Chapter 38

Light Regulation of Flowering Time in Arabidopsis

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Introduction

Plant development is dependent on not only endogenous conditions but also environmental factors. One of the best examples of environmental regulation of plant development is photoperiodic flowering, by which plant flower in response to changes of day length (Garner and Allard 1920). The predictability conferred by the seasonal changes in photoperiod enables plants to flower at the most favorable time of the year. The question of what photoreceptors mediate photoperiodic flowering has been one of the focuses in our efforts to understand the underlying mechanisms of photoperiodism. An action spectrum for the photoperiodic regulation of flowering time was reported in as early as 1945, which showed that red light was the most effective spectrum of light used in the night-break experiments to inhibit flowering of SD plants, suggesting a red light-absorbing pigment in the photoperiodic response (Parker et al 1945). It was later found that the red light effect could be reversed by far-red light which, together with a similar effect of light on germination, contributed to the discovery of phytochrome (Borthwick et al 1952). In addition to red light, blue/UV-A light has also been found to affect flowering time in some of the early works, but most of these light effects were attributed to phytochromes (Parker et al 1946, Meijer 1959, Brown and Klein 1971). We now know that, in addition to phytochromes, blue/UV-A light receptors also play important roles in the light regulation of flowering time (Guo et al 1998, Imaizumi et al 2003). In the last 5 years, significant progress has been made in the study of plant photoreceptors and the molecular mechanisms underlying light regulation of flowering time. Most of these studies were carried out in the model plant *Arabidopsis thaliana*. Although it has been clearly shown in the earlier physiological studies that photoperiodic flowering in different plant species responds to light in different ways, the studies in Arabidopsis nevertheless provides a good framework of how photoreceptors generally work, and it is likely that the observed variations among different plants

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may represent modifications of the basic mechanisms revealed in Arabidopsis. In this short review, we focus on our current understanding of how photoreceptors regulate flowering time in Arabidopsis. Readers are suggested to also read recent review articles covering the related topics (Lin 2000, Mouradov et al 2002, Yanovsky and Kay 2003), and other chapters in this volume for additional discussions of phytochromes, cryptochromes, and other photoreceptors.

Photoreceptors

The Arabidopsis genome encodes at least ten different photosensory receptors, including five phytochromes (phyA to phyE), three cryptochromes (cry1 to cry3), and two phototropins (Cashmore 1997, Briggs and Huala 1999, Nagy and Schafer 2002, Quail 2002, Lin and Shalitin 2003). This list is likely to grow as more LOV-domain proteins other than phototropins may also act as photoreceptors (Imaizumi et al 2003). All of these photoreceptors, except phototropins, have been shown to play roles in the regulation of flowering time. Our current view with respect to how different photoreceptors regulate flowering time in Arabidopsis has been shaped largely by the physiological and genetics studies of Arabidopsis mutants. Several recent review articles have provided a detailed account of these studies (Koornneef et al 1998, Lin 2000, Mouradov et al 2002, Yanovsky and Kay 2003). Mutations in a photoreceptor gene may cause delayed or accelerated flowering. Among different Arabidopsis photoreceptor mutants, *phyB*, *phyC*, *phyE*, and *phyD* mutants showed accelerated flowering under various experimental conditions tested, and they are most likely negative regulators of floral initiation. On the other hand, *phyA*, *cry1*, and *cry2* mutants exhibit delayed flowering phenotype, so they are positive regulators of flowering. Using different combinations of these photoreceptor mutations to test flowering time in plants grown under different light conditions, it has been shown that different phytochromes and cryptochromes act antagonistically as well as redundantly to influence the developmental transition from vegetative growth to reproductive development (Mockler et al 2003) (Figure 1).

The complex interactions of different phytochromes and cryptochromes are interpreted in a model in Figure 1. One may expect that in young seedlings, the major function of photosensory receptors should be to promote vegetative growth and accumulation of photosynthetic products. In doing so, these photoreceptors may also act to suppress reproductive development until plants are mature enough. This view is certainly consistent with the finding that most phytochromes are negative regulators of floral initiation. For example, mutations of *PHYB*, *PHYC*, *PHYD*, and *PHYE* genes all cause the mutant plants to flower earlier than the wild type (Reed et al 1993, Devlin et al 1998, 1999, Franklin et al 2003). Like its function in the regulation of stem elongation, the phyB function in the regulation of flowering time is dependent on red light (Lin 2000, Quail 2002). It is possible that phyC, phyD, and phyE also mediate red light inhibition of floral initiation. The action of phyB in the suppression of floral initiation is

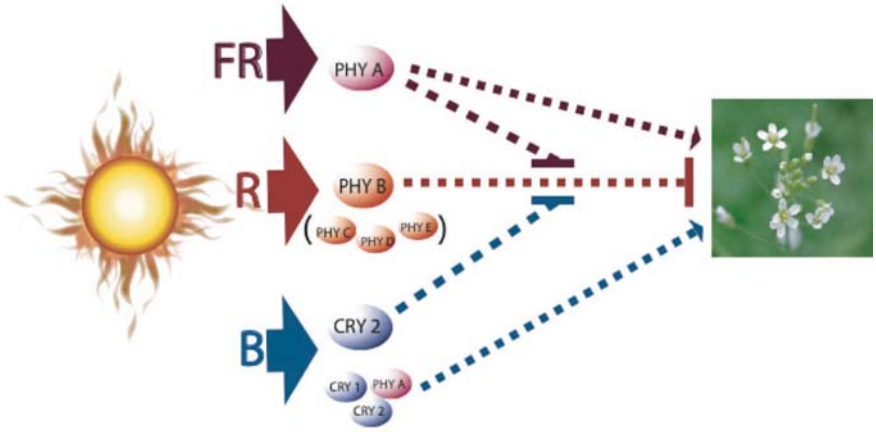

FIG. 1. A model depicting roles of different photoreceptors and their interactions. *Arrows* indicate positive effect on floral initiation, *bar-headed lines* depict negative effect on flowering. *Dashed lines* indicate incomplete understanding of the molecular mechanisms

antagonized by two other photoreceptors, phytochrome A and blue light receptor cry2. PhyA mediates FR light promotion of flowering that is antagonistic to the phyB function; cry2 mediates blue light suppression of phyB activity. In addition, cry1, cry2, and phyA also mediate, in a partially redundant manner, blue light promotion of flowering that is independent from their activity in the suppression of the phyB function (Mockler et al 1999, 2003) (Figure 1). The interactions among different photoreceptors responsive to different spectra of light would presumably allow plants to fine-tune the timing of their developmental transition in adaptation to different light environments. For example, phyB, phyD, and phyE can apparently act in response to a decreased R/FR ratio of light received by plants grown under the shade of canopies of neighboring plants (Devlin et al 1998, 1999, Franklin et al 2003). In the absence of shade, these three phytochromes promote vegetative growth and suppress flowering. In the presence of shade, the activity of these photoreceptors decreases, allowing floral initiation to take place. There seems an apparent advantage for a plant that grows under the shade from the canopies of surrounding plants to complete its life cycle before deprivation of light, water, and nutrients by its competing neighbors. Different photoreceptors sensing different spectral regions of light may also help discriminate photoperiods. For example, it is known that the relative light spectral composition changes throughout a day: blue and far-red spectra are relatively more abundant in twilight, whereas the red spectrum is relatively more abundant in daylight (Hart 1988). Therefore, different photoreceptors acting in response to different spectra of light may provide a more accurate measurement of the day length, although it is not immediately obvious what adaptive advantages plants may have by possessing different photoreceptors that act antagonistically. One outcome of the antagonistic actions between phyB, phyA, and cry2 has been dis-

covered recently in the control of protein stability of a flowering-time regulator, CO (*CONSTANS*), as discussed later. Interactions among different photoreceptors acting in different spectral ranges of solar radiation may also help plants to adapt to certain light conditions yet to be recognized. It will be interesting to examine whether such antagonistic interactions between phytochromes and cryptochromes are also present in plant species other than Arabidopsis.

Mechanisms

Photoreceptors may exert a different effect on light regulation of reproductive development in plants through their roles in the regulation of photosynthetic gene expression, metabolite partitioning, nutrient uptake and distribution, and hormone biosynthesis. However, the question of how light regulates flowering time has been traditionally focused on its role in sensing the change of day length. How plants "recognize," "remember," and respond to day-length changes have challenged plant biologists for the last 80 years or so. Among various hypotheses based on early physiological studies, the external coincidence model has gained most of the experimental support in recent years (Thomas and Vince-Prue 1997). According to this hypothesis, photoperiodism is governed by two interacting mechanisms: one controlled by the circadian clock and the other regulated by the photoreceptors (Yanovsky and Kay 2003). The circadian clock is entrained according to environmental signals such as light and temperature. Phytochromes and cryptochromes are apparently the major photoreceptors mediating light entrainment of the circadian clock in plants (Somers et al 1998). The role of light in the photoperiodic flowering is more than the entrainment of the clock. It is the interactions between the circadian clock-dependent processes called photoperiodic response rhythm (PRR) and the photoreceptor-dependent reactions independent of the clock that allow plants to distinguish a long day from a short day and to trigger or suppress floral initiation. The molecular nature of the PRR and how PRR interact with the photoreceptor-regulated reactions have remained elusive until recently (also see Chapters 39 and 41 by Izawa and Somers, respectively). Several studies have demonstrated that the photoperiod-dependent circadian rhythm of mRNA expression of the flowering-time gene *CO* and the photoreceptor-dependent light regulation of CO protein level form a basis for the external coincidence mechanism underlying photoperiodism in Arabidopsis (Figure 2). It is now clear that the expression of certain flowering-time genes such as *FT* is, at least partially, controlled by light regulation of the amount of CO protein (Valverde et al 2004). The CO protein is ubiquitinated and degraded by the 26S proteosome in darkness, but CO protein is relatively stable in white light. Analysis of the CO protein in the photoreceptor mutants demonstrates that cry1/cry2 and phyA stabilize CO protein in response to blue light and far-red light, respectively, and that phyB promotes CO degradation in red light. As described previously, phyB mediates red light suppression of flowering, whereas cry2 and phyA mediate blue and far-red light promotion, respectively (Figure 1).

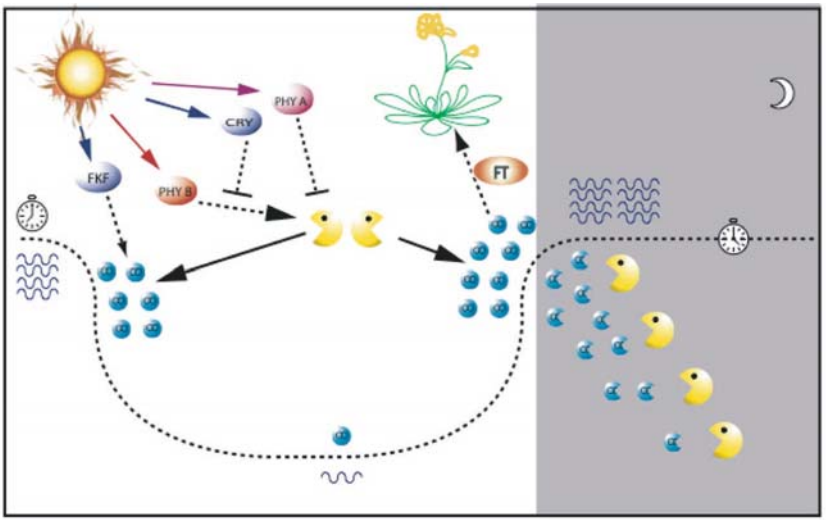

FIG. 2. Photoreceptors and the circadian clock exert functional interaction in the regulation of cellular level of CO protein**.** *CO* mRNA levels (depicted by the number of *wavy lines*) is regulated by the circadian clock, which is entrained via the action of phytochromes and cryptochromes. The peak of the circadian rhythm of *CO* mRNA expression (*dashed curve*) runs from the late afternoon to the early morning. CO protein levels (depicted by the number of *spheres*) are determined by not only its mRNA expression, but also protein degradation by the proteosome. CO degradation is promoted by phyB (*oval and arrow*), but inhibited by cryptochromes and phyA during the day. During the night, the CO mRNA level remains high, but little CO protein accumulates due to proteolysis. During long days depicted, phyA and cryptochromes help maintain higher CO protein level to promote flowering. *Arrows* indicate stimulatory actions, *lines with bar-heads* represent inhibitory actions, and *dashed lines* suggest the involvement of additional proteins

The discovery of different roles of the three photoreceptors in the control of CO protein stability revealed an important mechanism by which photoreceptors regulate flowering time. The photoreceptor regulation of CO degradation, coupled with the photoperiodic response rhythms of *CO* transcription, enables plants to decrease the amount of CO protein in short days, and to gradually increase the level of CO protein when the day length gets longer. Similar mechanisms are probably also used by rice, a short-day plant, wherein CO acts as a transcription suppressor of FT, to inhibit flowering in long days (Hayama et al 2003).

Perspective

How photoreceptors mediate light regulation of CO protein stability is apparently one of the questions that remains to be answered. Genes that are known to be involved in the light regulation of FT expression but do not affect the

expression of *CO* mRNA expression would likely play a role in the light regulation of CO degradation. *PFT1* (*PHYTOCHROME AND FLOWERING TIME 1*) is apparently a good candidate (Cerdan and Chory 2003). PFT1 mediates phyB regulation of FT expression independent from regulation of CO mRNA expression. It will be interesting to see whether *pft1* mutation affects CO protein stability. On the other hand, E3 ubiquitin ligase must play critical role in the ubiquitin/proteosome-mediated degradation of CO in darkness. E3 ubiquitin ligase is responsible for the substrate recognition of the ubiquitin-proteosome apparatus. Among different types of E3 ubiquitin ligase, the RNIG E3 and SCF (SKP1, Cullin, F-box) E3 are the most versatile families. The Arabidopsis genome encodes over 400 RING proteins, or 21 SKP1-like, 10 Cullin-like, and over 700 F-box-containing proteins (Vierstra 2003). It is not known what type of E3 ligase may be involved in the ubiquitination and degradation of CO in darkness. However, both a RING E3 protein (COP1) and an F-box protein (ZTL) have been found to be involved in protein degradation in the absence of light as well as in the control of flowering time (Osterlund et al 2000, Mas et al 2003). *COP1* encodes a RING-finger protein with WD-40 repeats that was originally identified in the constitutive photomorphogenesis mutant *cop1* (Deng et al 1989). Mutations in the *COP1* gene caused accelerated flowering, in addition to its well-known constitutive photomorphogenesis phenotypes. COP1 has been found to act as the E3 ubiquitin ligase in the proteosome-mediated degradation of the transcription factor HY5 in darkness (Osterlund et al 2000). COP1 may also be involved in the light-dependent degradation of photoreceptors such as cry2 and phyA (Shalitin et al 2002, Seo et al 2004). It will be interesting to find out whether COP1, ZTL, or related proteins might be involved in the degradation of CO.

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