Plant Biotechnology 25, 123–129 (2008)

Invited Review

Novel blue light receptors with an F-box: their direct control of the circadian clock and the flowering timing in *Arabidopsis*

Sumire Fujiwara*

Department of Plant Cellular and Molecular Biology, Ohio State University, 038 Rightmire Hall, 1060 Carmack Road, Columbus, OH 43210, USA
* E-mail: fujiwara.5@osu.edu Tel: +1-614-292-2533 Fax: +1-614-292-5379

Received December 19, 2007, accepted December 28, 2007 (Edited by H. Tsuji)

Abstract Most plants sense their environmental changes such as photoperiod by using an endogenous circadian clock to regulate their developmental phases. The core oscillator of the clock has been reported to be composed of a negative feed back loop of transcription. Meanwhile, recent biochemical studies have been shedding light on the importance of post-translational regulations for the circadian mechanisms. ZEITLUPE (ZTL) family members are unique proteins which have three characteristic domains; a LIGHT, OXYGEN AND VOLTAGE domain, an F-box domain and six kelch repeats. Recently, two of the three family members, ZTL and FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 were reported to be novel types of blue light receptors which post-translationaly regulate the circadian clock and photoperiodic flowering, respectively. In this review, studies about ZTL family members making substantial progress are summarized and discussed.

Key words: *Arabidopsis*, blue light receptor, circadian clock, protein degradation, ZTL family.

A circadian clock is an endogenous oscillator which generates rhythms with approximately 24 h period. The clock is entrained by environmental stimuli, such as photoperiod and thermoperiod, so that organisms can coordinate their endogenous biological activities to external daily rhythms and seasonal changes (Dunlap 1999). In plants, many physiological phenomena are under circadian clock regulation, such as photoperiodic flowering, leaf movement, hypocotyl elongation and photosynthesis (McClung 2006; Yanovsky and Kay 2003; Niinuma et al. 2007). Microarray analyses suggest that ~10% of *Arabidopsis* genes show circadian oscillation of mRNA levels (Harmer et al. 2000). Most of the genes which participate in clock regulation show circadian oscillation of both mRNA and protein levels. In *Arabidopsis*, numerous genes have been reported to be involved in the circadian mechanisms, including two myb transcription factors *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*), pseudo response regulators which likely act as transcriptional co-factors [*TIMING OF CAB EXPRESSION 1* (*TOC1*)/*PSEUDO RESPONSE REGULATOR 1* (*PRR1*), *PRR3*, *PRR5*, *PRR7*, *PRR9*], and classic photoreceptors (phytochromes and cryptochromes) (McClung 2006; Mizuno and Nakamichi 2005). In particular, LHY, CCA1 and TOC1 have been identified as candidate core factors of the negative feed back loop of the central oscillator (Alabadi et al. 2001; Mizoguchi et al. 2002). Other genes that influence the clock regulation and flowering time include *ZEITLUPE* (*ZTL*) family members, *ZTL*, *FLAVIN-BINDING*, *KELCH REPEAT*, *F-BOX 1* (*FKF1*) and *LOV KELCH PROTEIN 2* (*LKP2*) (Somers et al. 2000; Nelson et al. 2000; Schultz et al. 2001). While many of *Arabidopsis* circadian clock studies have focused on the transcriptional regulation, ZTL family members have been one of the most characterized proteins for their molecular functions as a result of recent biochemical studies. Kim et al. (2007) and Sawa et al. (2007) reported that ZTL and FKF1 can function as blue light receptors for regulating the central clock oscillation and photoperiodic flowering response, respectively. In this review, mainly focusing on the recent progress on biochemical approaches, studies about ZTL family members are summarized and discussed.

ZTL family members are unique F-box proteins which regulate light mediated protein degradation

The members of ZTL family share very high degree of

Abbreviations: ASK, *Arabidopsis* Skp1-like protein; CDF, CYCLING DOF FACTOR; CO, CONSTANS; GI, GIGANTEA; FT, FLOWERING LOCUS T; FKF1, FLAVIN-BINDING, KELCH REPEAT, F-BOX 1; FMN, flavin mononucleotide; LOV, LIGHT, OXYGEN AND VOLTAGE; LKP, LOV KELCH PROTEIN; OX, overexpressor; PHY, PHYTOCHROME; PRR, PSEUDO RESPONSE REGULATOR; SCF, Skp/Cullin/F-box; TOC1, TIMING OF CAB EXPRESSION 1; ZTL, ZEITLUPE

This article can be found at http://www.jspcmb.jp/

amino acid sequence homology; 70–80% identity throughout the entire protein (Somers et al. 2000; Nelson et al. 2000; Schultz et al. 2001). Each member of ZTL protein family has a LOV, an F-box and six kelch repeat domains.

Their LOV domain structures are similar to those of the blue light receptors, phototropins (phot1 and phot2) (Briggs and Christie 2002; Cheng et al. 2003). Their LOV domains can bind flavin mononucleotide (FMN) and show typical spectral properties of blue light photoreceptors indicating the blue light related functions (Imaizumi et al. 2003). All the ZTL family member proteins show light-induced difference spectra of the LOV domains which have been observed in formation of the long-lived photo-intermediate of the phototropin LOV domains (Imaizumi et al. 2003; Sakai et al. 2001). This photo-intermediate involves the formation of a cysteinyl adduct between a cysteine and FMN chromophore (Salomon et al. 2000). Replacing the cysteine of FKF1 with alanine abolishes the all photochemistry suggesting that FKF1 has a property of a blue light receptor (Imaizumi et al. 2003). In contrast to the phototropin LOV domains, the LOV domains of FKF1, LKP2 and ZTL don't show detectable dark recovery. Taken together, these data indicate that ZTL family members could be previously unknown photoreceptors (Imaizumi et al. 2003).

The F-box is a motif found in F-box proteins which specifically interacts with SKP1, a component of the Skp/Cullin/F-box (SCF) complexes that recruit substrate specific proteins for ubiquitination and subsequent proteolysis by the 26S proteasome in an ATP dependent manner (Deshaies 1999; Vierstra 2003).

Protein–protein interaction domains exist C-terminal to the F-box to define the specificity of the F-box protein. Six kelch repeats are this region for ZTL family members, which forms a β-propeller structure which is similar to the one formed by WD40 repeats (Garcia-Higuera et al.1998).

The combination of these three domains of ZTL family members suggests that they may mediate the light dependent protein degradation by forming SCF complexes. Yasuhara et al. (2004) performed yeast two hybrid analysis testing the interactions between ZTL family members and components of the clock and the SCF complexes. ZTL family members interacted with similar *Arabidopsis* Skp1-like proteins (ASK) and their F-box domains are sufficient for the interactions. *In vivo* studies showed that ZTL associates with known core components of SCF complexes (Han et al. 2004). Mutations in the F-box of ZTL disrupt the association with core components of the complex and cause the circadian disfunction. ZTL protein is stabilized in the F-box mutant due to lack of the SCF^{ZTL} complex formation (Han et al. 2004).

ZTL, a novel blue light receptor connecting light input and the circadian clock

ztl-1 was isolated as a long period mutant from a period mutant screening using a luminescence assay (Millar et al. 1995). *ztl-1* shows a long period phenotype both under red and blue light (Somers et al. 2000). Interestingly, the period effects of *ztl-1* are strongly fluence rate dependent, showing much longer period under low fluence, implicating ZTL function in a light input pathway to the clock. Further characterization showed that the free running period of two different clock regulated genes (*CHLOROPHYLL A*/*B BINDING PROTEIN 2* and *COLD CIRCADIAN RHYTHM–RNA BINDING2*) and cotyledon leaf movement are lengthened by the *ztl-1* mutation. The *ztl-1* mutant (C24 ecotype background) also shows a slight late flowering phenotype under long days but not under short days, indicating that *ztl-1* affects the photoperiodic flowering time regulation at least in this ecotype. Hypocotyl elongation in *ztl* mutants is hypersensitive to red light, but little affected by blue light. Taken together, these results indicate that ZTL participates in the regulation of wide range of clock related phenomena (Somers et al. 2000). *ztl-2*, which shows similar phenotypes to *ztl-1*, and *ztl-1* both have a single amino acid substitution in kelch domain and their mRNA and protein levels are similar to those of wild type plants (Somers et al. 2000, 2004). A null T-DNA insertion line *ztl-3* was isolated (originally reported as *adagio 1*, Jarillo et al. 2001) that lacks detectable ZTL mRNA and protein (Somers et al. 2004). This line shows an overall similar phenotype to *ztl-1* and *ztl-2*, and *ztl-1* was confirmed to be a null allele by genetic analysis (Somers et al. 2004). Interestingly, *ztl* mutants show a long period phenotype even in continuous dark, suggesting that ZTL is not simply functioning in the light input pathway but is closely related to the clock oscillator.

Mas et al. (2003b) found that ZTL and TOC1 interact *in vitro* and *in vivo*, and TOC1 is a substrate of targeted degradation by ZTL through a proteasome-dependent pathway. The *in vivo* interaction is abolished by the *ztl-1* mutation in the kelch domain. This mutation doesn't change the diurnal oscillation of *TOC1* mRNA, but results in constitutively high TOC1 protein accumulation. Knockout or decreased levels of *TOC1* shorten the free running period of the circadian clock, while TOC1 overexpression results in long period (Somers et al. 1998; Mas et al. 2003a; Makino et al. 2002). *ztl-1*/TOC1-RNAi line shows short period phenotype similar to that of TOC1-RNAi line (Mas et al. 2003a, 2003b). Taken together, these data suggest that the long period phenotype of *ztl* mutants is due to the constitutive accumulation of TOC1 protein.

Somers et al. (2004) generated *ZTL* overexpressor lines with different ZTL expression levels and found that

circadian period is highly sensitive to the ZTL expression level. The circadian period in the light becomes increasingly shorter at higher levels of ZTL, and strong ZTL overexpression causes arrhythmicity. This ZTL level dependent period phenotype might be due to the changes in TOC1 protein level. TOC1 is a substrate of targeted degradation of ZTL (Mas et al. 2003b), so TOC1 level might be low in ZTL overexpressors.

The strong overexpressor of ZTL shows late flowering under long days (Kiyosue and Wada 2000; Somers et al. 2004). RNA levels of floral activators *CONSTANS* (*CO*) and *FLOWERING LOCUS T* (*FT*) were significantly low in the line (Somers et al. 2004). According to the recent studies concerning FKF1, the late flowering phenotype of ZTL-OX might be due to the competitive interaction of ZTL with FKF1 to GIGANTEA (GI). The FKF1-GI interaction is suggested to be important for releasing the *CO* transcription from repression by CYCLING DOF FACTOR 1 (CDF1) (Sawa et al. 2007, see the FKF1 section of this review). High levels of ZTL proteins may competitively interact with GI, resulting in fewer GI-FKF1 parings that may phenocopy an *fkf1* knockout, like late flowering.

Kiba et al. (2007) found that ZTL targets not only TOC1 but also PRR5 for degradation through proteasome pathway by direct interaction. PRR7 and PRR9 don't interact with ZTL *in vivo* (Kiba et al. 2007). These interaction data are consistent with the yeast two hybrid data by Yasuhara et al. (2004) showing that ZTL interacts with TOC1 and PRR5, but not with PRR3, PRR7 and PRR9. PRR5 protein is less stable under dark and stable under blue light due to the direct interaction with ZTL (Kiba et al. 2007). The PR domain of PRR5 is critical for the interaction with ZTL, and the deletion of the domain results in stabilized PRR5 protein as in *ztl* mutants. Overexpression of the PR domain alone phenocopies the hypocotyl and flowring features of the long period *ztl-21* mutant (Kevei et al. 2006; Matsushika et al. 2007) suggesting that excess PR domains might sequester ZTL away from the normal ZTL-PRR5 association (Kiba et al. 2007).

Red light hypersensitivity for hypocotyl elongation and the long period of *ztl* were only partially suppressed by the *prr5* mutation (Kiba et al. 2007). In contrast, the *toc1* mutation, which shows a much shorter period than *prr5*, completely suppressed the long period phenotype of *ztl* (Mas et al. 2003a, 2003b; Yamamoto et al. 2003). Further genetic analyses are important to clarify their contribution for the circadian mechanisms.

As described above, TOC1 protein is degraded by ZTL (Mas et al. 2003b). However, ZTL and TOC1 proteins show similar oscillation pattern; both peak in the evening. Therefore, there should be other factors or mechanisms which regulate the TOC1 stability and/or ZTL activity for the degradation. Para et al. (2007) found that PRR3 might function to stabilize TOC1 protein by competing with ZTL. TOC1 and PRR3 show *in vivo* and *in vitro* interaction, and PRR3 positively regulate the TOC1 protein level posttranscriptionally. PRR3 doesn't interact with ZTL in yeast (Yasuhara et al. 2004; Para et al. 2007), so it might function as a TOC1 stabilizer by protecting from degradation by ZTL.

Different from most clock related genes, *ZTL* mRNA level doesn't show any diurnal oscillation (Somers et al. 2000; Figure 1). Nonetheless, ZTL protein level shows a

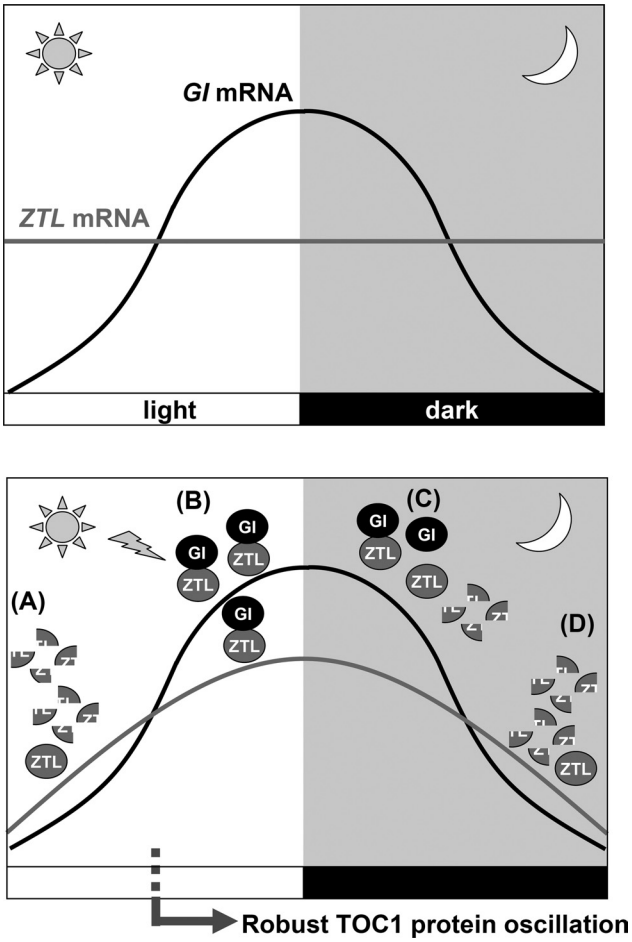

Figure 1. GI posttranslationaly generates ZTL diurnal oscillation. Under light/dark cycles, *GI* mRNA level shows a robust oscillation (black line), while *ZTL* mRNA level is constant (gray line) (upper panel) (Fowler et al. 1999; Somers et al. 2000). Lower panel shows the protein level of GI (black line, David et al. 2006) and ZTL (gray line, Kim et al. 2003) and their status. ZTL protein levels show diurnal oscillation due to the blue-light-dependent interaction with GI (Kim et al. 2007). (A) In the morning, ZTL level is low due to the low level of GI. (B) GI accumulates during the photoperiod and directly interacts with ZTL. This GI-ZTL interaction is enhanced by blue light absorption by ZTL and protects ZTL from proteasome dependent degradation. (C) In the dark, the GI-ZTL interaction is diminished, and ZTL released from the interaction is degraded. GI protein level also goes down due to its mRNA oscillation. (D) ZTL drops to low level due to the low level of GI. This oscillation of ZTL protein generated by the interaction with GI is needed for the robust oscillation of TOC1, a key component of the circadian clock (Kim et al. 2007).

clear diurnal and circadian oscillation (Kim et al. 2003; Figure 1). ZTL protein itself is degraded by the proteasome pathway, and the degradation is circadian phase dependent; labile in the morning and stable in the evening. These data indicate the possible posttranslational regulation of ZTL stability. Kim et al. (2007) reported that ZTL is a novel type of blue light receptor which is stabilized by direct interaction with GI specifically in blue light (Figure 1). GI has been well known to be a major positive regulator of photoperiodic flowering and reported to function as a peripheral circadian clock regulator by genetic analysis (Fowler et al. 1999; Yanovsky and Kay 2003; Mizoguchi et al. 2005; Fujiwara et al. 2005a, 2005b; Niinuma et al. 2007). However, its biochemical function has been unclear because GI encodes a large plant specific unknown protein (Fowler et al. 1999). Kim et al. (2007) found that in GI overexpressor and knock out lines, ZTL protein level damps to high or low level, respectively, with no *ZTL* mRNA level change. *In vitro* degradation assays showed that ZTL protein is stabilized in GI overexpressors. GI interacts strongly with the LOV domain of ZTL *in vivo* and *in vitro*. The interaction of these proteins is enhanced by blue light, and having mutations in LOV domain diminishes the interaction whereas mutations in other domains do not. Intriguingly, a mutation in LOV domain (C82A), implicated in the flavin-dependent photochemistry, eliminates the blue-light dependency of GI-ZTL interaction. Due to the clock controlled *GI* mRNA transcription, GI protein shows cyclic accumulation that confers a post-translational rhythm of ZTL protein (Figure 1). This mechanism of establishing the oscillation of ZTL results in the robust oscillation of TOC1 rhythms which is necessary for proper circadian clock regulation. Taken together, these findings also define a novel type of LOV domain function; blue light dependent protein–protein interaction which confers circadian protein oscillation by post-translational stabilization by the interactor.

Kevei et al. (2006) conducted a large scale genetic screen of clock affecting loci from EMS mutagenized populations. They characterized new *ztl* alleles and the previously isolated alleles (*ztl-1*, *2*, and *3*) which cover each of the ZTL protein domains. All of the alleles tested show long period phenotype regardless of the light conditions even in dark grown seedlings. Mutants except *ztl-3* (null) and *ztl-31* (expressing a truncated protein) have significant levels of ZTL protein, thus all the protein domains contribute to the circadian system. A LOV domain mutant *ztl-21* retains wild type response to red light both for circadian period and for hypocotyl elongation. Jarillo et al. (2001) demonstrated that ZTL interacts with PHYTOCHROME B (PHYB) in yeast suggesting that defect in red light response of *ztl* mutants is possibly through ZTL-PHYB interaction, but this interaction is unaffected by any *ztl* mutations tested (Kevei et al. 2006). Mutations of kelch repeat affect protein binding at the LOV and the F-box domains in yeast. Taken together, the complexity of *ztl* mutant phenotypes is due to the functions of several target proteins which require interaction with ZTL differently.

Is LKP2 an alternate for ZTL?

A second member of ZTL family, LKP2 was identified by BLAST searches (Shultz et al. 2001). *LKP2* mRNA is detected in wide variety of tissues by RT-PCR and doesn't show diurnal oscillation (Shultz et al. 2001). GFP associated fluorescence is detected in nuclei in the 35S:LKP2-GFP plant (Yasuhara et al. 2004), while CFP-LKP2 signals are localized both in the nucleus and cytosol when transiently expressed in onion epidermal cells (Fukamatsu et al. 2005). According to the yeast two hybrid assay, LKP2 interacts with TOC1 and PRR5 through its LOV domain and ASK proteins through its F-box domain (Yasuhara et al. 2004). Di19 and CONSTANS LIKE 1 (COL1) were also identified as interactors of LKP2 by yeast two hybrid screening to find candidate substrates of LKP2 (Fukamatsu et al. 2005). COL1 and other CO/COL family members also interact with ZTL, FKF1 or LKP2. CFP-LKP2 form nuclear bodies only when it is co-expressed with YFP-CO or YFP-COL1, suggesting the possibility that LKP2 and CO/COL family members function together in the nuclear bodies. CDF1, CDF2 and CDF3 interact with the kelch repeats of LKP2 in yeast (Imaizumi et al. 2005; See the FKF1 section of this review). GI also interacts with LKP2 *in vivo* (Kim et al. 2007).

Although many interactors have been reported, the function of LKP2 is still unclear. So far, no phenotypic difference with wild type has been reported for the *lkp2* knockouts. An *lkp2* knockout line shows wild type flowering phenotype under long day and short day conditions (Imaizumi et al. 2005). On the other hand, LKP2-OX shows various phenotypes which imply its function in the circadian mechanisms (Shultz et al. 2001). The overexpression of LKP2 results in arrhythmicity for various clock outputs both under continuous light and dark conditions, and the expression of the clock components also show arrhythmicity. LKP2-OX also shows a long hypoctyl phenotype under white, red and blue light and late flowering under long days.

Taken together, the conclusion of these studies is the LKP2 might function in clock regulation but how is still not clear. There might be redundant factors working with LKP2 that mask the phenotype of the *lkp2* single knockout. Overall phenotypes of LKP2-OX are similar to those of ZTL-OX, suggesting that LKP2 may have redundant function with ZTL. Yeast two hybrid data by Yasuhara et al. (2004) showed that ZTL family members homo- or hetero-dimerize between LKP2 and ZTL,

LKP2 and LKP2, LKP2 and FKF1, but don't between ZTL and ZTL, or ZTL and FKF1. These interaction data also support the idea that LKP2 and ZTL (and FKF1) may have redundant functions. *ztl* single mutants show strong phenotype which is different from an *lkp2* knockout line, suggesting the possibility that LKP2 may function as an alternate or supplement of ZTL.

FKF1, another blue light receptor which directly regulates photoperiodic flowering

Different from ZTL and LKP2, FKF1 doesn't appear to participate in circadian clock regulation but has an important role for photoperiod recognition for proper flowering time regulation.

An *fkf1* mutant was originally isolated as a late flowering mutant, which is rescued by vernalization or gibberellin treatment (Nelson et al. 2000). *fkf1* mutants and FKF1-OX lines don't show defects in circadian period, suggesting that they don't participate in the clock regulation (Nelson et al. 2000; Imaizumi et al. 2003). Different from *ZTL* and *LKP2*, *FKF1* mRNA shows robust circadian oscillation (Nelson et al. 2000). These data suggest that FKF1 functions for controlling flowering under the regulation of the circadian clock.

Arabidopsis plants sense seasonal changes by measuring the daily photoperiods, which then determine when to flower. Photoperiodic flowering regulation is mediated by complex interactions between environmental signals and time keeping mechanisms associated with the circadian clock (Yanovsky and Kay 2003; Imaizumi and Kay 2006). The crucial step for the day length measurement is the proper regulation of circadian CO expression and CO protein stability and activity by light. The coincidence of high levels of CO and light induces the expression of *FT*. The FT protein and the rice ortholog of FT (Heading Date 3a) protein are suggested to be mobile flowering induction signals "florigen" (Corbesier et al. 2007; Tamaki et al. 2007).

A number of factors including GI, FKF1 and CDF1 proteins are known to regulate *CO* transcription (Fowler et al. 1999; Suarez-Lopez et al. 2001; Imaizumi et al. 2003; Mizoguchi et al. 2005; Imaizumi et al. 2005). GI and FKF1 are positive regulators and CDF1 is a negative regulator of *CO* transcription. In the *gi* mutant and GI-OX, overall *CO* mRNA levels damp to low and high levels, respectively (Suarez-Lopez et al. 2001; Mizoguchi et al. 2005). In contrast, in *fkf1* mutants, the daytime peak of *CO* is absent but night time *CO* expression is not changed in long day and short day (Imaizumi et al. 2003). High levels of daytime *CO* expression can be detected only when high levels of FKF1 protein and light coincides, suggesting that FKF1 protein regulates *CO* transcription in a light-dependent manner. Imaizumi et al. (2005) reported that FKF1 regulates *CO* expression in part by degrading CDF1, a Dof transcription factor which directly binds to the *CO* promoter and suppresses *CO* transcription. CDF1 was isolated with other Dof transcription factors (CDF2 and CDF3) by a yeast two-hybrid screen to find substrate proteins of targeted degradation by FKF1 interacting with its kelch repeats. These three transcription factors also interacted with LKP2, but not with ZTL. Within the three CDFs, only CDF1 showed late flowering when overexpressed which is similar to *fkf1*, suggesting that CDF1 is a substrate of FKF1 participating in flowering time regulation.

GI and FKF1 show a similar rhythmic oscillation pattern of RNA and protein (Fowler et al. 1999; Imaizumi et al. 2003; David et al. 2006; Imaizumi et al. 2005). Furthermore, both positively regulate photoperiod dependent flowering by regulating the expression of floral activator genes, including *CO* and *FT*, under the regulation of the circadian clock (Suarez-Lopez et al. 2003; Imaizumi et al. 2003; Mizoguchi et al. 2005; Fujiwara et al. 2005a, 2005b; Imaizumi and Kay 2006). Those reports imply the possible functional interaction between GI and FKF1. Kim et al. (2007) found that GI and FKF1 proteins interact *in planta*. Sawa et al. (2007) also found that GI and FKF1 directly interact *in vivo* and *in vitro*. Interestingly, their interaction occurs differentially throughout the day, peaking in the afternoon both under short day and long day conditions, and diminishing at night. Similar to the findings in the GI-ZTL study, the FKF1-GI interaction is induced by blue light and the LOV domain is responsible for the interaction. The N terminus of GI is sufficient for the light dependent interaction with FKF1. The light dependency is abolished by blue light blind mutations in the LOV domain, suggesting that FKF1 is a blue light receptor, like ZTL. The timing of this GI-FKF1 interaction likely regulates the timing of *CO* expression in part. The *CO* repressor CDF1 interacts with GI. The associations of GI and FKF1 with *CO* chromatin were detected in the evening and the association of CDF1 was detected in the morning. These data suggest that the regulation of *CO* transcription by GI, FKF1 and CDF1 is required for the day-length measurement for flowering. The authors suggest that GI may interact with CDF1 that has already bound to the *CO* promoter to repress the transcription in the morning. Then, in the afternoon, FKF1 may interact with the GI-CDF1 complex and degrade the CDF1 to release the *CO* repression. However, it is still not clear whether GI-FKF1-CDF1 form a complex or not. Both FKF1 and CDF1 interact with the N terminus of GI (amino acids 1-391). Hence, it is possible that a GI-FKF1-CDF1 complex is not formed but GI-FKF1, FKF1-CDF1 and GI-CDF1 interactions occur independently to regulate *CO* transcription.

Genetic analyses suggest that FKF1 function is largely dependent on GI, but GI function is only partially

dependent on FKF1 function (Sawa et al. 2007). This suggests that GI would regulate not only FKF1 function but also other proteins or pathways in the photoperiodic flowering pathway. These are consistent with the report by Mizoguchi et al. (2005) showing that GI promote flowering through *CO* dependent and independent pathways. In addition, *CDF1* RNAi cause a very weak early flowering phenotype and only slightly suppresses the late flowering phenotype of *fkf1* (Imaizumi et al. 2005), suggesting that there are other important factors regulating flowering time under FKF1.

Conclusion

A circadian clock has pivotal roles for many physiological phenomena in *Arabidopsis*, including photoperiodic flowering and hypocotyl elongation. Fine tuning of the circadian mechanisms responding to environment is important for the regulation. Recent biochemical studies have made substantial progress in revealing the importance of post-transcriptional/transclational regulations of central clock machinery and its related input and output pathways. A major breakthrough was the finding that ZTL family members comprise a novel type of blue light receptor which directly regulates the clock and photoperiodic flowering. They function with other proteins for the proper targeted degradation of substrate proteins in a light dependent manner. However, the precise mechanisms are still not clear. ZTL family members interact with many kinds of proteins. Some of the interactors possibly regulate ZTL family members and the other might be degraded by them or function together. How might they coordinate so many factors? Protein modifications such as phosphorylation would be important for the proper recognition of interactors and the regulation of stabilization and degradation. Changing the forms and the members of complex formation might allow them to regulate many kinds of events depending on their circumstances. Further biochemical analyses combined with genetic approaches will lead us towards further understanding.

Acknowledgement

I would like to thank Dr. David E Somers for critical reading of the manuscript. I am funded by NSF grant MCB 0544137 to Dr. David E Somers and Support for Long-term Visit from Yamada Science Foundation.

References

Alabadi D, Oyama T, Yanovsky MJ, Harmon FG, Mas P, Kay SA (2001) Reciprocal regulation between TOC1 and LHY/CCA1 within the Arabidopsis circadian clock. *Science* 293: 880–883

Briggs WR, Christie JM (2002) Phototropins 1 and 2: versatile plant blue-light receptors. *Trends Plant Sci* 7: 204–210

Cheng P, He Q, Yang Y, Wang L, Liu Y (2003) Functional conservation of light, oxygen, or voltage domains in light sensing. *Proc Natl Acad Sci USA* 100: 5938–5943

Corbesier L, Vincent C, Jang S, Fornara F, Fan Q, Searle I, Giakountis A, Farrona S, Gissot L, Turnbull C, Coupland G (2007) FT protein movement contributes to long-distance signaling in floral induction of Arabidopsis. *Science* 316: 1030–1033

David KM, Armbruster U, Tama N, Putterill J (2006) Arabidopsis GIGANTEA protein is post-transcriptionally regulated by light and dark. *FEBS Lett* 580: 1193–1197

Deshaies RJ (1999) SCF and Cullin/Ring H2-based ubiquitin ligases. *Annu Rev Cell Dev Biol* 15: 435–467

Dunlap JC (1999) Molecular bases for circadian clocks. *Cell* 96: 271–290

Fowler S, Lee K, Onouchi H, Samach A, Richardson K, Morris B, Coupland G, 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

Fujiwara S, Oda A, Kamada H, Coupland G, Mizoguchi T (2005) Circadian clock components in *Arabidopsis* II. LHY/CCA1 regulate the floral integrator gene *SOC1* in both GI-dependent and -independent pathways. *Plant Biotechnol* 22: 319–325

Fujiwara S, Nakagawa M, Kamada H, Mizoguchi T (2005) Circadian clock components in *Arabidopsis* III. LHY/CCA1/GI in regulating the floral integrator genes *LFY*/*SOC1*/*FT* to control flowering time and shoot architecture. *Plant Biotechnol* 22: 327–331

Fukamatsu Y, Mitsui S, Yasuhara M, Tokioka Y, Ihara N, Fujita S, Kiyosue T (2005) Identification of LOV KELCH PROTEIN2 (LKP2)-interacting factors that can recruit LKP2 to nuclear bodies. *Plant Cell Physiol* 46: 1340–1349

Garcia-Higuera I, Gaitatzes C, Smith TF, Neeri EJ (1998) Folding a WD Repeat Propeller. *J Bio Chem* 273: 9041–9049

Han L, Mason M, Risseeuw EP, Crosby WL, Somers DE (2004) Formation of an SCF complex is required for proper regulation of circadian timing. *Plant J* 40: 291–301

Harmer SL, Hogenesch JB, Straume M, Chang HS, Han B, Zhu T, Wang X, Kreps JA, Kay SA (2000) Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. *Science* 290: 2110–2113

Imaizumi T, Kay SA (2006) Photoperiodic control of flowering: not only by coincidence. *Trends Plant Sci* 11: 550–558

Imaizumi T, Schultz TF, Harmon FG, Ho LA, Kay SA (2005) FKF1 F-box protein mediates cyclic degradation of a repressor of CONSTANS in Arabidopsis. *Science* 309: 293–297

Imaizumi T, Tran HG, Swartz TE, Briggs WR, Kay SA (2003) FKF1 is essential for photoperiodic-specific light signalling in Arabidopsis. *Nature* 426: 302–306

Jarillo JA, Capel J, Tang RH, Yang HQ, Alonso JM, Ecker JR, Cashmore AR (2001) An Arabidopsis circadian clock component interacts with both CRY1 and phyB. *Nature* 410: 487–490

Kevei E, Gyula P, Hall A, Kozma-Bognár L, Kim WY, Eriksson ME, Tóth R, Hanano S, Fehér B, Southern MM, Bastow RM, Viczián A, Hibberd V, Davis SJ, Somers DE, Nagy F, Millar AJ (2006) Forward genetic analysis of the circadian clock separates the multiple functions of *ZEITLUPE*. *Plant Physiol* 140: 933–945

Kiba T, Henriques R, Sakakibara H, Chua NH (2007) Targeted

Degradation of PSEUDO-RESPONSE REGULATOR5 by an SCF^{ZTL} Complex Regulates Clock Function and Photomorphogenesis in *Arabidopsis thaliana*. *Plant Cell* 19: 2516–2530

Kim WY, Fujiwara S, Suh SS, Kim J, Kim Y, Han L, David K, Putterill J, Nam HG, Somers DE (2007) ZEITLUPE is a circadian photoreceptor stabilized by GIGANTEA in blue light. *Nature* 449: 356–360

Kim WY, Geng R, Somers DE (2003) Circadian phase-specific degradation of the F-box protein ZTL is mediated by the proteasome. *Proc Natl Acad Sci USA* 100: 4933–4938

Kiyosue T, Wada M (2000) LKP1 (LOV kelch protein 1): a factor involved in the regulation of flowering time in Arabidopsis. *Plant J* 23: 807–815

Makino S, Matsushika A, Kojima M, Yamashino T, Mizuno T (2002) The APRR1/TOC1 quintet implicated in circadian rhythms of Arabidopsis thaliana: I. Characterization with APRR1-overexpressing plants. *Plant Cell Physiol* 43: 58–69

Mas P, Alabadi D, Yanovsky MJ, Oyama T, Kay SA (2003a) Dual role of TOC1 in the control of circadian and photomorphogenic responses in Arabidopsis. *Plant Cell* 15: 223–236

Mas P, Kim WY, Somers DE, Kay SA (2003b) Targeted degradation of TOC1 by ZTL modulates circadian function in Arabidopsis thaliana. *Nature* 426: 567–570

Matsushika A, Kawamura M, Nakamura Y, Kato T, Murakami M, Yamashino T, Mizuno T (2007) Characterization of Circadian-Associated Pseudo-Response Regulators: II. The Function of PRR5 and Its Molecular Dissection in *Arabidopsis thaliana*. *Biosci Biotechnol Biochem* 71: 535–544

McClung CR (2006) Plant circadian rhythms. *Plant Cell* 18: 792–803

Millar AJ, Carre IA, Strayer CA, Chua N-H, Kay SA (1995) Circadian clock mutants in *Arabidopsis* identified by luciferase imaging. *Science* 267: 1161–1163

Mizoguchi T, Wheatley K, Hanzawa Y, Wright L, Mizoguchi M, Song HR, Carre IA, Coupland G (2002) LHY and CCA1 are partially redundant genes required to maintain circadian rhythms in Arabidopsis. *Dev Cell* 2: 629–641

Mizoguchi T, Wright L, Fujiwara S, Cremer F, Lee K, Onouchi H, Mouradov A, Fowler S, Kamada H, Putterill J, Coupland G (2005) Distinct Roles of GIGANTEA in Promoting Flowering and Regulating Circadian Rhythms in Arabidopsis. *Plant Cell* 17: 2255–2270

Mizuno T, Nakamichi N (2005) Pseudo-Response Regulators (PRRs) or True Oscillator Components (TOCs). *Plant Cell Physiol* 46: 677–685

Nelson DC, Lasswell J, Rogg LE, Cohen MA, Bartel B (2000) FKF1, a clock-controlled gene that regulates the transition to flowering in Arabidopsis. *Cell* 101: 331–340

Niinuma K, Nakagawa M, Calvino M, Mizoguchi T (2007) Dance of plants with circadian clock. *Plant Biotechnol* 24: 87–97

Para A, Farré EM, Imaizumi T, Pruneda-Paz JL, Harmon FG, Kay SA (2007) PRR3 Is a Vascular Regulator of TOC1 Stability in the Arabidopsis Circadian Clock. *Plant Cell* 19: 3462–3473

Sakai T, Kagawa T, Kasahara M, Swartz TE, Christie JM, Briggs WR, Wada M, Okada K (2001) Arabidopsis nph1 and npl1: blue light receptors that mediate both phototropism and chloroplast relocation. *Proc Natl Acad Sci USA* 98: 6969–6974

Salomon M, Christie JM, Knieb E, Lempert U, Briggs WR (2000) Photochemical and mutational analysis of the FMN-binding domains of the plant blue light receptor, phototropin. *Biochemistry* 39: 9401–9410

Sawa M, Nusinow DA, Kay SA, Imaizumi T (2007) FKF1 and GIGANTEA Complex Formation Is Required for Day-Length Measurement in *Arabidopsis*. *Science* 318: 261–265

Schultz TF, Kiyosue T, Yanovsky M, Wada M, Kay SA (2001) A role for LKP2 in the circadian clock of Arabidopsis. *Plant Cell* 13: 2659–2670

Suarez-Lopez P, Wheatley K, Robson F, Onouchi H, Valverde F, Coupland G (2001) CONSTANS mediates between the circadian clock and the control of flowering in Arabidopsis. *Nature* 410: 1116–1120.

Somers DE, Kim WY, Geng R (2004) The F-Box Protein ZEITLUPE Confers Dosage-Dependent Control on the Circadian Clock, Photomorphogenesis, and Flowering Time. *Plant Cell* 16: 769–782

Somers DE, Schultz TF, Milnamow M, Kay SA (2000) ZEITLUPE encodes a novel clock-associated PAS protein from *Arabidopsis*. *Cell* 101: 319–329

Somers DE, Webb AAR, Pearson M, Kay S (1998) The short-period mutant, *toc1-1*, alters circadian clock regulation of multiple outputs throughout development in *Arabidopsis thaliana*. *Development* 125: 485–494

Tamaki S, Matsuo S, Wong HL, Yokoi S, Shimamoto K (2007) Hd3a protein is a mobile flowering signal in rice. *Science* 316: 1033–1036

Vierstra RD (2003) The ubiquitin/26S proteasome pathway, the complex last chapter in the life of many plant proteins. *Trends in Plant Sci* 8: 135–142

Yamamoto Y, Sato E, Shimizu T, Nakamich N, Sato S, Kato T, Tabata S, Nagatani A, Yamashino T, Mizuno T (2003) Comparative genetic studies on the APRR5 and APRR7 genes belonging to the APRR1/TOC1 quintet implicated in circadian rhythm, control of flowering time, and early photomorphogenesis. *Plant Cell Physiol* 44: 1119–1130

Yasuhara M, Mitsui S, Hirano H, Takanabe R, Tokioka Y, Ihara N, Komatsu A, Seki M, Shinozaki K, Kiyosue T. (2004) Identification of ASK and clock-associated proteins as molecular partners of LKP2 (LOV kelch protein 2) in *Arabidopsis*. *J Exp Bot* 55: 2015–2027

Yanovsky MJ, Kay SA (2003) Living by the calendar: how plants know when to flower. *Nat Rev Mol Cell Biol* 4: 265–275