called annual plants and plants that live more than one year, known as biennials or perennials. The first ones flower only once in their life cycle. However, among perennial plants there are species that flower only once in their life cycle (monocarpic) or species that flower every year, once maturity is reached (polycarpic). In annual species like *Arabidopsis*, flower initiation, defined as the morphological changes that make meristems to specify flower meristems, is immediately followed by the development of flowers. Therefore flower initiation can be considered the crucial regulatory point on which selection acts to ensure flowering and fruiting on time. Nonetheless, in polycarpic perennials is common to find a delay between flower initiation and flower development and therefore both, the time of floral meristem

Abbreviations used in this paper: B, blue; FR, far red; GA, gibberellins; LD, long day; R, red; SD, short day.

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initiation and bud burst are probably targets of selective pressure. In addition, in polycarpic and monocarpic perennial plants, sensitivity or responsiveness to environmental signals must change along the development of the plant to prevent flowering before maturity is reached.

Despite the relevance of the environmental signals on flowering induction, the molecular mechanisms underlying signal perception, transduction, integration as well as responsiveness are generally unknown in most species. Only in model *Arabidopsis* plants and more recently in rice and a few other species, molecular genetic analyses are providing a way to identify some of the genes involved in those processes. Analyses of gene sequences and their molecular function are helping to infer some of the molecular mechanisms involved in the environmental regulation of flowering.

There have been numerous recent excellent reviews on the molecular genetics aspects of flowering time control in *Arabidopsis* (Mouradov *et al.,* 2002; Simpson and Dean, 2002; Putterill *et al.*, 2004; Boss *et al.*, 2004). To complement them, here we aim to summarize current discoveries on the mechanisms that plants use to perceive environmental signals (specifically light and temperature), to interpret and integrate those signals and to finally regulate flowering time. This review is mainly focused on annual plants and especially on *Arabidopsis* because most of the available gene functional data come from this species. However, additional information currently arising in other plant systems is also considered.

I. Genetic regulation of flowering time in *Arabidopsis*

Arabidopsis is an annual plant that germinates and flowers within a year. *Arabidopsis* is considered a facultative long day plant since all known wild type strains do not show any obligate photoperiod requirement to flower but their flowering is accelerated by long days (LD). *Arabidopsis* plants behave differentially under distinct light wave conditions, being red (R), far-red (FR) and blue (B) lights, the most important on flowering time regulation. Low ratios of R/FR light as well as B light have a promotive effect on flowering. Temperature also affects flowering time in several ways. Many *Arabidopsis* strains show different degrees of response to prolonged exposure to low non-freezing temperatures (0ºC-10ºC) during their vegetative development, treatment known as vernalization. The vernalization caused by low winter temperatures accelerates flowering in many *Arabidopsis* strains (Napp-Zinn, 1987). Ambient temperature, above 10ºC, also affects flowering, with higher temperatures causing a moderate acceleration of flowering time (Westerman and Lawrence, 1970; Blázquez *et al.*, 2003). Finally, flowering time in *Arabidopsis* is also sensitive to mineral nutrition and to different types of biotic and abiotic stresses. However, these responses are so far poorly described and understood (Pigliucci *et al.*, 1995; Pigliucci and Kolodynska, 2002).

Genetic analyses in *Arabidopsis* have allowed the identification of many genes involved in the regulation of flowering time (Mouradov *et al.*, 2002; Simpson and Dean, 2003; Putterill *et al.*, 2004). The results of these studies are consistent with a model in which several pathways regulate the expression of a few key genes known as flowering signal integrators whose main function is to regulate the expression of genes specifying flower meristem identity (Kardailsky *et al.*, 1999; Moon *et al.*, 2005). Five partially independent pathways have been described based on the differential phenotype of flowering time mutants under distinct environmental conditions of temperature and photoperiod. These pathways are the photoperiod pathway, the autonomous pathway, the vernalization pathway, the light quality pathway and the gibberellin pathway (Figure 1).

The photoperiod pathway groups genes whose mutants show a late flowering phenotype under LD photoperiods that is not rescued by vernalization treatments. This phenotype suggests that their major role is floral promotion in response to the inductive LD photoperiods (Koornneef *et al.*, 1991). This pathway includes genes encoding photoreceptors such as *FHA* / *CRY2*, components of the circadian clock, clock associated genes such as *GIGANTEA* (*GI*) (Park *et al.*, 1999; Fowler *et al.*, 1999) and the downstream transcriptional regulator *CONSTANS* (*CO*) (Puterill *et al.*, 1995). Flowering signal integrators such as *FT* (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999) and *SUPRESSOR OF OVEREXPRESSION OF CO 1* (*SOC1*) are targets of *CO* (Samach *et al.*, 2000). Most genes in this pathway show a circadian regulation of their expression (Fowler *et al.*, 1999; Devlin and Kay 2000; Suarez-López *et al.,* 2001) (Figure 1).

The autonomous pathway includes genes whose mutants show a photoperiod-independent late flowering phenotype that can be reverted by vernalization (Martínez-Zapater and Somerville, 1990). These features indicate that the pathway is required to promote flowering independently of day length and its function is redundant with vernalization. Genes included in this pathway are *FCA*, *FY*, *FPA*, *LUMINIDEPENDENS* (*LD*), *FLD*, *FVE* and *FLK* (Redei, 1962; Koornneef *et al.*, 1991; Sanda y Amasino, 1996; Lim *et al.*, 2004; Mockler *et al.*, 2004). Molecular analyses have revealed that they down-regulate the expression of *FLC,* a major flowering repressor in *Arabidopsis*, although, as described later, not in a linear fashion (Michaels and Amasino, 1999; Sheldon *et al.*, 2000; Rouse *et al.*, 2002) (Figure 1). *FLC* reduces the expression of flowering signal integrators such as *FT* and *SOC1* (Hepworth *et al.*, 2002) (Figure 1). Consistent with the role of the autonomous pathway in the repression of *FLC*, null *flc* mutations suppress the late flowering phenotype of autonomous flowering mutants (Michaels and Amasino, 2001). In addition, some members of this pathway could participate in the integration of signals from environmental factors, such as ambient temperature (Blázquez *et al.*, 2003; Kim *et al.*, 2004).

The vernalization pathway includes genes whose mutations block the acceleration of flowering caused by vernalization. This phenotype suggests a role in the establishment and/or maintenance of the vernalized state, which at the molecular level is reflected by suppression of *FLC* expression. Genes included in this pathway are, *VERNALIZATION INDEPENDENT3* (*VIN3*) involved in the establishment of suppression of *FLC* expression (Sung and Amasino, 2004) and *VERNALIZATION1* (*VRN1*) (Levy *et al.*, 2002) and *VERNALIZATION2* (*VRN2*) (Gendall *et al.,* 2001), that are required for maintenance of *FLC* suppression during the development of vernalized plants. The functional redundancy observed between the autonomous and vernalization pathways is explained at the molecular level by their coincidence on *FLC* repression (Figure 1).

In addition to those three pathways, there are two other interacting pathways with a less defined function such as the

gibberellin (GA) (Moon *et al.*, 2003) and the light quality pathways (Cerdan and Chory, 2003) (Figure 1). Exogenous application of physiologically active GAs have a flowering promoting effect on *Arabidopsis* (Langridge, 1957), whereas GA deficient and insensitive mutants have a late flowering phenotype (Wilson *et al.*, 1992). This late flowering phenotype is more extreme under non inductive SD than under LD suggesting a partial redundancy between the photoperiod pathway and the effect of gibberellins in flowering promotion (Wilson *et al.*, 1992). Gibberellins have been shown to positively regulate the expression of flowering signal integrator genes such as *SOC1* and *LFY* (Blazquez and Weigel 2000; Moon *et al.*, 2003) (Figure 1). Furthermore, photoreceptors like PHYB could directly regulate flowering as part of the shade avoidance syndrome by modulating the expression of the flowering signal integrator *FT* in a photoperiod-independent pathway mediated by the product of the *PHYTOCHROME and FLOWERING TIME1* (*PFT1*) locus (Cerdán and Chory, 2003).

Signals originating from these five flowering regulatory pathways are integrated at different levels by the set of integrator genes (Figure 1). *FLC* integrates signals from the autonomous and vernalization pathways (Michaels and Amasino, 2001) and itself regulates two additional integrator genes, *FT* and *SOC1*, the expression of which also integrates signals from the photoperiod pathway gene *CO* (Samach *et al.*, 2000; Onouchi *et al.,* 2000). In addition, *SOC1* integrates signals from gibberellins (Moon *et al.*, 2003), whereas *FT* does so from *PFT1* (Cerdan and Chory, 2003) and together are responsible for the activation of flower meristem identity genes, (Kardailsky *et al.*, 1999; Samach *et al.*, 2000) such as *LEAFY* (*LFY*), *APETALA1* (*AP1*), *APETALA2* (*AP2*), *CAULIFLOWER* (*CAL*) and *FRUITFUL* (*FUL*). Furthermore, some of the later genes such as *LFY* could also integrate flowering signals from specific flowering regulatory pathways (Blázquez *et al.*, 2002). In addition, there must be an upper level of pathway interaction since different pathways integrate environmental signals mediated by the same photoreceptors or that are outputs of the circadian clock such as the photoperiod and the GA pathways (Blázquez and Weigel, 2002) (Figure 1).

II. Light effects on floral transition

Light is a complex environmental factor with multiple signal components such as light quality, light intensity, photoperiod and directionality which differentially modulate many aspects of plant development. Light is one of the most important factors influencing flowering time, light quality and photoperiod being the components with a more relevant effect. White light consists of different light wavelengths whose ratios show diurnal and seasonal variation and are strongly influenced by the plant canopy (Ballare, 1999). The length of the light period (photoperiod) also shows seasonal differences with a specific pattern of annual variation in different latitudes. How plants are able to sense this environmental variation and use it to trigger specific developmental responses such as flowering is something that we are starting to understand in a few model systems such as *Arabidopsis* and rice.

Photoreceptor and light quality regulation of flowering time

The diversity of light wavelengths and intensities are sensed in plants by small families of specialized photoreceptors. Phytochromes and cryptochromes are the major photoreceptor families involved in light perception. Phytochromes belong to a family of chromoproteins bearing a linear tetrapyrrole chromophore that in *Arabidopsis* includes five homologous members, PHYA, PHYB, PHYC, PHYD and PHYE (Quail, 2002). A general effect of phytochromes in flowering can be inferred from mutants *hy1* and *hy2*, impaired in the synthesis of the chromophore of all phytochromes. These mutants show a severe early flowering phenotype independently of photoperiod (Goto *et al.*, 1991) indicating that phytochromes generally function to repress flowering. The five *Arabidopsis* phytochromes show distinct but partially overlapping functions. The light labile PHYA is the most sensitive to FR light and *phyA* mutants display late flowering phenotype specially when plants are grown under SD photoperiod enriched with FR light, which suggests that the positive effect of FR light on flowering acceleration is mainly mediated by PHYA (Reed *et al.*, 1994; Johnson *et al.*, 1994). The light stable phytochromes, PHYB to PHYE, are more sensitive to R light. The main effect of R light on flowering repression seems to be mediated by *PHYB*, since mutants in this gene show early flowering phenotype regardless of photoperiod conditions. *PHYD* and *PHYE* would function redundantly with *PHYB* on flowering repression, since *phyD* or *phyE* mutations do not cause early flowering but

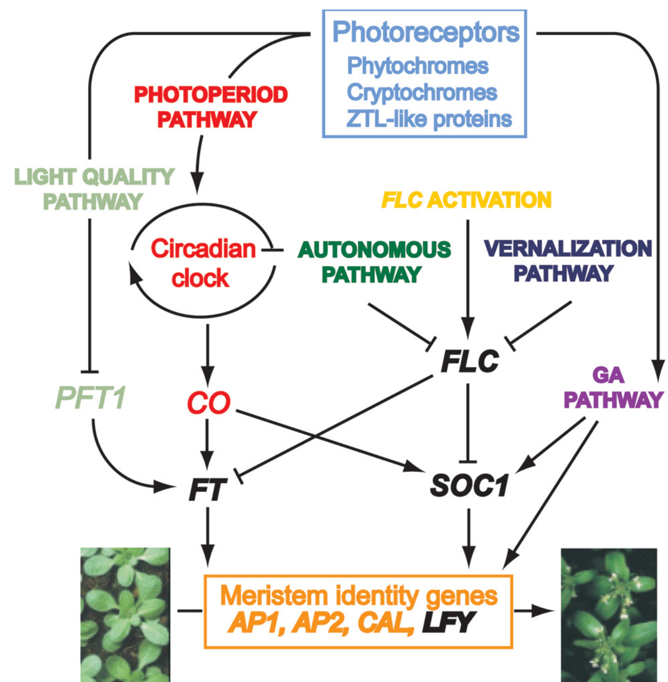

Fig. 1. Genetic control of flowering time in *Arabidopsis*. *The scheme shows the known pathways involved in flowering regulation and the interactions between them and the floral integrator genes. Photoreceptors act at least at three different levels: entraining the circadian clock, repressing* PFT1 *expression and probably modulating GA biosynthesis.* FLC *integrates signals from several pathways and repress* SOC1 *and* FT. CO, *a circadian clock regulated gene, activates both* FT *and* SOC1. SOC1 *also integrates signals from* FLC, CO *and the GA pathway. Both* FT *and* SOC1 *activate meristem identity genes causing the transition from vegetative to reproductive development at the shoot apical meristem. Floral integrator genes are represented in black bold fonts. Arrows indicate activation and bars depict inhibition.*

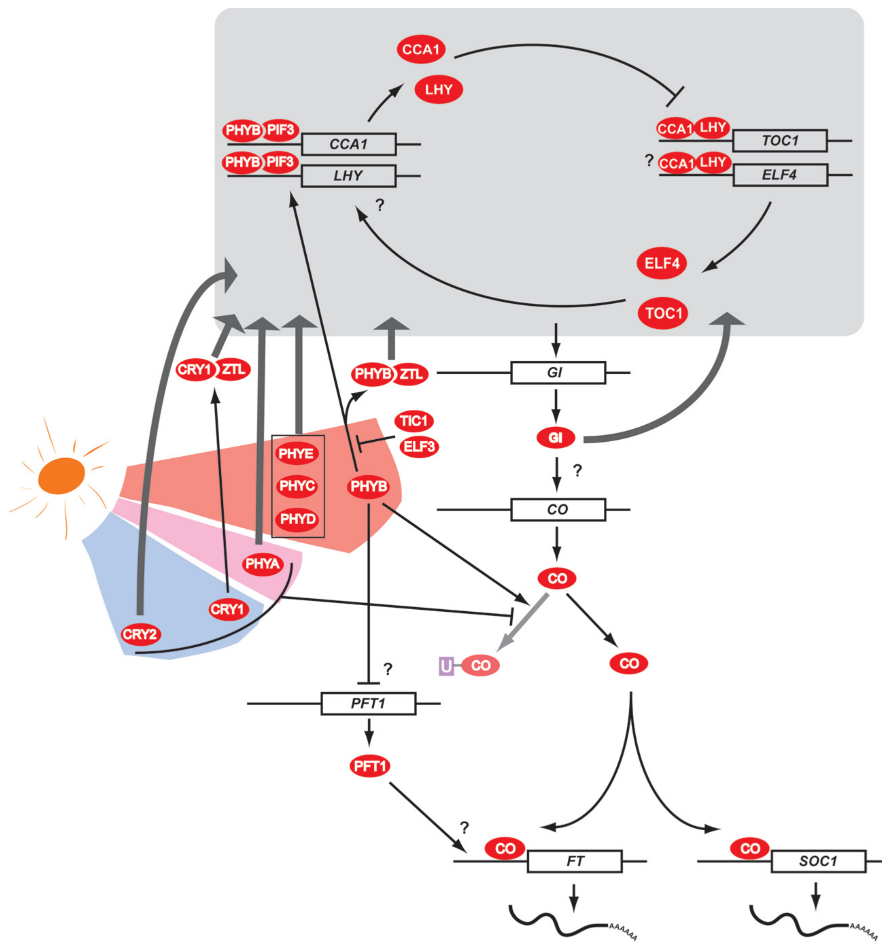

Fig. 2. Light effects on *Arabidopsis* flowering. *Different light wavelengths are perceived by distinct photoreceptors, which act on the regulation of flowering time at several levels. Photoreceptors entrain the circadian clock (light grey box);* CCA1 *and* LHY *could be activated by the complex PHYB-PIF3. Once these genes are translated CCA and LHY could bind* TOC1 *and* ELF4 *promoters repressing their expression. The latter genes activate* LHY *and* CCA *by an unknown mechanism.* GI *is one of the clock outputs, although may also act as an input and activates* CO*. CO is ubiquitinated and degraded by proteasome, photoreceptors playing a key role in this process. PHYB promotes degradation, whereas PHYA, CRY1 and CRY2 inhibit this process. CO directly activates the expression of* FT *and* SOC1*.* FT *is activated by PFT1 independently of photoperiod but depending on red light in a PHYB mediated mechanism. Thin arrows indicate activation, thicker arrows indicate clock entraining, bars depict repression and purple U box represent ubiquitin.*

enhance the early flowering phenotype of *phyB* plants (Halliday *et al.,* 1994; Aukerman *et al.*, 1997; Devlin *et al.*, 1998, 1999). In contrast, *phyC* mutants flower early under SD conditions but at the same time as wild type under LD conditions suggesting a PHYC role in photoperiod responsiveness (Monte *et al.*, 2003). Nevertheless, this PHYC role might be dependent on PHYB activity since double mutants *phyC phyB* flower as early as *phyB* (Monte *et al.*, 2003).

Blue/UV-A light photoreceptors, known as cryptochromes, are flavoproteins with similarity to bacterial DNA photolyases bearing flavin and pterin chromophores. In *Arabidopsis*, these proteins are encoded by two genes *CRYPTOCHROME1* (*HY4/CRY1*) and *CRYPTOCHROME2* (*FHA/CRY2*) (Ahmad and Cashmore, 1993; Lin *et al.*, 1998). The effects of blue light on flowering time acceleration are mainly mediated by *CRY2*, since *cry2* mutants show late flowering phenotype under different photoperiodic conditions (Lin, 2000). However, *CRY1* appears to function redundantly with *CRY2* in the regulation of flowering time since *cry1 cry2* double mutants flower much later than *cry2* plants (Mockler *et al.*, 2003). Thus, flowering acceleration promoted by far-red and blue lights are mediated by far red photoreceptor PHYA and blue light photoreceptors CRY1 and CRY2 (Figures 1 and 2).

Phytochromes and cryptochromes are found in all organs throughout the plant life cycle (Ahmad and Cashmore, 1993; Lin *et al.*, 1998; Sharrock and Clack, 2002). However, leaves are likely the major site for light detection, given the larger detection surface.

At the subcellular level, GFP fusions to phytochromes have revealed that PHYA and PHYB are located in the cytoplasm during dark periods and move to the nucleus in response to red light (Kircher *et al.*, 1999). In contrast, PHYC, PHYD and PHYE seem localized in the nucleus under any light regime (Kircher *et al.*, 2002). Similarly, based on protein fusion studies and cell fractionation analyses, CRY2 has been found to be constitutively located in the nucleus whereas CRY1 is nuclear located primarily in darkness (Guo *et al.*, 1999; Kleiner *et al.*, 1999; Mas *et al.*, 2000).

Phytochrome and cryptochrome mediated light effects on flowering time result from the interaction with different flowering regulatory pathways at several levels (Figures 1 and 2). Apart from the participation of photoreceptors in the photoperiod pathway (described below) light quality could also affect flowering time through a photoperiod independent pathway. Indeed, compact plant canopies or high plant densities produce a decrease in the ratio of red to far-red light reaching the shadowed plants. This change in light quality triggers a series of developmental responses, including flowering acceleration, aimed to increase light competition ability and known as the shade avoidance syndrome (Ballare, 1999). Shade avoidance responses are mediated by phytochromes and particularly by PHYB since *phyB* mutants are early flowering and show a constitutive avoidance phenotype (Halliday *et al.*, 1994). Mutations at the *PFT1* locus cause a slight delay in flowering time and suppress the early phenotype of *phyB* mutants (Cerdán and Chory, 2003). In addition *pft1 phyB* double mutants abolish the increased levels of expression of *FT* observed in *phyB* mutants under LD and SD conditions indicating that PHYB requires PFT to regulate *FT*. In contrast *CO* expression is not affected in *pft* mutants, indicating that the effects of *PFT1* on flowering time are mediated directly by *FT* and independently of the photoperiodic pathway (Figure 2). PFT could also act downstream of PHYA based on the non additive late flowering phenotype of *pft phyA* double mutants, with the effect of PHYA being mediated through its interaction with PHYB (Mockler *et al.*, 2003). Thus, expression of the flowering signal integrator *FT* could be modulated by the perception of light quality by phytochromes

through a regulatory pathway mediated by PFT independently of photoperiod.

Photoperiodic regulation of flowering time

Photoperiod is the length of the light period of the daily light/dark cycle and it is one of the major environmental factors regulating flowering time. Plants are classified in terms of their photoperiod responses in LD, SD and neutral species. Some plants flower only or faster when photoperiod is shorter than some critical value (SD plants like rice), other when photoperiod is longer (LD plants as *Arabidopsis*) while certain plants flower independently of day length (neutral plants). Plants use the photoperiod length to sense seasonal progression along the year by measuring the daily variation through their internal circadian rhythm. Thus, plants translate circadian into circannual information.

Circadian rhythms, with a period length close to 24 hours are commonly observed in many plant processes such as leaf movement or stomata aperture (Salome and McClung, 2004). These rhythms are generated by an internal mechanism, which is organized in three components: The clock core or central oscillator that generates the 24h timing mechanism, the input pathways that synchronise the clock oscillator to daily cycles of light and dark and the output pathways that regulate specific processes (Strayer and Kay, 1999). Two basic *Arabidopsis* observations support a major role of circadian rhythms in the photoperiodic regulation of flowering. First, most mutants affected in the circadian clock show flowering time alterations in response to photoperiod (Schaffer *et al.*, 1998; Wang and Tobin, 1998; Strayer *et al.*, 2000; Doyle *et al.*, 2002). Second, most *Arabidopsis* genes involved in the photoperiod flowering pathway show a circadian regulation of their expression (Figure2) (Fowler *et al.*, 1999; Kardailsky *et al.*, 1999; Suarez-López *et al.,* 2001).

The core of the circadian oscillator is based on the function of at least four proteins, CIRCADIAN CLOCK ASSOCIATED1 (CCA1), LATE ELONGATED HYPOCOTYL (LHY), TIMING OF CAB1 EXPRESSION (TOC1) and EARLY FLOWERING4 (ELF4). LHY and CCA1 are MYB transcription factors and both proteins as well as their corresponding mRNAs show circadian expression with a peak at dawn. Furthermore, ectopic expression of these proteins causes a severe reduction of each other expression (Schaffer *et al.*, 1998; Wang and Tobin, 1998). TOC1 shares similarity to bacterial response regulators while ELF4 has no homology with proteins of known function. Their mRNAs are also expressed in a circadian fashion but, unlike *LHY* and *CCA1* mRNAs, they peak at dusk. In addition, *toc1* and *elf4* mutants show a reduction of *LHY* and *CCA1* mRNAs levels (Strayer *et al.*, 2000; Doyle *et al.*, 2002). Together these data have led to a model in which the four proteins create a feedback loop of approximately 24h (Alabadi *et al.*, 2001). *CCA1* and *LHY* expression is higher during the morning, translation of LHY being also enhanced by light (Martinez-Garcia *et al.*, 2000) and CCA1 and LHY repress the expression of *TOC1* and *ELF4*. CCA1 and LHY bind *TOC1* promoter *in vitro* through a short sequence called evening element (Harmer *et al.*, 2000), which is also found in the *ELF4* promoter, although CCA1 and LHY binding has not been demonstrated in this case. Since TOC1 and ELF4 are required for normal expression of *CCA1* and *LHY*, their reduction is followed by a decrease in *CCA1* and *LHY* mRNA levels. At the evening, LHY and CCA1 protein levels are very low allowing *TOC1* and *ELF4* mRNA levels to rise again. The increased expression of these evening genes acts to positively regulate the expression of *CCA1* and *LHY*, thus closing the regulatory loop. The mechanism underlying *CCA1* and *LHY* activation by TOC1 and ELF4 is still unknown. Mutations in these genes cause a loss of circadian rhythms or alterations in the circadian period and consequently reduction of photoperiodic flowering responses (Schaffer *et al.*, 1998; Wang and Tobin, 1998; Strayer *et al.*, 2000; Doyle *et al.*, 2002).

Input pathways synchronize the clock to natural daily light-dark cycles and are mediated by photoreceptors. Phytochromes, cryptochromes and one additional group of putative blue-light receptors, the ZTL family, are predicted to participate in clock entrainment with different involvement depending on wavelength and light intensity. At least four of the five phytochromes and the cryptochromes are involved in clock entrainment. PHYA is required for clock adjustment under very low and high light intensity conditions, whereas PHYB, PHYD and PHYE function under high intensity (Somers *et al.*, 1998; Devlin and Kay, 2000: Yanovsky *et al.*, 2000). On the other hand CRY1 and CRY2 also function at low or intermediate light intensities (Somers *et al.*, 1998; Devlin and Kay, 2000). In *Arabidopsis*, PHYA and CRY2 seem to be the major photoperiodic light receptors (Yanovsky and Kay, 2002), but other species show different light preferences. For example, the main light photoperiod receptor in rice is PHYB (Izawa *et al.*, 2002), while it could be PHYA in pea (Weller *et al.*, 2001). Nonetheless, *Arabidopsis phyA phyB cry1 cry2* quadruple mutants are still sensitive to the light signals regulating the clock (Yanovsky *et al.*, 2000) suggesting that other photoreceptors control clock entrainment. This could be the role of the proteins ZTL, FKF1, LKP2 of the ZTL family, which contain PAS/LOV and F-box domains as well as Kelch repeats (Somers *et al.*, 2000: Nelson *et al.*, 2000: Schultz *et al.*, 2001, Jarillo *et al.*, 2001). Mutations in the corresponding genes lead to a late flowering phenotype under LD but not under SD conditions and abolish a variety of circadian rhythms. These proteins could function as photoreceptors in clock entraining through phytochrome and cryptochrome signaling pathways since ZTL interacts *in vitro* with PHYB and CRY1 (Jarillo *et al.*, 2001).

Which are the photoreceptor-mediated mechanisms responsible for clock entrainment? Some of these mechanisms could involve the direct interaction of photoreceptors with components in the transcriptional regulatory machinery of core clock components. In this way, it has been shown that red light induces PHYB activation and relocation to the nucleus where it interacts with PHYTOCHROME INTERACTING FACTOR3 (PIF3) in a reversible fashion (Ni *et al.*, 1999). Recent evidence indicates that this PHYB-PIF3 interaction is followed by a rapid degradation of the PIF3 protein (Bauer *et al.*, 2004), which is a basic helix-loop-helix transcription factor that can bind specifically DNA elements present in several light regulated genes including *CCA1* and *LHY* (Martínez-García *et al.*, 2000). Other genes such as *GI*, *ELF3* and *TIC* could also participate in light signaling to the clock. Mutants at either *EARLY FLOWERING 3* (*ELF3*) or *TIME FOR COFEE (TIC)* are early flowering and show alterations of circadian rhythms. ELF3 has also been shown to interact *in vitro* with PHYB, suggesting that ELF3 may act attenuating the light input provided by PHYB (Liu *et al.*, 2001). Double mutants *tic elf3* completely eliminate circadian rhythmicity of *CAB* (Hall *et al.*, 2003). Thus, ELF3 and TIC have been proposed as components of the bridge between photoreceptors and the clock. In addition, ZTL-like photoreceptors contain F-

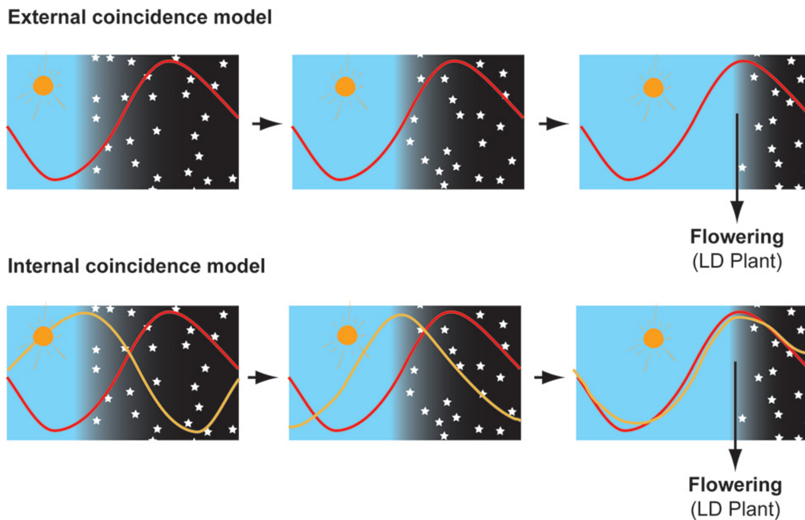

Fig. 3. Proposed models for photoperiod measurement. *The upper panel illustrates the external coincidence model, which postulates that an internal clock entrained by light creates an endogenous rhythm with a light sensitive phase. When this sensitive phase overlaps with the light period, photoperiodic responses are promoted (in LD plants) or inhibited (in SD plants). The lower panel illustrates the internal coincidence model, which proposes that light acts entraining at least two different clocks that lead to two different internal rhythms. Photoperiodic responses are triggered when these two rhythms coincide.*

box domains involved in targeting proteins for degradation (Kipreos y Pagano, 2000) and kelch repeats which mediate specific protein-protein interactions (Adams *et al.*, 2000). This structure suggest that they could participate in clock entrainment through the light dependent targeting of clock components for degradation (Kim *et al.*, 2003; Yanovsky and Kay, 2003; Imaizumi *et al.*, 2003) (Figure 2).

The circadian clock generates a series of rhythmic outputs, one of them being involved in the photoperiod regulation of flowering. Genetic analysis has shown that within the photoperiod pathway, *GI* activates *CO*, which then activates *FT* (Figure 2). *GI* encodes a nuclear protein (Huq *et al.*, 2000) whose molecular function has not yet been established, but its expression shows a circadian pattern peaking at the middle of the light period. Mutations in *GI* cause a late flowering phenotype under LD conditions and a reduced expression of *CCA1* and *LHY* (Park *et al.*, 1999; Fowler *et al.*, 1999). In addition, the expression pattern of *GI* is altered in *lhy cca1* double mutant background (Mizoguchi *et al.*, 2002). Nonetheless *GI* seems not to be a member of the clock since a variety of clock regulated responses are not affected in *gi* mutants. In contrast, its LHY and CCA1 expression dependence suggests that GI is more likely part of the clock output.

The effects of *GI* on flowering time are mediated by *CO* since *gi* mutants show reduced levels of *CO* mRNA compared to wild type and overexpression of *CO* compensates their late flowering phenotype (Suárez-López *et al.*, 2001). *CO* encodes a protein containing two B-type zinc-finger domains (Puterill *et al.*, 1995) and its expression is under circadian regulation. Similar to *gi*, *co* mutants do not show alterations of clock regulated responses like *CAB1* expression (Ledger *et al.*, 2001), supporting the role of *CO* as a clock output. Circadian regulation of *CO* expression results in *CO* mRNA levels peaking during the night under SD conditions but at dusk when plants are grown under LD inductive photoperiods. In these conditions, CO directly activates the expression of the floral integrators *FT* (showing circadian regulated expression peaking at dusk, like *CO*) and *SOC1* (peaking at dawn) (Kardailsky *et al.*, 1999; Kobayashi *et al.*, 1999; Samach *et al.*, 2000) (Figures 1 and 2). The molecular mechanism of FT action is not yet defined but, once *FT* reaches a certain threshold, flower meristem identity genes are transcriptionally activated promoting the switch from vegetative to reproductive development at the apex (Kardailsky *et al.*, 1999) (Figure 1).

Overall, the CO and GI proteins of the flowering photoperiod pathway work as a circadian clock regulated output pathway controlling the expression of flowering signal integrator genes under inductive photoperiods. But how this pathway measures the length of the day period and activates transcription under LD? Two different models have been classically proposed to explain how plants measure seasonal progression (Figure 3). The first model was proposed by Bünning (1936), who postulated that an internal clock leads to an endogenous rhythm with a light sensitive phase and that photoperiodic responses are promoted (in the case of LD plants) or repressed (in the case of SD plants) when the light part of the day coincides with the sensitive phase of this endogenous rhythm (Figure 3). In contrast with this external coincidence model, Pittendrigh (1960) hypothesized the internal coincidence model. This model proposes the existence of two endogenous rhythms entrained by light to ensure that they coincide under inductive conditions (Figure 3). In *Arabidopsis*, high levels of *CO* expression only have effects on *FT* expression and flowering induction when occurring during the light period. Furthermore, plants ectopically expressing *CO,* where its transcription is not clock dependent, still maintain certain rhythmicity in CO protein levels suggesting that there is an additional rhythmic factor regulating the CO level (Valverde *et al.,* 2004). In fact, CO protein is more effectively ubiquitinated and degraded by the proteasome complex in the dark than under light. It has been shown that photoreceptors play a key role in this regulation, different photoreceptors acting in antagonistic ways. CRY1, CRY2 and PHYA appear to stabilize CO protein under blue and far-red light, while PHYB activates CO degradation under red light (Valverde *et al.*, 2004). Therefore light and clock act on CO level, whose regulation integrate both signals to measure photoperiod (Figure 2). The role of CO in photoperiodic promotion of flowering supports the external coincidence model, since flowering occurs when *CO* expression overlaps with the inductive light period that stabilizes CO protein. Thus, *Arabidopsis* plants discriminate flowering inductive photoperiods (LD) from repressive ones (SD) at the level of CO activity.

Flowering time regulation in short day plants

The long photoperiodic conditions that promote flowering in *Arabidopsis* have opposite effect in SD plants such as rice. This contrasting situation raised the question of whether plant species with different photoperiod requirements to flower would

use the same or different molecular mechanisms for photoperiod detection and response. The answer to this question has been provided by the results of an important effort to map and clone the major QTLs responsible for flowering time in rice (or heading date as they are known in rice). The first three genes identified, *Heading date1* (*Hd1*), *Heading date3a* (*Hd3a*) and *Heading date6* (*Hd6*) are homologues of the *Arabidopsis CO* (Yano *et al.*, 2000), *FT* (Kojima *et al.*, 2002) and *CK2,* a kinase regulating circadian clock function in *Arabidopsis* (Takahashi *et al.*, 2001). Rice phytochromes are also involved in photoperiodic control of flowering, as shown by the photoperiod insensitive early flowering displayed by rice mutants in chromophore biosynthesis. Furthermore, *OsGI*, the rice *GI* homologue, regulates flowering time in response to photoperiodic conditions, since lines with reduced levels of *OsGI* mRNA show a delayed flowering under inductive SD conditions whereas under LD conditions they flower only slightly later than wild type (Hayama *et al.*, 2003). Thus, rice shares common components with *Arabidopsis* in the control of flowering in response to photoperiod but these components regulate an opposite photoperiodic response.

Several studies have shown that rice *Hd1*, as *Arabidopsis CO*, shows circadian regulation of its expression, with a peak at dusk under LD conditions and a peak in the middle of the night under SD conditions (Izawa *et al.*, 2002, Kojima *et al.*, 2002 and Hayama *et al.*, 2003) (Figure 4). Furthermore, as *FT* in *Arabidopsis*, *Hd3a* is a strong flowering promoter in rice, since *35S::Hd3a* transgenic plants show an extreme early flowering phenotype (Kojima *et al.*, 2002) that is photoperiod independent. However, in contrast to *Arabidopsis CO*, *Hd1* shows a dual role in the regulation of *Hd3a* expression, repressing under LD conditions and promoting under SD conditions. Consistently, *photoperiod sensitivity1* (*se1*), a mutant of *Hd1*, shows early flowering under long days and late flowering under short days (Yano *et al.*, 2000), which correlated with elevated mRNA levels of *Hd3a* under LD conditions and decreased *Hd3a* mRNA levels under SD (Izawa *et al.*, 2002, Kojima *et al.*, 2002). Thus, this mechanism maintains a strong parallelism with *Arabidopsis* (Figure 4). *Hd1* peaks at dusk under long days, resulting in a signal that inhibits *Hd3a* mRNA expression and repress flowering. However, the same molecular elements lead to opposite responses under LD and SD by changing the *CO* / *Hd1* effect on *FT* / *Hd3a* function, indicating that other light regulated components participate in this process. One such component might be the recently isolated *Early heading date 1* (*Ehd1*), which acts as another SD promotor of *Hd3a*. *Ehd1* encodes a B-type response regulator not found in *Arabidopsis* that functions independently of *Hd1* (Doi *et al.*, 2004). Further analysis of the regulatory mechanisms in other plant species with different photoperiodic responses will reveal the degree of conservation of the photoperiod flowering pathway in the plant kingdom.

Molecular mechanisms in the light regulation of flowering time

A combination of transcriptional and post-transcriptional regulation is used by plants to control flowering responses to different light components. Transcriptional regulation is the basis of the generation of the circadian rhythm and the output circadian pathways. Post-transcriptional regulation at the level of protein stability appears related to photoreceptor mediated responses. It has been described at the level of the input pathways entraining the clock where protein ubiquitination and proteasome mediated degradation has been suggested. Stabilization of CO protein is also part of photoperiod measurement by providing the molecular component for the light sensitive phase. The light stability of photoreceptors has been also associated with flowering time variation in different plant species. In *Arabidopsis* this is the case of the B light photoreceptor CRY2, which is light labile and rapidly disappears when plants are exposed to B light (Ahmad *et al.*, 1998; Lin *et al.*, 1998). *EDI*, a naturally occurring allele of *CRY2* present in the Cvi strain, strongly affects the photoperiodic regulation of flowering. This *CRY2- Cvi* allele carries one amino acid substitution that confers higher light stability, allowing its faster accumulation during the night. Accumulation of CRY2-Cvi in transgenic L*er* plants results in photoperiod insensitivity (El-Assal *et al.*, 2001) what could be explained by the effect of CRY2-Cvi in the stabilization of CO protein. In barley, another LD plant species, a barley maturity day length response-1 (BMDR-1) mutant line shows early flowering under short days. This severe earliness results from a higher stability of phytochrome B in the dark but not in the light

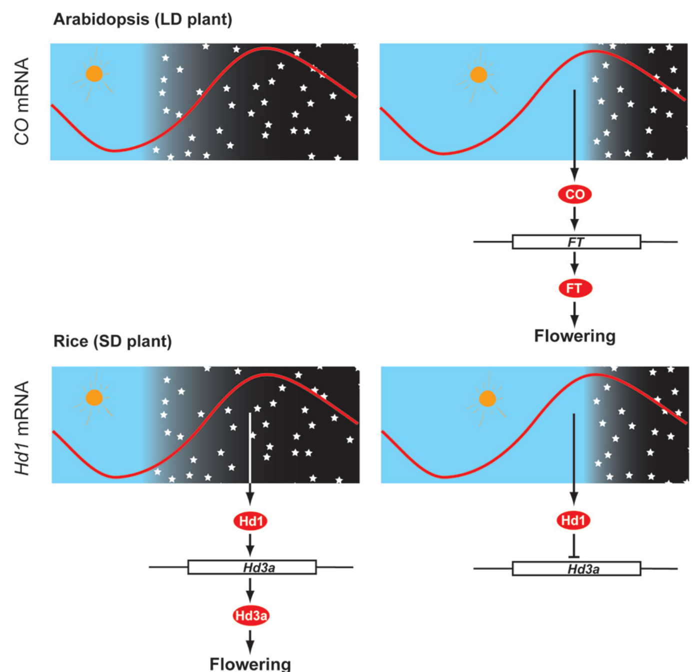

Fig. 4. Model for photoperiod flowering induction in LD and SD plants. *In Arabidopsis* CO *shows circadian regulation of expression, accumulating at midnight in SD and at dusk in LD; the rice* CO *homologue,* Hd1 *is expressed in a similar fashion. In Arabidopsis under SD conditions, CO high midnight levels do not activate* FT *expression whereas high levels of Hd1 activate the expression of the rice* FT *homologue* Hd3a, *which promotes flowering. Finally in Arabidopsis the coincidence of high CO levels with the light period (under LD) activates* FT *expression which promotes flowering. In rice, high levels of* Hd1 *in the light period suppresses* Hd3a *expression inhibiting flowering.*

(Hanumappa *et al.*, 1999), confirming the relevance of protein stability in flowering responses to light.

III. Temperature effects on floral transition

Light quality and day length information are not enough for the plant to ensure its developmental adjustment to environment and, for many species, temperature constitutes a major predictable environmental factor regulating flowering time. As light, temperature also shows diurnal and seasonal variation along the year and this variation not only conditions plant growth rate but regulates the timing of many developmental transitions such as germination, bud dormancy and bursting, or flowering initiation (Henderson *et al.*, 2003). Regarding flowering time, both ambient temperature and long time exposure to low winter temperatures have been shown to have an important effect on flowering time (Henderson *et al.*, 2003). In many species from temperate climates a requirement of low winter temperatures ensures spring flowering. This prolonged chilling treatment required to promote or accelerate flowering is known as vernalization (Chouard, 1960). Vernalization effects on flowering are quantitative, so longer cold exposures promotes flowering more than shorter ones till the response is saturated. Furthermore, after vernalization, plants do not necessarily initiate flowering but acquire the competence to do so (Chouard, 1960).

Temperature is perceived in the entire plant body (Lang, 1965). It has been demonstrated that plant cells react to cold-shock by an immediate rise of free cytoplasmic calcium (Knight *et al.*, 1991), sensing cooling rates rather than absolute temperature (Plieth *et al.*, 1999). Furthermore the rising of free cytoplasmic calcium is attenuated by repeated short stimuli. This feature implies that this first signal is only adequate for short-term responses. However several cold-shock responses, such as the acquisition of freezing tolerance or the vernalization response, require long exposure periods (several days to acquire freezing tolerance and several weeks or months to acquire a vernalized state). Thus, medium and long-term responses would require a more lasting mechanism of low temperature sensing that so far is unknown.

Regarding vernalization, although all plant cells can sense and react to low temperatures, studies involving grafting and localized chilling treatments have shown that only cold exposure perceived at the meristems can generate a vernalization response, implying a requirement for active cell proliferation (Lang, 1965; Wellensiek 1962). Once meristems have been exposed to prolonged chilling temperatures, they "remember" this exposure by acquiring a vernalized state that is stably maintained through mitosis, suggesting the existence of epigenetic mechanisms to generate this memory. For instance, explants regenerated from cold exposed tissues of *Lunaria biennis* will only flower when they originate from tissues containing dividing cells (Wellensiek, 1964). Similarly, when vernalized apices from the biennial plant *Hyoscyamus niger* were grafted to a non-vernalized plant, they maintained their vernalized state and were able to flower when plants were exposed to inductive photoperiodic conditions. In contrast, non-vernalized apices grafted to a vernalized plant were not able to initiate flowering under the correct photoperiods (Lang, 1965). The molecular mechanisms by which plants are able to remember past exposures to low temperatures are starting to be uncovered in different plant species.

Molecular basis of the vernalization requirement

Many species include spring or summer-annual genotypes, which flower rapidly without a vernalization requirement and genotypes requiring exposure to low winter temperatures to flower in the spring, which are known as winter annual or biennials. This vernalization requirement can be more or less absolute generating true biennial types or late flowering types with a positive vernalization response. A wide variation for vernalization requirement is found within *Arabidopsis*, where it is distinguished between summer-annual strains (most laboratory strains like Columbia and L*er* are summer-annuals) and winter-annual strains. Summer-annual strains germinate in early spring to flower and set fruit in summer and then spend the cold part of the year as dormant seeds. In contrast, winter-annuals accessions germinate in fall, stand the winter in a vegetative state and initiate their reproductive phase in the spring. Genetic analyses in *Arabidopsis* has shown that the winter-annual habit in many *Arabidopsis* accessions requires dominant alleles at two loci, *FLOWERING LOCUS C* (*FLC*) and *FRIGIDA* (*FRI*) (Lee *et al.*, 1993; Clarke and Dean, 1994; Koornneef, *et al.*, 1994). *FLC* encodes a MADS-box transcription factor which functions as a flowering repressor (Michaels and Amasino, 1999; Sheldon *et al.*, 2000) whose expression level negatively correlates with flowering time. Dominant alleles at *FRI* enhance the expression of *FLC* whereas vernalization overcomes the effect of *FRI* and represses *FLC* expression proportionally to the duration of the cold treatment. This 'vernalized' estate is mitotically stable when plants return to warmer growing conditions during the rest of their life (Michaels and Amasino, 1999; Sheldon *et al.*, 2000). Genetic analyses have shown that *FLC* represses flowering by repressing the transcriptional expression of the flowering signal integrators *FT* and *SOC1* (Onouchi *et al.*, 2000). For *SOC1*, this *FLC* mediated repression has been shown to require a MADS-box binding site in its promoter region (Hepworth *et al.*, 2002). Naturally occurring weak alleles of *FLC* are altered in their transcriptional regulation, being *FLC* first intron a key region in the regulation of its expression (Sheldon *et al.*, 2002). A well known low expression allele, the *FLC*-L*er*, results from the insertion of a transposable element within this first *FLC* intron (Michaels *et al.*, 2003).

Although *FLC* is the major gene responsible for the generation of the vernalization requirement, *flc-3* null mutants still maintain certain response to vernalization, specially under SD conditions, (Michaels and Amasino, 2001). This indicates that other genes also contribute to the vernalization requirement. In fact, five additional *FLC* homologous genes have been found in the *Arabidopsis* genome and named as *MADS AFFECTING FLOWERING* (*MAF1-5*) (Ratcliffe *et al.*, 2001; Ratcliffe *et al.*, 2003). When these genes are over expressed in transgenic plants, all but *MAF5* produce a delay of flowering similar to *FLC* overexpression (Ratcliffe *et al.*, 2001; Ratcliffe *et al.*, 2003), suggesting that they could also function as flowering repressors (Scortecci *et al.*, 2001; Ratcliffe *et al.*, 2003). In contrast with the other *MAF* genes, expression of *MAF5* increases with vernalization treatment suggesting an opposite role to *FLC* on the vernalization response

(Ratcliffe *et al.*, 2003). In addition, *MAF2* shows reduction in its expression only under very prolonged vernalization (Scortecci *et al.*, 2001; Ratcliffe *et al.*, 2003), suggesting that it could generate a requirement for long vernalization periods.

The correlation between the level of *FLC* expression and flowering time and the drastic effect of vernalization in the suppression of *FLC* expression indicate that *FLC* is a key point in the regulation of flowering time by vernalization. Furthermore, multiple mutant analyses of flowering time have identified genes involved in the positive or negative regulation of *FLC*. In the next sections we will describe the information available on the positive and negative regulation of *FLC* expression.

Positive regulation of FLC

Natural functional alleles of *FRIGIDA* are major enhancers of *FLC* expression, since their late flowering effects are suppressed by lesions in *FLC* (Michaels and Amasino, 1999). *FRI*, encodes a protein of unknown molecular function (Johanson *et al.*, 2000) and many of the recessive *fri* alleles present in summer-annual *Arabidopsis* strains correspond to loss of function alleles involving protein structure alteration (Johanson *et al.*, 2000). There are six *FRI* homologues in the *Arabidopsis* genome, one of them, *FRIGIDA LIKE1* (*FRL1*) being required for *FRI*-mediated activation of *FLC* and vice versa (Michaels *et al.*, 2004). Interestingly, another *FRI* homologue has a histone binding domain, suggesting that *FRI*-like genes might activate *FLC* expression through chromatin modification.

Several screens for mutations that convert winter-annual, *FRI*-containing lines, into early flowering plants, have led to the identification of additional positive regulators of *FLC* expression (Zhang and Van Nocker, 2002; Zhang *et al.*, 2003; Noh and Amasino, 2003; He *et al.*, 2004a). Many of these genes have been molecularly characterized and found to encode *Arabidopsis* homologues of subunits of the yeast PAF1 complex. This complex is involved in the establishment and maintenance of transcription promotive chromatin modifications and is thought to recruits SET1 (a H3-K4 methyltransferase) to the 5' end of target genes (Krogan *et al.*, 2003; Ng *et al.*, 2003). Methylation of lysine 4 in histone 3 is a tag of actively transcribed genes in yeast (Santos–Rosa *et al.*, 2002). The yeast PAF1 complex consists of PAF1, CTR9, LEO1, CDC73 and RTF1 proteins. In *Arabidopsis*, genes like *VERNALIZATION INDEPENDENCE4 (VIP4), VIP6/EARLY FLOWERING8* (*ELF8*) and *VIP5* encode the homologues of LEO1 (Zhang *et al.*, 2003), RTF1 and CTR9, respectively (Oh *et al.*, 2004; He *et al.*, 2004a). Another member of this complex, *EARLY FLOWERING7* (*ELF7*) encodes the homologue of yeast PAF1, the RNA polymerase II associated factor. Mutations in these genes suppress *FLC* expression not only in winter annuals but also in autonomous-pathway late flowering mutants (see below) indicating that the *Arabidopsis* PAF1 complex is generally required to achieve high levels of *FLC* expression (He *et al.*, 2004a). The high expression level of *FLC* in winter annuals and autonomous late flowering mutants is related to an increase in H3-K4 trimethylation in *FLC* chromatin around the transcription start site, the first exon and the 5' portion of the first intron (He *et al.*, 2004a). *MAF* genes are also targets of the PAF1 complex and expression of *FLM* and *MAF2* is reduced in *vip5, vip6/elf8 and elf7* mutants (Oh *et al.*, 2004; He *et al.*, 2004a).

The *Arabidopsis* homologue of SET1, the yeast H3-K4 methyltransferase, could be encoded by *EARLY FLOWERING IN SHORT DAYS* (*EFS*). This gene, initially identified by the mutants early flowering phenotype under short days (Soppe *et al.*, 1999) encodes a SET domain protein, whose mutation cause suppression of FRI-mediated late flowering and reduction of trimethylation levels of *FLC* chromatin and of *FLC* expression (He and Amasino, 2005). Finally, mutants at the *PHOTOPERIOD INDEPENDENT EARLY FLOWERING1* (*PIE1*) locus also display a similar early flowering phenotype under short days as *efs*, *elf7* or *elf8* and is required for *FLC* expression (Noh and Amasino, 2003). PIE is the *Arabidopsis* homologue of ISW1p, a yeast ATP-hydrolyzing chromatin-remodeling protein that preferentially binds di- and trimethylated H3-K4 and it could be binding methylated H3-K4 and remodeling chromatin of *FLC* and its *MAF* relatives to enhance their expression (He and Amasino, 2005) (Figure 5).

Negative regulation of FLC

Summer-annual *Arabidopsis* genotypes flower rapidly because often carry putative null *fri* alleles and *FLC* is also repressed by the autonomous pathway (Michaels and Amasino, 1999). This pathway is not linear but genes can be grouped in two different epistatic groups defined by genetic analyses (Koornneef *et al.*, 1991), the *FY* (Simpson *et al.*, 2003) and *FCA* (Macknight *et al.,* 1997) group and the *FPA* (Schomburg *et al.,* 2000) and *FVE* (Ausin *et al.,* 2004) one. Three other loci, *LUMINIDEPENDENS* (*LD*) (Lee *et al.,* 1994), *FLOWERING LOCUS D* (*FLD*) (He *et al.*,2003) and *FLOWERING LOCUS K* (*FLK*) (Lim *et al.,* 2004, Mockler *et al.*, 2004) have not been included in any of these groups.

FVE and *FLD* encode proteins with similarity to components of the mammalian histone deacetylase complex (HDAC). *FVE* encodes a retinoblastoma associated protein (RbAp) homologue (Ausin *et al.*, 2004). These proteins function as histone chaperons and have been found in different chromatin interacting complexes (Quian and Lee, 1995; Tyler *et al.*,1996 Ridgway y Almouzni, 2000; Rossi *et al.*, 2003). Interestingly, *Arabidopsis fve* mutants show increased levels of H3 and H4 acetylation at *FLC* chromatin suggesting a role of FVE in HDAC complexes (Ausin *et al.*, 2004). *FLD* encodes a homologue of the human KIAA0610: which is another component of mammalian HDACs (He *et al.*, 2003). In fact, *fld* mutants also show increased levels of histone acetylation in *FLC* chromatin (He *et al.*, 2003), together with higher H3-K4 methylation (He *et al.*, 2004a). Thus, both FVE and FLD repress *FLC* expression by a mechanism of histone deacetylation, although it is not known whether they participate in the same type of HDAC complex (Figure 5). Mutations at the *FCA* and *FY* genes do not alter *FLC* histone acetylation and these genes could regulate *FLC* expression through a different mechanism (He *et al.*, 2003; Ausin *et al.*, 2004). The molecular structure of FCA and FY suggest that they could regulate *FLC* expression at post–transcriptional levels. Indeed, *FCA* encodes a protein with two RNA binding domains and a WW protein interaction motif (Macknight *et al.*, 1997), whereas *FY* protein shares similarity to components of RNA 3' end processing complexes of yeast (Simpson *et al.*, 2003). In addition, it has been shown that both proteins interact physically *in vitro* and this interaction is required for the correct processing of *FCA* mRNA and for the regulation of *FLC* expression (Quesada *et al.*, 2003) (Figure 5). Whether *FLC*

pre-mRNA is targeted by the FCA/FY complex is not known yet. Other genes in the pathway like *FLK* and *FPA* encode putative RNA-binding proteins (Schomburg *et al.*, 2001; Lim *et al.*, 2004; Mockler *et al.*, 2004), whereas *LD* encodes a homeodomain protein (Lee *et al.*, 1994). Their molecular function in the repression of *FLC* expression remains unknown.

Molecular mechanism of vernalization

The mitotic stability of the vernalized state is characteristic of an epigenetic regulation. Epigenetic silencing of genes is mediated by covalent modifications of chromatin elements, including DNA methylation and histone deacetylation and methylation (Bird, 2002). Although, the study of epigenetic mechanisms of *FLC* regulation was initially focused on the role of DNA methylation, the effects of DNA demethylation on flowering time are contradictory (Ronemus *et al.*, 1996; Finnegan *et al.*, 1996) and independent of the vernalization effect (Genger *et al.*, 2003). Recently, the role of histone modification in this process has been demonstrated since vernalization increases methylation of H3K9 and H3K27 in specific regions within the *FLC* locus (Bastow *et al.*, 2004). In animals, such histone modifications are characteristic of silenced chromatin states because they promote the recruitment of HETEROCHROMATIN PROTEIN1 (HP1) and the formation of stable heterochromatin (Kuzmichev *et al.*, 2002). Thus, these modifications would convert active *FLC* chromatin into a heterochromatin-like state (Bastow *et al.*, 2004; Sung and Amasino 2004) (Figure 5). A similar suppression of *FLC* expression has also been proposed to take place in the case of the natural partially silenced *FLC* allele. In this case, the presence of a DNA methylated transposable element in the *FLC* intron 1 causes localized H3K9 histone methylation in the surrounding *FLC* chromatin, which correlates with reduction of its mRNA expression (Liu *et al.*, 2004).

To identify the genetic components of the vernalization pathway, several mutant screenings where carried out in genetic backgrounds with elevated *FLC* expression. Thus, mutants that specifically fail to repress *FLC* expression and lack the early flowering phenotype caused by vernalization treatments were isolated (Chandler *et al.*, 1996; Sung and Amasino, 2004). Mutants at the *VERNALIZATION INDEPENDENT3* (*VIN3*) gene are affected in the establishment of the vernalized estate of *FLC* since *FLC* mRNA levels were higher in *vin3* than in wild type plants after a vernalization treatment. Interestingly, *vin3* mutants do not display any of the histone modifications observed in *FLC* chromatin after vernalization, suggesting that VIN3 is involved in a crucial early step for those modifications (Sung and Amasino, 2004). *VIN3* encodes a PHD-finger containing protein and its expression increases proportionally with the length of cold treatment, suggesting that it could be involved in the measurement of cold treatment (Sung and Amasino, 2004). How *VIN3* is regulated by long-term cold exposure or factors functioning upstream of *VIN3* remains unknown, but it would be of great interest to identify such factor/s that perceive long-term cold exposure signals and trigger the vernalization response.

Mutants in the *VERNALIZATION1* (*VRN1*) and *VERNALIZATION2* (*VRN2*) genes are blocked in their vernalization response because they are unable to maintain the vernalized state (Levy *et al.*, 2002; Bastow *et al.*, 2004). *FLC* expression in these mutants is strongly reduced at the end of the vernalization treatment but increases again several days after transferring the vernalized plants to warm growing conditions (Bastow *et al.*, 2004). In these mutants vernalization induced downregulation of *FLC* correlates with histone H3 deacetylation of *FLC* chromatin. However such deacetylation is not maintained when plants are transferred to warm growing conditions. In addition, *vrn1* mutants show H3 methylation at K27 but not at K9 whereas *vrn2* H3 do not show methylation at any of those lysines (Bastow *et al.*, 2004; Sung and Amasino, 2004). Together, these results suggest that VIN3 could function in the establishment of the vernalized estate of *FLC* by histone H3 deacetylation, whereas VRN1 and VRN2 would be required to maintain this vernalized estate through methylation of H3K9 (VRN2) and H3K27 (Figure 5). In *Arabidopsis*, histone methylation can lead to DNA methylation and subsequent gene silencing (Jackson *et al.*, 2002), so a tantalizing idea is that *FLC* could be heavily methylated during the vernalization process. However differences in the *FLC* DNA methylation between vernalized and non-vernalized plants have never been found (Finnegan *et al.*, 2004).

Vernalization mechanisms in other plant species

Similarly to what has been described for *Arabidopsis*, *FLC* orthologues seem to be flowering repressors in *Brassica* species (Sheldon *et al.*, 2000; Schranz *et al.*, 2002) and the main effects of vernalization on flowering time are also mediated through downregulation of *FLC* (Sheldon *et al.*, 2000). *FLC* orthologues have not been found in the genome of rice, although this species does not show a vernalization requirement. In wheat, barley and other temperate grasses, the two major loci generating the vernalization requirement, *VRN1* and *VRN2* (not related to the *Arabidopsis VRN1* and *VRN2*) show epistatic interactions and seem to be part of the same regulatory pathway (Tranquilli *et al.*, 2000). Dominant alleles at *VRN1* are responsible for spring growth habit while recessive alleles at this locus generate a winter habit and vernalization requirement. In contrast, functional alleles at *VRN2* generate a winter growth habit. Recently, the corresponding wheat genes have been isolated and none of them encodes an *FLC* homologue. *VRN1* encodes an *Arabidopsis APETALA1* (*AP1*) homologue that, contrary to the role of *FLC*, functions as a floral inducer rather than repressor. Vernalization treatments are required to increase the levels of *VRN1* expression (Yan *et al.*, 2003). *VRN2* encodes a CCT domain and a zinc–finger containing protein termed ZCCT1. A homologous gene is absent in *Arabidopsis* and ZCCT1 appears to be specific of grass species. Analogously to FLC, VRN2 functions as a flowering repressor and its mRNA expression decreases during cold exposure. Genetic variation at *VRN1* and *VRN2* could explain the growth habit of most cultivated spring wheat accessions (Yan *et al.*, 2004). Nothing is known yet about the molecular mechanisms responsible for the generation and maintenance of the vernalized state in grasses. However, the similarities of the response with *Arabidopsis* suggest the involvement of epigenetic mechanisms.

In contrast to the observed conservation of the mechanisms for the photoperiod regulation of flowering across different plant taxa such as monocots and dicots, current evidence on the vernalization mechanisms suggest the recruitment of distinct gene sets for generation of vernalization requirements in different plant families. Temperate grasses have their origin in subtropical grasses which likely had no vernalization requirement. Thus *Arabidopsis*

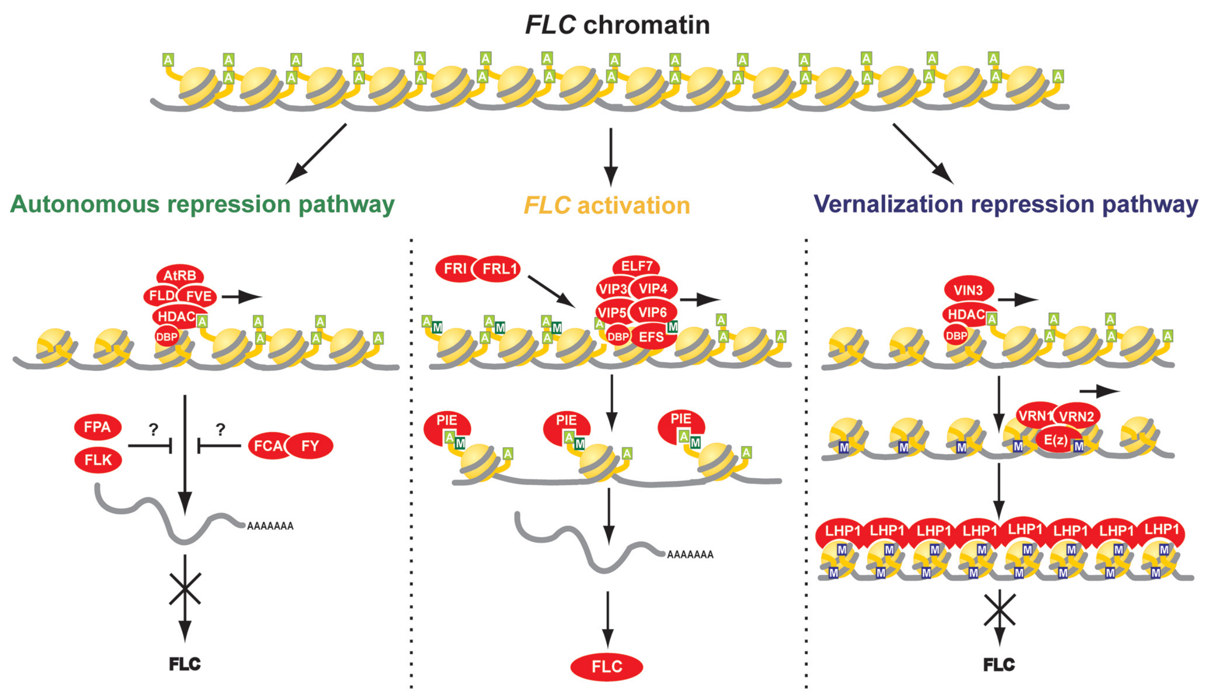

Fig. 5. A molecular model for the regulation of *FLC* expression. *Left panel schematically shows possible mechanisms of regulation carried out by the autonomous pathway. FVE and FLD could repress FLC expression by deacetylating histones in specific regions of* FLC *chromatin. These proteins could be part of a complex involving a histone deacetylase, a DNA binding protein (DBP) and a retinoblastoma homologous protein. The complex FCA-FY, FPA and FLK could act directly or indirectly at the post-transcriptional level repressing* FLC *expression. Central panel shows the possible mechanism for* FLC *activation in winter annual strains and late flowering mutants. A putative FRI-FRL1 complex activates* FLC *expression by an unknown mechanism, while ELF7, VIP3, VIP4, VIP5 and VIP6 could be part of the same complex activating* FLC *through H3-K4 methylation which could be performed by the SET domain containing protein EFS. After H3-K4 methylation the yeast ISWI homologue PIE, would recognize methylated H3-K4 and facilitate* FLC *chromatin accessibility. The right panel shows the possible mechanism of* FLC *repression carried out by the vernalization pathway. First VIN3 together with a HDAC and a DNA recognition protein will deacetylate specific regions of* FLC *chromatin. Thereafter a protein complex including VRN1, VRN2 and a metyltransferase activity (E[z] type) might cause H3-K9 and H3-K27 methylation. This chromatin conformation could be recognized by LIKE HETEROCHROMATIN PROTEIN1 (LHP1) to stably repress* FLC *expression. Light green A box depicts acetyl groups, dark green M box depicts methyl groups in H3-K4 position and blue M box represent methyl groups in H3-K9 or H3-K27 position*

and grasses have independently developed distinct vernalization pathways recruiting different genes for analogous function *(FLC/ZCCT)*, or homologous genes for different functions *(AP1/VRN1)*.

Effect of ambient temperature

Ambient temperature significantly influences the flowering time of plants. Wild type *Arabidopsis* strains flower earlier at 23ºC than at 16ºC (Blázquez *et al.*, 2003). Whether this is a general effect related to the temperature sensitivity of specific chemical reactions or there is a specific pathway to sense and react to ambient temperature in plants is still unknown. Interestingly, the flowering repression caused by low ambient temperatures seems to be independent of the repression produced by several photo-receptors in *Arabidopsis* (Halliday *et al.*, 2003). In this way the early flowering phenotype of *phyB* mutants under standard temperatures is suppressed at 16ºC, suggesting the existence of a flowering repression mechanism not affected by *phyB* mutations (Halliday *et al.*, 2003). This mechanism also blocks the function of phytochromes PHYA and PHYD since *phyA phyB phyD* triple mutants that are earlier than *phyB* mutants at 22º C do not show phenotypic differences when compared at 16ºC (Halliday *et al.*, 2003). At this temperature, only *PhyE* seems to have a non redundant function because quadruple mutants *phyA phyB phyD phyE* flower earlier than *phyA phyB phyD* triple mutants (Halliday *et al.*, 2003). The low temperature suppression of PHYA function could also explain the extremely temperature sensitive phenotype displayed by *fha-1* mutants (an allele of *CRY2*). At 23ºC *fha-1* mutants are late flowering but they show an extreme delay in flowering time at 16ºC (Blázquez *et al.*, 2003). Since PHYA and CRY2 function redundantly at 23ºC to promote flowering, *fha-1/cry2* mutants are only slightly delayed. However, at 16ºC, the lack of function of PHYA, makes *fha-1* mutants to show a phenotype as extreme as the double *phyA,fha-1* mutants (Blazquez *et al.*, 2003). The effect of ambient temperature on flowering time, like the effects of photoreceptor mutations, are mediated at the level of transcriptional regulation of *FT*, since there is a correlation between *FT* mRNA levels and flowering time in all phytochrome single and multiple mutant combinations at 16ºC or 23ºC. These effects were not observed on the expression of other floral integrators such as *SOC1* or *FLC* (Halliday *et al.*, 2003). Although phytochrome sensitivity to ambient temperature has been suggested as responsible for the ambient temperature effects on phytochrome mutants the results can also be explained by a temperature dependent repression mechanism preventing

the effects caused by phytochrome mutations.

The effect of ambient temperature on flowering time has also been analyzed in late flowering mutants of *Arabidopsis*. Most mutants show a similar behavior as wild type plants with low temperatures causing an additional delay in their flowering time. However *fve* and *fca* mutants of the autonomous pathway show little difference in flowering time at 16ºC or 23ºC. Based on these results, FVE and FCA have been proposed to function in a pathway that mediates ambient temperature response (Blázquez *et al.*, 2003). It is possible that the effect of *fve* and *fca* mutations on the response to ambient temperature could be mediated by *FLC,* a clear target of these genes. Consistent with this hypothesis *FLC* shows increased mRNA levels with moderate temperature decreases (Blázquez *et al.*, 2003). However, these observed increases were rather small and might be irrelevant in mutant backgrounds such as *fve* and *fca* with constitutive high *FLC* levels. Moreover, *fve* mutations also block the delay in flowering time caused by intermittent cold treatments (2-5 h at 4ºC) that seems to be mediated by *FLC* (Kim *et al.*, 2004). FLC represses *FT* expression and could suppress any temperature effect of the light quality pathway on the expression of this flowering signals integrator (Figure 1). The fact that *flc* null mutants still show a flowering time delayed when exposed to low ambient temperatures (Blázquez *et al.*, 2003) indicates that other genes besides *FLC* could participate in this response, other *MAF* genes being good candidates.

Molecular mechanisms in the temperature regulation of flowering time

Flowering responses to low temperatures can take place in the short and long terms and transcriptional regulation of *FLC* could be involved in both of them. In the short term, *FLC* transcript levels increase as a response to intermittent exposures to low temperatures (Kim *et al.*, 2004) correlating with a flowering delay. This role of *FLC* in short term temperature responses would delay flowering in cold spring and could explain why all summer annual accessions caused by mutations at *FLC* carry partially but not fully silenced alleles. Furthermore, the suppression of these temperature effects by *fve* mutations suggests that they are also mediated by histone acetylation changes in *FLC* chromatin. In long term temperature responses, histone methylation seems to be the mechanism to generate active or inactive *FLC* chromatin states and similar regulatory mechanisms on other *MAF* genes, such as *MAF2,* could contribute to create long term responses to longer low temperature exposures. Similar mechanisms could be the basis of vernalization response in other plant species as well as of other long term plant responses to environment. For sessile organisms like plants, epigenetic mechanisms provide an efficient tool to adapt the genotype to specific long term seasonal changes in environmental conditions.

Participation of *FLC* in short term temperature responses has only been investigated in summer annual strains and it could be interesting to confirm this role in winter annuals. In these strains, the *FLC* states of active expression derived from H3-K4 trimethylation or suppression as a result of vernalization and H3-K9 and H3-K27 methylation could hardly allow additional fluctuations in its expression. However, even in vernalized winter annual, there is a basal level of *FLC* that could be involved in short term low temperatures responses, either because a basal expression is maintained in all cells or because the efficiency of transcriptional suppression is depending on cell type. In this way, vernalized winter annuals could still use *FLC* to integrate short term low temperature responses during the spring. Additionally, other elements and other molecular mechanisms could also contribute to these short term flowering responses to temperature.

IV. Other environmental factors affecting floral transition

Flowering time is also affected by many other environmental factors such as mineral nutrition and different biotic and abiotic stresses, although the regulatory pathways involved are poorly understood. In general, *Arabidopsis* plants grown under low mineral nutrition tend to flower later (Pigliucci *et al.*, 1995). Consistently with this observation, mutations at *CHL1*, likely affecting nitrate uptake from the soil and import into nascent organs (including floral organs), cause late flowering (Guo *et al.*, 2001).

Plants exposed to unfavorable environmental conditions can trigger flowering prematurely as a response to obtain reproductive success. Different types of stress promote the synthesis of secondary messengers such as salicylic acid (SA) and nitric oxide (NO) involved in defense responses. In *Arabidopsis* SA is present at very low basal concentrations but greatly increases with pathogen infection and UV-C irradiation. UV-C irradiation causes an acceleration of flowering time that is SA-dependent and correlates with an increase of *FT* expression (Martínez *et al.*, 2004). Consistently with a role of SA as an elicitor of the flowering response, *sid1* and *sid2* mutants (unable to synthesize SA) and transgenic nahG plants (unable to accumulate SA) flower later than their respective wild type strains under any photoperiod tested (Martínez *et al.*, 2004). Late flowering of SA mutants correlates with increased levels of *FLC* and decreased levels of *FT and SOC1*, independently of photoperiod (Martínez *et al.*, 2004). NO is also produced as a response to biotic and abiotic stimuli such as salt stress and pathogen infection (Lamattina *et al.*, 2003). Exogenous NO treatments delay flowering and this delay correlates with elevated levels of *FLC* expression and a decrease in *CO* expression. Consistent with those observations, *nox1*, a mutant that overproduces NO, displays a late flowering phenotype and *nos1*, a mutant with reduced levels of NO, is early flowering. The late flowering phenotype of *nox1* mutant seems to be caused by elevated levels of *FLC* and a decrease in *CO,* mainly during the night. Moreover, *nos1* early flowering correlates with reduced *FLC* transcript levels and elevated *CO* expression (He *et al.*, 2004b).

Water imposes a strong selective pressure and influences almost all aspects of plant biology. Both water deficiency (drought) and water excess (flooding) cause stress to plants that in the case of flooding is due to anoxic conditions in the root. In *Arabidopsis*, different drought and flooding treatments do not seem to cause a significant effect on flowering time in different wild genotypes (Pigliucci *et al.*, 1995; Pigliucci and Kolodynska, 2002). However, *Arabidopsis* displays two genetic strategies to avoid water stress that seem correlated with flowering time: dehydration avoidance and drought escape (flowering acceleration). Thus, ecotypes flowering later tend to have a better water use efficiency. Interestingly, introgression lines of *FRI* and /or *FLC* in L*er* background,

apart from their lateness, showed an increased water use efficiency compared to L*er* suggesting pleiotropic effects for the *FRI* and *FLC* active alleles (McKay *et al.*, 2003).

Finally, even continuous or repetitive touch or wind provokes changes in plant development. These mechanically-induced developmental changes are known as thigmomorphogenesis (Ennos, 1997). Effects on flowering time are part of the responses to wind and *Arabidopsis* plants exposed to daily wind treatments flower later than untreated controls (Johnson *et al.*, 1998). Whether these effects function through the already described regulatory pathways or not, remains to be shown.

V. Conclusions and future prospects

The genetic analysis of flowering time in *Arabidopsis* has been very efficient in the identification of the genes involved in the regulation of this complex developmental process. Research is now focused on the understanding of the molecular mechanisms involved. Judging from the complexity observed within the different environmental signaling mechanisms, the different scale plant responses to distinct signals as well as the variation in the regulatory responses uncovered in different plant species we are far from understanding the process at the molecular level. In spite of this, common elements as well as specific ones can be observed for different aspects of the flowering response.

In this way, different flowering regulatory pathways seem to share several interaction domains not only at the level of integrator genes, but at the levels of signal perception as well as circadian regulation. Several types of photoreceptors participate in different pathways affecting flowering time such as the photoperiod pathway, the light quality pathway or the GA pathway. Furthermore, the circadian clock is not only entrained by light signals but also by temperature signals and some components of a putative entraining mechanism have been recently identified (Salome and McClung, 2005). More complex experimental designs are required to understand the specific function of every molecular component in these interactive networks and new models need to be developed that incorporate spatial information, since different interactions can take place in different cell types.

Plant flowering responses in the short term and long term ranges share transcriptional regulation as a basic molecular response mechanism. However, in short term responses, transcriptional regulation is found frequently coupled with post-transcriptional regulation of protein stability to generate rapid changes of protein abundance and activity. Mechanisms based in the regulation of protein stability could be widely used in plants to respond to rapid changing environment conditions. Indeed, approximately 5% of the *Arabidopsis* genes encode proteins predicted to be involved in the ubiquitine-proteasome system and regulation of protein degradation by ubiquitination is relevant in many plant processes (Hellmann and Estelle, 2002). For long-term responses such as the generation of the vernalization requirement or the vernalized state, *Arabidopsis* uses epigenetic modifications of chromatin similar to those described in mammals and *Drosophila*. These mechanisms confer mitotically heritable changes that are reversed at meiosis and are crucial for the environmental adaptation of the plant plastic development. Interestingly, many of these plants chromatin modifications are environmentally regulated providing a mechanism for long term modulation of plant development by the environment. In this context, one relevant question is whether plants use more intensively particular mechanisms for the environmental regulation of gene expression or they have developed new regulatory mechanisms in some cases. In addition, the role of different mechanisms of post-translational protein modification is still to be described in the regulation of short term flowering responses and they could be more intensively used in plants than in mobile organisms.

Finally, comparison of flowering regulatory mechanisms in different species points out the conservation of the molecular mechanisms regulating flowering responses to environmental conditions in different plant taxa. The extent of conservation will likely depend on the distribution of the environmental factor as well as the evolutionary history of the taxa considered. In this way, circadian regulation as well as responses to photoperiod are expected to use conserved mechanisms in quite divergent plant taxa. Indeed, this is the case for the central photoperiod pathway of flowering time regulation, which seems to be conserved among monocot and dicots suggesting that its origin predated the divergence of those major groups of angiosperms and it could be widely distributed in different plant families. However, different species still show specific features to discern the light preference and specific pathways such as that mediated by *Hde1* in rice (Doi *et al.*, 2004). On the other side, when adaptation to a given environmental factor takes place independently in different taxa, it might be expected that different genes and mechanisms could have been recruited. This seems to be the case for the vernalization response, given the differences found between monocot and dicot species, which could have independently evolved different strategies to sense and respond to seasonal temperature variation. Apart from these basic questions on how photoperiodic or temperature flowering responses have been generated in different plant taxa, many specific questions on specific adaptations to particular flowering stimuli remain to be answered. Fortunately, the wealth of information generated in a number of plant model species and the strength of high throuput technologies now provide the tools to address those questions.

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References

ADAMS, J., KELSO, R. and COOLEY, L. (2000). The kelch repeat superfamily of proteins: propellers of cell function. *Trends Cell. Biol.* 10: 17-24.

AHMAD, M. and CASHMORE, A.R. (1993). HY4 gene of *Arabidopsis thaliana* encodes a protein with characteristics of a blue-light photoreceptor. *Nature.* 366: 162-166.

AHMAD, M., JARILLO, J.A., SMIRNOVA, O. and CASHMORE, A.R. (1998). The CRY1 blue light photoreceptor of *Arabidopsis* interacts with phytochrome A in vitro. *Mol. Cell.* 1: 939-948.

ALABADI, D., OYAMA, T., YANOVSKY, M.J., HARMON, F.G., MAS, P. and KAY, S.A. (2001). Reciprocal regulation between TOC1 and LHY/CCA1 within the *Arabidopsis* circadian clock. *Science.* 293: 880-883.

AUKERMAN, M.J., HIRSCHFELD, M., WESTER, L., WEAVER, M., CLACK, T., AMASINO, R.M. and SHARROCK, R.A. (1997). A deletion in the PHYD gene of the *Arabidopsis* Wassilewskija ecotype defines a role for phytochrome D in red/

far-red light sensing. *Plant Cell.* 9: 1317-1326.

AUSIN, I., ALONSO-BLANCO, C., JARILLO, J.A., RUIZ-GARCIA, L. and MARTINEZ-ZAPATER, J.M. (2004). Regulation of flowering time by FVE, a retinoblastoma-associated protein. *Nat. Genet.* 36: 162-166

BALLARE C.L. (1999). Keeping up with the neighbours: phytochrome sensing and other signalling mechanisms. *Trends Plant Sci.* 4: 201.

BASTOW, R., MYLNE, J.S., LISTER, C., LIPPMAN, Z., MARTIENSSEN, R.A. and DEAN, C. (2004). Vernalization requires epigenetic silencing of FLC by histone methylation. *Nature.* 427: 164-167.

BAUER, D., VICZIAN, A., KIRCHER, S., NOBIS, T., NITSCHKE, R., KUNKEL, T., PANIGRAHI, K.C., ADAM, E., FEJES, E., SCHAFER, E. and NAGY, F. (2004). Constitutive photomorphogenesis 1 and multiple photoreceptors control degradation of phytochrome interacting factor 3: a transcription factor required for light signaling in *Arabidopsis*. *Plant Cell.* 16: 1433-1445

BERNIER, G. and PÉRILLEUX, C. (2005) A physiological overview of the genetics of flowering time control. *Plant Biotech. J.* 31: 3-16

BIRD, A. (2002). DNA methylation and epigenetic memory. *Genes Dev.* 16: 6-21.

BLAZQUEZ, M.A. and WEIGEL, D. (2000). Integration of floral inductive signals in *Arabidopsis*. *Nature.* 404: 889-892.

BLAZQUEZ, M.A., TRENOR, M. and WEIGEL, D. (2002). Independent control of gibberellin biosynthesis and flowering time by the circadian clock in *Arabidopsis*. *Plant Physiol.* 130: 1770-1775.

BLAZQUEZ, M.A., AHN, J.H. and WEIGEL, D. (2003). A thermosensory pathway controlling flowering time in *Arabidopsis thaliana*. *Nat. Genet.* 33: 168-171.

BOSS, P.K., BASTOW, R.M., MYLNE, J.S. and DEAN, C. (2004). Multiple pathways in the decision to flower: enabling, promoting and resetting. *Plant Cell.*16: S18-31.

BÜNNING, E. (1936) Die endogene Tagesrhthmik als Grundlage der photoperiodischen Reaktion. *Ber. Dtsch. Bot. Ges.* 54: 590-607

CERDAN, P.D. and CHORY, J. (2003). Regulation of flowering time by light quality. *Nature.* 423: 881-885.

CHANDLER, J., WILSON, A. and DEAN, C. (1996). *Arabidopsis* mutants showing an altered response to vernalization. *Plant J.* 10: 637-644.

CHOUARD, P. (1960) Vernalization and its relations to dormancy. *Annu. Rev. Plant Physiol.* 11:191-238.

CLARKE, J.H., Y DEAN, C. (1994). Mapping *FRI*, a locus controlling flowering time and vernalization response in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 242: 81-8

DEVLIN, P.F. and KAY, S.A. (2000). Cryptochromes are required for phytochrome signaling to the circadian clock but not for rhythmicity. *Plant Cell.* 12: 2499-2510.

DEVLIN, P.F., PATEL, S.R. and WHITELAM, G.C. (1998). Phytochrome E influences internode elongation and flowering time in *Arabidopsis*. *Plant Cell.* 10: 1479-1487.

DEVLIN, P.F., ROBSON, P.R.H., PATEL, S.R., GOOSEY, L., SHARROCK, R.A. and WHITELAM, G.C. (1999). Phytochrome D acts in the shade-avoidance syndrome in *Arabidopsis* by controlling elongation growth and flowering time. *Plant Physiol.* 119: 909-915.

DOI, K., IZAWA, T., FUSE, T., YAMANOUCHI, U., KUBO, T., SHIMATANI, Z., YANO, M. and YOSHIMURA, A. (2004). Ehd1: a B-type response regulator in rice, confers short-day promotion of flowering and controls FT-like gene expression independently of Hd1. *Genes Dev.* 18: 926-936

DOYLE, M.R., DAVIS, S.J., BASTOW, R.M., MCWATTERS, H.G., KOZMA-BOGNAR, L., NAGY, F., MILLAR, A.J. and AMASINO, R.M. (2002). The *ELF4* gene controls circadian rhythms and flowering time in *Arabidopsis thaliana*. *Nature.* 419: 74-77.

EL-ASSAL, EL-DIN S., ALONSO-BLANCO, C., PEETERS, A.J., RAZ, V. and KOORNNEEF, M. (2001). A QTL for flowering time in *Arabidopsis* reveals a novel allele of CRY2. *Nat. Genet.* 29: 435-440.

ENNOS, A. (1999). The aerodynamics and hydrodynamics of plants. *J. Exp. Biol.* 202: 3281-3284.

FINNEGAN, E.J., PEACOCK, W.J. and DENNIS, E.S. (1996). Reduced DNA methylation in *Arabidopsis thaliana* results in abnormal plant development. *Proc. Natl. Acad. Sci. USA.* 93: 8449-8454.

FINNEGAN, E.J., SHELDON, C.C., JARDINAUD, F., PEACOCK, W.J. and DENNIS, E.S. (2004). A cluster of *Arabidopsis* genes with a coordinate response to an environmental stimulus. *Curr. Biol.* 14: 911-916.

FOWLER, S., LEE, K., ONOUCHI, H., SAMACH, A., RICHARDSON, K., MORRIS, B., COUPLAND, G. and PUTTERILL, 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.

GENGER, R.K., PEACOCK, W.J., DENNIS, E.S. and FINNEGAN, E.J. (2003). Opposing effects of reduced DNA methylation on flowering time in *Arabidopsis thaliana*. *Planta.* 216: 461-466.

GENDALL, A.R., LEVY, Y.Y., WILSON, A. and DEAN, C. (2001). The VERNALIZATION 2 gene mediates the epigenetic regulation of vernalization in *Arabidopsis*. *Cell.* 107: 525-535.

GOTO, N., KUMAGAI, T. and KOORNNEEF, M. (1991). Flowering responses to light-breaks in photomorphogenic mutants of *Arabidopsis thaliana*, a long-day plant. *Physiol. Plantarum.* 83: 209-215

GUO, H., DUONG, H., MA, N. and LIN, C. (1999). The *Arabidopsis* blue light receptor cryptochrome 2 is a nuclear protein regulated by a blue light-dependent post-transcriptional mechanism. *Plant J.* 19: 279-287.

GUO, L.P., ZHANG, F.S., WANG, X.R., MAO, D.R. and CHEN, X.P. (2001). Effect of long-term fertilization on soil nitrate distribution. *J. Environ. Sci. (China.)* 13: 58-63.

HALL, A., BASTOW, R.M., DAVIS, S.J., HANANO, S., MCWATTERS, H.G., HIBBERD, V., DOYLE, M.R., SUNG, S., HALLIDAY, K.J., AMASINO, R.M. and MILLAR, A.J. (2003). The *TIME FOR COFFEE* gene maintains the amplitude and timing of *Arabidopsis* circadian clocks. *Plant Cell.* 15: 2719-2729.

HALLIDAY, K.J., SALTER, M.G., THINGNAES, E. and WHITELAM, G.C. (2003). Phytochrome control of flowering is temperature sensitive and correlates with expression of the floral integrator FT. *Plant J.* 33: 875-885.

HALLIDAY, K.J., KOORNNEEF, M. and WHITELAM, G.C. (1994). Phytochrome B and at least one other phytochrome mediate the accelerated flowering response of *Arabidopsis thaliana* L. to low red/far-red ratio. *Plant Physiol.* 104: 1311-1315.

HALLIDAY, K.J. and WHITELAM, G.C. (2003). Changes in photoperiod or temperature alter the functional relationships between phytochromes and reveal roles for phyD and phyE. *Plant Physiol.* 131: 1913-1920.

HANUMAPPA, M., PRATT, L.H., CORDONNIER-PRATT, M.M. and DEITZER, G.F. (1999). A photoperiod-insensitive barley line contains a light-labile phytochrome B. *Plant Physiol.*119: 1033-1040.

HARMER, S.L., HOGENESCH, J.B., STRAUME, M., CHANG, H.-S., HAN, B., ZHU, T., WANG, X., KREPS, J.A. and KAY, S.A. (2000). Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. *Science.* 290: 2110-2113.

HAYAMA, R., YOKOI, S., TAMAKI, S., YANO, M. and SHIMAMOTO, K. (2003). Adaptation of photoperiodic control pathways produces short-day flowering in rice. *Nature.* 422: 719-722.

HE, Y., MICHAELS, S.D. and AMASINO, R.M. (2003). Regulation of flowering time by histone acetylation in *Arabidopsis*. *Science.* 302: 1751-1754.

HE, Y., DOYLE, M.R. and AMASINO, R.M. (2004a). PAF1-complex-mediated histone methylation of *FLOWERING LOCUS C* chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. *Genes Dev.* 18: 2774-2784.

HE, Y. and AMASINO, R.M. (2005). Role of chromatin modification in flowering-time control. *Trends Plant. Sci.* 10: 30-35

HE, Y., TANG, R.-H., HAO, Y., STEVENS, R.D., COOK, C.W., AHN, S.M., JING, L., YANG, Z., CHEN, L., GUO, F., FIORANI, F., JACKSON, R.B., CRAWFORD, N.M. and PEI, Z.-M. (2004b). Nitric oxide represses the *Arabidopsis* floral transition. *Science.* 305: 1968-1971.

HELLMANN, H. and ESTELLE, M. (2002). Regulation by Protein Degradation. *Science.* 297: 793-797.

HENDERSON, I.R., SHINDO, C. and DEAN, C. (2003). The need for the winter in the switch to flowering. *Ann. Rev. Genet.* 37: 371-392

HEPWORTH, S.R., VALVERDE, F., RAVENSCROFT, D., MOURADOV, A. and COUPLAND, G. (2002). Antagonistic regulation of flowering-time gene *SOC1* by CONSTANS and FLC via separate promoter motifs. *EMBO J.* 21: 4327-4337.

HUQ, E., TEPPERMAN, J.M. and QUAIL, P.H. (2000). GIGANTEA is a nuclear protein involved in phytochrome signaling in *Arabidopsis. Proc. Natl. Acad. Sci. USA.* 97: 11673-11673

IMAIZUMI, T., TRAN, H.G., SWARTZ, T.E., BRIGGS, W.R. and KAY, S.A. (2003).

FKF1 is essential for photoperiodic-specific light signalling in *Arabidopsis*. *Nature.* 426: 302-306

IZAWA, T., OIKAWA, T., SUGIYAMA, N., TANISAKA, T., YANO, M. and SHIMAMOTO, K. (2002). Phytochrome mediates the external light signal to repress FT orthologues in photoperiodic flowering of rice. *Genes Dev.* 16: 2006-2020.

JACKSON, J.P., LINDROTH, A.M., CAO, X. and JACOBSEN, S.E. (2002). Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature.* 416: 556-560.

JARILLO, J.A., CAPEL, J., TANG, R.H., YANG, H.Q., ALONSO, J.M., ECKER, J.R. and CASHMORE, A.R. (2001). An *Arabidopsis* circadian clock component interacts with both CRY1 and PHYB. *Nature.* 410: 487-490.

JOHANSON, U., WEST, J., LISTER, C., MICHAELS, S., AMASINO, R. and DEAN, C. (2000). Molecular analysis of *FRIGIDA*, a major determinant of natural variation in *Arabidopsis* flowering time. *Science.* 290: 344-347.

JOHNSON, E., BRADLEY, M., HARBERD, N.P. and WHITELAM, G.C. (1994). Photoresponses of Light-Grown phyA Mutants of *Arabidopsis* Phytochrome A Is Required for the Perception of Daylength Extensions. *Plant Physiol.* 105: 141-149.

JOHNSON, K.A., SISTRUNK, M.L., POLISENSKY, D.H. and BRAAM, J. (1998). *Arabidopsis thaliana* responses to mechanical stimulation do not require ETR1 or EIN2. *Plant Physiol.* 116: 643-649.

KARDAILSKY, I., SHUKLA, V.K., AHN, J.H., DAGENAIS, N., CHRISTENSEN, S.K., NGUYEN, J.T., CHORY, J., HARRISON, M.J. and WEIGEL, D. (1999). Activation tagging of the floral inducer *FT*. *Science.* 286: 1962-1965.

KIM, H.J., HYUN, Y., PARK, J.Y., PARK, M.J., PARK, M.K., KIM, M.D., LEE, M.H., MOON, J., LEE, I. and KIM, J. (2004). A genetic link between cold responses and flowering time through FVE in *Arabidopsis thaliana*. *Nat. Genet.* 36: 167-171.

KIM, W.-Y., GENG, R. and SOMERS, D.E. (2003). Circadian phase-specific degradation of the F-box protein ZTL is mediated by the proteasome. *Proc. Natl. Acad. Sci. USA.* 100: 4933-4938.

KIPREOS, E.T. and PAGANO, M. (2000). The F-box protein family. *Genome Biol.* 1: 30021-7.

KIRCHER, S., KOZMA-BOGNAR, L., KIM, L., ADAM, E., HARTER, K., SCHAFER, E. and NAGY, F. (1999). Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. *Plant Cell.* 11: 1445-1456.

KIRCHER, S., GIL, P., KOZMA-BOGNAR, L., FEJES, E., SPETH, V., HUSSELSTEIN-MULLER, T., BAUER, D., ADAM, E., SCHAFER, E. and NAGY, F. (2002). Nucleocytoplasmic partitioning of the plant photoreceptors phytochrome A, B, C, D and E is regulated differentially by light and exhibits a diurnal rhythm. *Plant Cell.* 14: 1541-1555.

KLEINER, O., KIRCHER, S., HARTER, K. and BATSCHAUER, A. (1999). Nuclear localization of the *Arabidopsis* blue light receptor cryptochrome 2. *Plant J.* 19: 289-296.

KNIGHT MR, CAMPBELL AK, SMITH SM and TREWAVAS AJ. (1991) Transgenic plant aequorin reports the effects of touch and cold-shock and elicitors on cytoplasmic calcium *Nature*. 8;352(6335):524-6

KOBAYASHI, Y., KAYA, H., GOTO, K., IWABUCHI, M. and ARAKI, T. (1999). A pair of related genes with antagonistic roles in mediating flowering signals. *Science.* 286: 1960-1962.

KOJIMA, S., TAKAHASHI, Y., KOBAYASHI, Y., MONNA, L., SASAKI, T., ARAKI, T. and YANO, M. (2002). Hd3a, a rice ortholog of the *Arabidopsis FT* gene, promotes transition to flowering downstream of *Hd1* under short-day conditions. *Plant Cell Physiol* 43: 1096-1105.

KOORNNEEF, M., HANHART, C.J. and VAN DER VEEN, J.H. (1991). A genetic and physiological analysis of late flowering mutants in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 229: 57-66.

KOORNNEEF, M., BLANKESTIJN DE VRIES, C. J. HANHART, W. SOPPE and A. J. M. PEETERS. (1994) The phenotype of some late-flowering mutants is enhanced by a locus on chromosome 5 that is not effective in the Landsberg *erecta* wild-type. *Plant J.* 6: 911-919

KROGAN, N.J., DOVER, J., WOOD, A., SCHNEIDER, J., HEIDT, J., BOATENG, M.A., DEAN, K., RYAN, O.W., GOLSHANI, A., JOHNSTON, M., GREENBLATT, J.F. and SHILATIFARD, A. (2003). The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol. Cell.* 11: 721-729.

KUZMICHEV, A., ZHANG, Y., ERDJUMENT-BROMAGE, H., TEMPST, P. and REINBERG, D. (2002). Role of the Sin3-histone deacetylase complex in growth regulation by the candidate tumor suppressor p33(ING1). *Mol. Cell. Biol.* 22: 835-848.

LAMATTINA, L., GARCIA-MATA, C., GRAZIANO, M. and PAGNUSSAT, G. (2003). Nitric oxide: the versatility of an extensive signal molecule. *Annu. Rev. Plant. Biol.* 54: 109-136.

LANG, A. (1965). Physiology of flower initiation. *Encyclopaedia of Plant Physiology*. Vol 15: W. Rhuland, ed. (Springer- Verlag, Berlín), 1380-1536

LANGRIDGE, J. (1957). Effect of day-length and gibberellic acid on the flowering of *Arabidopsis*. *Nature.* 180: 36–37.

LEDGER, S., STRAYER, C., ASHTON, F., KAY, S.A. and PUTTERILL, J. (2001). Analysis of the function of two circadian-regulated *CONSTANS-LIKE* genes. *Plant J.* 26: 15-22

LEE, I., BLEECKER, A. and AMASINO, R. (1993). Analysis of naturally occurring late flowering in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 237: 171-176

LEE, I., AUKERMAN, M.J., GORE, S.L., LOHMAN, K.N., MICHAELS, S.D., WEAVER, L.M., JOHN, M.C., FELDMANN, K.A. and AMASINO, R.M. (1994). Isolation of *LUMINIDEPENDENS:* a gene involved in the control of flowering time in *Arabidopsis*. *Plant Cell.* 6: 75-83

LEVY, Y.Y., MESNAGE, S., MYLNE, J.S., GENDALL, A.R. and DEAN, C. (2002). Multiple roles of *Arabidopsis VRN1* in vernalization and flowering time control. *Science.* 297: 243-246

LIM, M.H., KIM, J., KIM, Y.S., CHUNG, K.S., SEO, Y.H., LEE, I., HONG, C.B., KIM, H.J. and PARK, C.M. (2004). A new *Arabidopsis* gene, *FLK*, encodes an RNA binding protein with K homology motifs and regulates flowering time via *FLOWERING LOCUS C*. *Plant Cell.* 16: 731-740

LIN, C. (2000). Plant blue-light receptors. *Trends. Plant. Sci.* 5: 337-342.

LIN, C., YANG, H., GUO, H., MOCKLER, T., CHEN, J. and CASHMORE, A.R. (1998). Enhancement of blue-light sensitivity of *Arabidopsis* seedlings by a blue light receptor cryptochrome 2. *Proc. Natl. Acad. Sci. USA.* 95: 2686-2690.

LIU, J., HE, Y., AMASINO, R. and CHEN, X. (2004). siRNAs targeting an intronic transposon in the regulation of natural flowering behavior in *Arabidopsis*. *Genes Dev.* 18: 2873-2878.

LIU, X.L., COVINGTON, M.F., FANKHAUSER, C., CHORY, J. and WAGNER, D.R. (2001). *ELF3* encodes a circadian clock-regulated nuclear protein that functions in an *Arabidopsis* PHYB signal transduction pathway. *Plant Cell.* 13: 1293-1304.

MACKNIGHT, R., BANCROFT, I., PAGE, T., LISTER, C., SCHMIDT, R., LOVE, K., WESTPHAL, L., MURPHY, G., SHERSON, S., COBBETT, C. and DEAN, C. (1997). *FCA*, a gene controlling flowering time in *Arabidopsis*, encodes a protein containing RNA-binding domains. *Cell.* 89: 737-745

MARTINEZ, C., PONS, E., PRATS, G. and LEON, J. (2004). Salicylic acid regulates flowering time and links defence responses and reproductive development. *Plant J.* 37: 209-217.

MARTINEZ-GARCIA, J.F., HUQ, E. and QUAIL, P.H. (2000). Direct targeting of light signals to a promoter element-bound transcription factor. *Science.* 288: 859-863.

MARTINEZ-ZAPATER, J.M. and SOMERVILLE, C.R. (1990). Effect of light quality and vernalization on late flowering mutants of *Arabidopsis thaliana*. *Plant Physiol.* 92: 770–776

MAS, P., DEVLIN, P.F., PANDA, S. and KAY, S.A. (2000). Functional interaction of phytochrome B and cryptochrome 2. *Nature.* 408: 207-211.

MCKAY, J.K., RICHARDS, J.H. and MITCHELL-OLDS, T. (2003). Genetics of drought adaptation in *Arabidopsis thaliana*: I. Pleiotropy contributes to genetic correlations among ecological traits. *Mol. Ecol.* 12: 1137-1151.

MICHAELS, S.D. and AMASINO, R.M. (1999). *FLOWERING LOCUS C* encodes a novel MADS domain protein that acts as a repressor of flowering. *Plant Cell.*11: 949-956.

MICHAELS, S.D. and AMASINO, R.M. (2001). Loss of *FLOWERING LOCUS C* activity eliminates the late-flowering phenotype of *FRIGIDA* and autonomous pathway mutations but not responsiveness to vernalization. *Plant Cell.* 13: 935-941.

MICHAELS, S.D., BEZERRA, I.C. and AMASINO, R.M. (2004). *FRIGIDA*-related

genes are required for the winter-annual habit in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 101: 3281-3285.

MICHAELS, S.D., HE, Y.H., SCORTECCI, K.C. and AMASINO, R.M. (2003). Attenuation of *FLOWERING LOCUS C* activity as a mechanism for the evolution of summer-annual flowering behavior in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 100: 10102-10107.

MIZOGUCHI, T., WHEATLEY, K., HANZAWA, Y., WRIGHT, L., MIZOGUCHI, M., SONG, H.R., CARRE, I.A. and COUPLAND, G. (2002). *LHY* and *CCA1* are partially redundant genes required to maintain circadian rhythms in *Arabidopsis*. *Dev. Cell.* 2: 629-641.

MOCKLER, T., YANG, H., YU, X., PARIKH, D., CHENG, Y.C., DOLAN, S. and LIN, C. (2003). Regulation of photoperiodic flowering by *Arabidopsis* photoreceptors. *Proc. Natl. Acad. Sci USA.* 100: 2140-2145.

MOCKLER, T.C., YU, X., SHALITIN, D., PARIKH, D., MICHAEL, T.P., LIOU, J., HUANG, J., SMITH, Z., ALONSO, J.M., ECKER, J.R., CHORY, J. and LIN, C. (2004). Regulation of flowering time in *Arabidopsis* by K homology domain proteins. *Proc. Natl. Acad. Sci. USA.* 101: 12759-12764.

MONTE, E., ALONSO, J.M., ECKER, J.R., ZHANG, Y., LI, X., YOUNG, J., AUSTIN-PHILLIPS, S. and QUAIL, P.H. (2003). Isolation and characterization of phyC mutants in *Arabidopsis* reveals complex crosstalk between phytochrome signaling pathways. *Plant Cell.* 15: 1962-1980.

MOON, J., LEE, H., KIM, M. and LEE, I. (2005). Analysis of flowering pathway integrators in *Arabidopsis*. *Plant Cell Physiol.* 024.

MOON, J., SUH, S.S., LEE, H., CHOI, K.R., HONG, C.B., PAEK, N.C., KIM, S.G. and LEE, I. (2003). The *SOC1* MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. *Plant J.* 35: 613-623.

MOURADOV, A., CREMER, F. and COUPLAND, G. (2002). Control of flowering time: interacting pathways as a basis for diversity. *Plant Cell.* 14 Suppl: S111-130.

NAPP-ZINN, K. (1987). Vernalization environmental and epigenetic regulation. Manipulation of flowering. EdJ.G.Atherton. 123-132

NELSON, D.C., LASSWELL, J., ROGG, L.E., COHEN, M.A. and BARTEL, B. (2000). *FKF1:* a clock-controlled gene that regulates the transition to flowering in *Arabidopsis*. *Cell.* 101: 331-340.

NG, H.H., DOLE, S. and STRUHL, K. (2003). The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J. Biol. Chem.* 278: 33625-33628.

NI, M., TEPPERMAN, J.M. and QUAIL, P.H. (1999). Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature.* 400: 781-784.

NOH, Y.S. and AMASINO, R.M. (2003). *PIE1:* an ISWI family gene, is required for *FLC* activation and floral repression in *Arabidopsis*. *Plant Cell.* 15: 1671-1682.

OH, S., ZHANG, H., LUDWIG, P. and VAN NOCKER, S. (2004). A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the *Arabidopsis FLC/MAF* MADS box gene family. *Plant Cell.* 16: 2940-2953.

ONOUCHI, H., IGENO, M.I., PERILLEUX, C., GRAVES, K. and COUPLAND, G. (2000). Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among *Arabidopsis* flowering-time genes. *Plant Cell.* 12: 885-900.

PARK, D.H., SOMERS, D.E., KIM, Y.S., CHOY, Y.H., LIM, H.K., SOH, M.S., KIM, H.J., KAY, S.A. and NAM, H.G. (1999). Control of circadian rhythms and photoperiodic flowering by the *Arabidopsis GIGANTEA* gene. *Science.* 285: 1579-1582.

PIGLIUCCI, M. and KOLODYNSKA, A. (2002). Phenotypic plasticity and integration in response to flooded conditions in natural accessions of *Arabidopsis thaliana* (L.) Heynh (Brassicaceae). *Ann. Bot. (Lond).* 90: 199-207.

PIGLIUCCI, M., WHITTON, J. and SCHLICHTING, C.D. (1995). Reaction norms of *Arabidopsis*. I. Plasticity of characters and correlations across water, nutrient and light gradients. *J. Evol. Biol.* 8: 421-438.

PITTENDRIGH, C.S. (1960). Circadian rhythms and the circadian organization of living systems. *Cold Spring Harb. Symp. Quant. Biol.* 25: 159-184.

PLIETH, C., HANSEN, U.P., KNIGHT, H. and KNIGHT, M.R. (1999). Temperature sensing by plants: the primary characteristics of signal perception and calcium response. *Plant J.* 18: 491-497.

PUTTERILL, J., ROBSON, F., LEE, K., SIMON, R. and COUPLAND, G. (1995). The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell.* 80: 847-857.

PUTTERILL, J., LAURIE, R. and MACKNIGHT, R. (2004). It's time to flower: the genetic control of flowering time. *Bioessays.* 26: 363-373.

QUAIL, P.H. (2002). Photosensory perception and signalling in plant cells: new paradigms?. *Curr. Op. Cell Biol.*14: 180-188.

QUESADA, V., MACKNIGHT, R., DEAN, C. and SIMPSON, G.G. (2003). Autoregulation of *FCA* pre-mRNA processing controls *Arabidopsis* flowering time. *EMBO J.* 22: 3142-3152.

QIAN, Y.W. and LEE, E.Y. (1995). Dual retinoblastoma-binding proteins with properties related to a negative regulator of ras in yeast. *J. Biol. Chem.* 270: 25507-25513.

RATCLIFFE, O.J., NADZAN, G.C., REUBER, T.L. and RIECHMANN, J.L. (2001). Regulation of flowering in *Arabidopsis* by an *FLC* homologue. *Plant Physiol.* 126: 122-132.

RATCLIFFE, O.J., KUMIMOTO, R.W., WONG, B.J. and RIECHMANN, J.L. (2003). Analysis of the *Arabidopsis MADS AFFECTING FLOWERING* gene family: *MAF2* prevents vernalization by short periods of cold. *Plant Cell.* 15: 1159-1169.

REED, J.W., NAGATANI, A., ELICH, T.D., FAGAN, M. and CHORY, J. (1994). Phytochrome A and phytochrome B have overlapping but distinct functions in *Arabidopsis* development. *Plant Physiol.* 104: 1139-1149.

REDEI, G.P. (1962). Supervital mutants in *Arabidopsis*. *Genetics.* 47: 443-460

RIDGWAY, P. and ALMOUZNI, G. (2000). CAF-1 and the inheritance of chromatin states: at the crossroads of DNA replication and repair. *J. Cell. Sci.* 113: 2647-2658.

RONEMUS, M.J., GALBIATI, M., TICKNOR, C., CHEN, J. and DELLAPORTA, S.L. (1996). Demethylation-induced developmental pleiotropy in *Arabidopsis*. *Science.* 273: 654-657.

ROSSI, V., LOCATELLI, S., LANZANOVA, C., BONIOTTI, M.B., VAROTTO, S., PIPAL, A., GORALIK-SCHRAMEL, M., LUSSER, A., GATZ, C., GUTIERREZ, C. and MOTTO, M. (2003). A maize histone deacetylase and retinoblastoma-related protein physically interact and cooperate in repressing gene transcription. *Plant Mol. Biol.* 51: 401-413.

ROUSE, D.T., SHELDON, C.C., BAGNALL, D.J., PEACOCK, W.J. and DENNIS, E.S. (2002). *FLC*, a repressor of flowering, is regulated by genes in different inductive pathways. *Plant J.* 29: 183-191.

SALOME, P.A. and MCCLUNG, C.R. (2004). The *Arabidopsis thaliana* Clock. *J. Biol. Rhythms.* 19: 425-435.

SALOME, P.A. and MCCLUNG, C.R. (2005). *PSEUDO-RESPONSE REGULATOR* 7 and 9 are partially redundant genes essential for the temperature responsiveness of the *Arabidopsis* circadian clock. *Plant Cell.* 17: 791-803.

SAMACH, A., ONOUCHI, H., GOLD, S.E., DITTA, G.S., SCHWARZ-SOMMER, Z., YANOFSKY, M.F. and COUPLAND, G. (2000). Distinct roles of CONSTANS target genes in reproductive development of *Arabidopsis*. *Science.* 288: 1613-1616

SANDA, S.L. and AMASINO, R.M. (1996). Interaction of *FLC* and late-flowering mutations in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 251: 69-74.

SANTOS-ROSA, H., SCHNEIDER, R., BANNISTER, A.J., SHERRIFF, J., BERNSTEIN, B.E., EMRE, N.C., SCHREIBER, S.L., MELLOR, J. and KOUZARIDES, T. (2002). Active genes are tri-methylated at K4 of histone H3. *Nature.* 419: 407-411

SCHAFFER, R., RAMSAY, N., SAMACH, A., CORDEN, S., PUTTERILL, J., CARRE, I.A. and COUPLAND, G. (1998). The late elongated hypocotyl mutation of *Arabidopsis* disrupts circadian rhythms and the photoperiodic control of flowering. *Cell.* 93: 1219-1229.

SCHOMBURG, F.M., PATTON, D.A., MEINKE, D.W. and AMASINO, R.M. (2001). *FPA*, a gene involved in floral induction in *Arabidopsis*, encodes a protein containing RNA-recognition motifs. *Plant Cell.* 13: 1427-1436.

SCHRANZ, M.E., QUIJADA, P., SUNG, S.-B., LUKENS, L., AMASINO, R. and OSBORN, T.C. (2002). Characterization and effects of the replicated flowering time gene *FLC* in *Brassica rapa*. *Genetics.* 162: 1457-1468.

SCHULTZ, T.F., KIYOSUE, T., YANOVSKY, M., WADA, M. and KAY, S.A. (2001). A Role for *LKP2* in the circadian clock of *Arabidopsis*. *Plant Cell.* 13: 2659-2670.

SCORTECCI, K.C., MICHAELS, S.D. and AMASINO, R.M. (2001). Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. *Plant J.* 26: 229-236.

SHARROCK, R.A. and CLACK, T. (2002). Patterns of expression and normalized levels of the five *Arabidopsis* phytochromes. *Plant Physiol.* 130: 442-456.

SHELDON, C.C., CONN, A.B., DENNIS, E.S. and PEACOCK, W.J. (2002). Different regulatory regions are required for the vernalization-induced repression of FLOWERING LOCUS C and for the epigenetic maintenance of repression. *Plant Cell.* 14: 2527-2537.

SHELDON, C.C., ROUSE, D.T., FINNEGAN, E.J., PEACOCK, W.J. and DENNIS, E.S. (2000). The molecular basis of vernalization: the central role of *FLOWERING LOCUS C (FLC). Proc Natl Acad Sci USA.* 97: 3753-3758.

SIMPSON, G.G., DIJKWEL, P.P., QUESADA, V., HENDERSON, I. and DEAN, C. (2003). FY is an RNA 3' end-processing factor that interacts with FCA to control the *Arabidopsis* floral transition. *Cell.* 113: 777-787.

SIMPSON, G.G. and DEAN, C. (2002). *Arabidopsis*, the Rosetta stone of flowering time? *Science.* 296: 285-289.

SOMERS, D.E., DEVLIN, P.F. and KAY, S.A. (1998). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. *Science.* 282: 1488-1490

SOMERS DE, SCHULTZ TF, MILNAMOW M and KAY SA. (2000). *ZEITLUPE* encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell.*101(3):319-29.

SOPPE, W.J.J., BENTSINK, L. and KOORNNEEF, M. (1999). The early-flowering mutant *efs* is involved in the autonomous promotion pathway of *Arabidopsis thaliana. Development.* 126: 4763-4770.

STRAYER, C., OYAMA, T., SCHULTZ, T.F., RAMAN, R., SOMERS, D.E., MAS, P., PANDA, S., KREPS, J.A. and KAY, S.A. (2000). Cloning of the *Arabidopsis* clock gene *TOC1*: an autoregulatory response regulator homolog. *Science.* 289: 768-771.

STRAYER, C.A. and KAY, S.A. (1999). The ins and outs of circadian regulated gene expression. *Curr. Op. Plant Biol.* 2: 114-120.

SUAREZ-LOPEZ, P., WHEATLEY, K., ROBSON, F., ONOUCHI, H., VALVERDE, F. and COUPLAND, G. (2001). *CONSTANS* mediates between the circadian clock and the control of flowering in *Arabidopsis*. *Nature.* 410: 1116-1120.

SUNG, S. and AMASINO, R.M. (2004). Vernalization in *Arabidopsis thaliana* is mediated by the PHD finger protein VIN3. *Nature.* 427: 159-164.

TAKAHASHI, Y., SHOMURA, A., SASAKI, T. and YANO, M. (2001). Hd6: a rice quantitative trait locus involved in photoperiod sensitivity, encodes the alpha subunit of protein kinase CK2. *Proc. Natl. Acad. Sci. USA.* 98: 7922-7927.

TRANQUILLI, G. and DUBCOVSKY, J. (2000). Epistatic interaction between vernalization genes Vrn-Am1 and Vrn-Am2 in diploid wheat. *J. Hered.* 91: 304-306.

TYLER, J.K., BULGER, M., KAMAKAKA, R.T., KOBAYASHI, R. and KADONAGA, J.T. (1996). The p55 subunit of Drosophila chromatin assembly factor 1 is homologous to a histone deacetylase-associated protein. *Mol. Cell Biol.* 16: 6149-6159.

VALVERDE, F., MOURADOV, A., SOPPE, W., RAVENSCROFT, D., SAMACH, A. and COUPLAND, G. (2004). Photoreceptor regulation of CONSTANS protein in photoperiodic flowering. *Science.* 303: 1003-1006.

WANG, Z.Y. and TOBIN, E.M. (1998). Constitutive expression of the *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* gene disrupts circadian rhythms and suppresses its own expression. *Cell.* 93: 1207-1217.

WELLENSIEK, S.J. (1962). Dividing cells as the locus for vernalization. *Nature.* 195: 307–308

WELLENSIEK S.J. (1964) Dividing cells as the prerequisite for vernalization. *Plant Physiol.* 39: 832-835

WELLER, J.L., BEAUCHAMP, N., KERCKHOFFS, L.H., PLATTEN, J.D. and REID, J.B. (2001). Interaction of phytochromes A and B in the control of de-etiolation and flowering in pea. *Plant J.* 26: 283-294.

WESTERMAN, J.M. and LAWRENCE, M.J. (1970). Genotype-environment interaction and developmental regulation in *Arabidopsis thaliana* inbred lines. *Heredity.* 25: 609-627.

WILSON R.N., HECKMAN J.W. and SOMERVILLE C.R. (1992).Gibberellin is required for flowering under short days. *Plant Physiol.* 100 (1): 403-408

YAN, L., LOUKOIANOV, A., TRANQUILLI, G., HELGUERA, M., FAHIMA, T. and DUBCOVSKY, J. (2003). Positional cloning of the wheat vernalization gene VRN1. *Proc. Natl. Acad. Sci. USA.* 100: 6263-6268.

YAN, L., LOUKOIANOV, A., BLECHL, A., TRANQUILLI, G., RAMAKRISHNA, W., SANMIGUEL, P., BENNETZEN, J.L., ECHENIQUE, V. and DUBCOVSKY, J. (2004). The wheat *VRN2* gene is a flowering repressor down-regulated by vernalization. *Science.* 303: 1640-1644.

YANO, M., KATAYOSE, Y., ASHIKARI, M., YAMANOUCHI, U., MONNA, L., FUSE, T., BABA, T., YAMAMOTO, K., UMEHARA, Y., NAGAMURA, Y. and SASAKI, T. (2000). *Hd1:* a major photoperiod sensitivity quantitative trait locus in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell.* 12: 2473-2484.

YANOVSKY, M.J. and KAY, S.A. (2002). Molecular basis of seasonal time measurement in *Arabidopsis*. *Nature.* 419: 308-312.

YANOVSKY, M.J. and KAY, S.A. (2003). Living by the calendar: how plants know when to flower. *Nat. Rev. Mol. Cell. Biol.* 4: 265-276.

YANOVSKY, M.J., MAZZELLA, M.A. and CASAL, J.J. (2000). A quadruple photoreceptor mutant still keeps track of time. *Curr. Biol.* 10: 1013-1015.

ZHANG, H. and VAN NOCKER, S. (2002). The *VERNALIZATION INDEPENDENCE 4* gene encodes a novel regulator of *FLOWERING LOCUS C. Plant J.* 31: 663-673.

ZHANG, H., RANSOM, C., LUDWIG, P. and VAN NOCKER, S. (2003). Genetic analysis of early flowering mutants in *Arabidopsis* defines a class of pleiotropic developmental regulator required for expression of the flowering-time switch *FLOWERING LOCUS C. Genetics.* 164: 347-358.