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Mini Review

Molecular Mechanisms Underlying the *Arabidopsis* Circadian Clock

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A wide range of biological processes exhibit circadian rhythm, enabling plants to adapt to the environmental day–night cycle. This rhythm is generated by the so-called 'circadian clock'. Although a number of genetic approaches have identified >25 clock-associated genes involved in the Arabidopsis clock mechanism, the molecular functions of a large part of these genes are not known. Recent comprehensive studies have revealed the molecular functions of several key clock-associated proteins. This progress has provided mechanistic insights into how key clock-associated proteins are integrated, and may help in understanding the essence of the clock's molecular mechanisms.

Keywords: *Arabidopsis thaliana* • Circadian clock • Genetic circuit • Protein function.

Abbreviations: bHLH, basic helix–loop–helix; CCA1, CIRCADIAN CLOCK-ASSOCIATED 1; CCT, CONSTANS, CONSTANS-LIKE1 and TOC1; CHE, CCA1 HIKING EXPEDITION; CK2, CASEIN KINASE 2; CKB, CASEIN KINASE β SUBUNIT; ELF3, EARLY FLOWERING 3; ELF4, EARLY FLOWERING 4; FIO1, FIONA1; FKF1, FLAVIN BINDING, KELCH REPEAT, F-BOX1; GI, GIGANTEA; JMJ, JUMONJI; LHY, LATE ELONGATED HYPOCOTYL; LKP2, LOV KELCH PROTEIN2; LOV, light, oxygen, voltage; LUX, LUXARRHYTHMO; LWD, LIGHT-REGULATED WD; PCL1, PHYTOCLOCK 1; PR, Pseudo-receiver; PRMT5, PROTEIN ARGININE METHYL TRANSFERASE 5; PRR, PSEUDO-RESPONSE REGULATOR; RVE, REVEILLE; TCP, TEOSINTE BRANCHED1, CYCLOIDEA and PCF; TIC, TIME FOR COFFEE; TOC1, TIMING OF CAB EXPRESSION 1; ZTL, ZEITLUPE.

Introduction

Circadian rhythm is the temporal oscillation of genetic, metabolic and physiological processes based on the 24 h cycle, allowing organisms to anticipate day–night changes in the environment (Bunning 1967, Pittendrigh 1993). A wide variety of organisms from cyanobacteria to mammals display circadian rhythms at the level of metabolism, physiology and behavior under conditions in which there are no external time cues, indicating that these rhythms are driven by an endogenous timekeeping mechanism, the so-called 'circadian clock'.

The circadian clock in Arabidopsis plants regulates a number of biological processes, such as rhythmic leaf movement (Bunning 1967, Millar et al. 1995), petal opening (Bunning 1967), the elongation rate of stems, hypocotyls and roots (Lecharny 1985, Dowson-Day and Millar 1999, Nozue et al. 2007, Yazdanbakhsh et al. 2011), circumnutation of stems (Niinuma et al. 2005), central and secondary metabolite biosynthesis (Warren and Wilkins 1961, Kolosova et al. 2001, Blasing et al. 2005, Fukushima et al. 2009), hormone biosynthesis and responses (Thain et al. 2004, Covington and Harmer 2007, Covington et al. 2008, Michael et al. 2008, Mizuno and Yamashino 2008), water stress responses (Fowler et al. 2005, Bieniawska et al. 2008, Kidokoro et al. 2009, Legnaioli et al. 2009, Nakamichi et al. 2009), stomatal opening (Holmes 1986, Somers et al. 1998), Ca^{2+} concentrations in certain cellular compartments (Johnson et al. 1995, Xu et al. 2007), water uptake (Takase et al. 2011), seed dormancy (Penfield and Hall 2009) and defence against pathogens (W. Wang et al. 2011). In addition, the clock is used in some plants to measure the environmental photoperiod to induce inflorescence meristems (Bunning 1967), so that flowering occurs during the correct season (photoperiodic flowering) (Ganner and Allard 1920). These phenomena coordinately contribute to fitness (or adaptive advantage) in 24 h day–night cycles (Green et al. 2002, Dodd et al. 2005, Yerushalmi et al. 2011).

Mutant screening and genetic mapping–cloning approaches have been taken in Arabidopsis in order to understand the molecular mechanisms of the plant clock (Millar et al. 1995). At least 25 genes associated with clock function have been identified by classical genetics strategies, as well as by reverse genetics (**Fig. 1**). A number of recent studies have revealed the molecular functions of clock-associated proteins, which have long been undetermined. These findings provide us for the first

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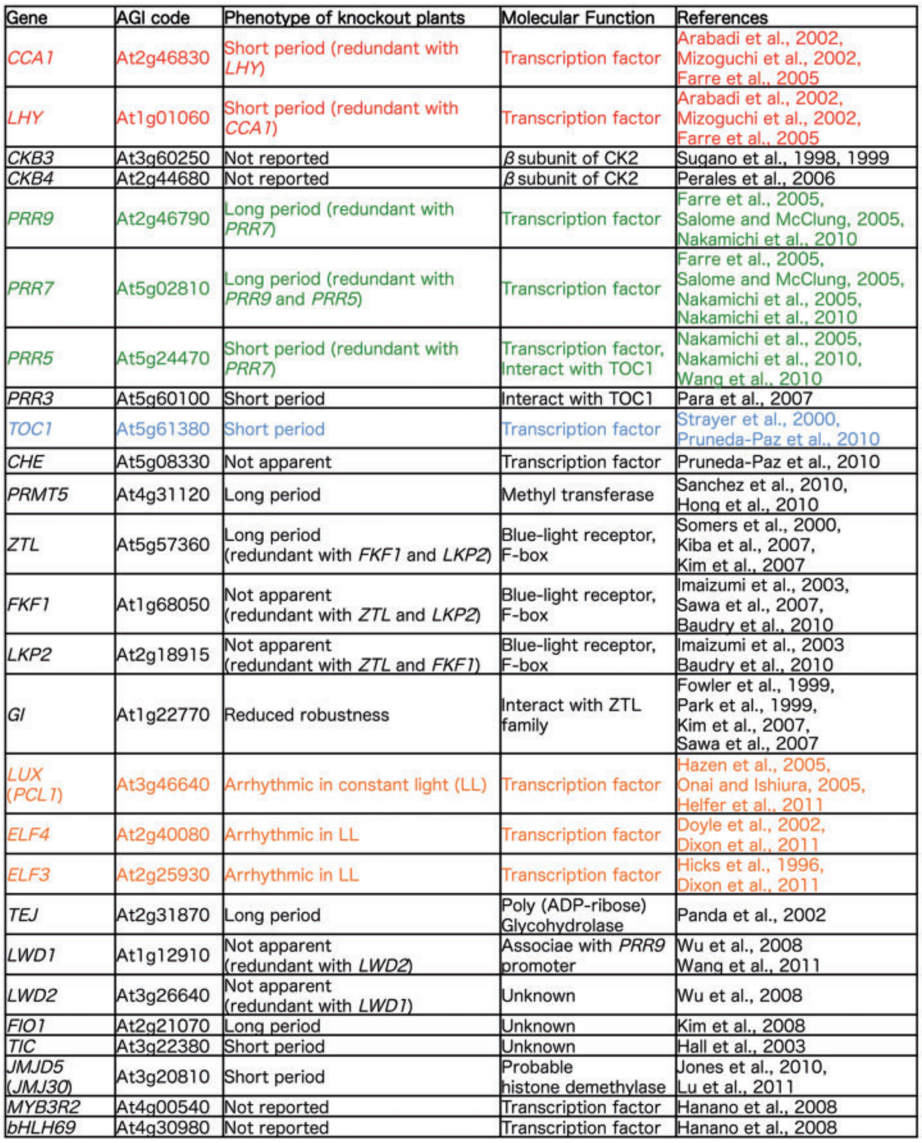

Gene	AGI code	Phenotype of knockout plants	Molecular Function	References
CCA1	At2g46830	Short period (redundant with *LHY*)	Transcription factor	Arabadi et al., 2002, Mizoguchi et al., 2002, Farre et al., 2005
LHY	At1g01060	Short period (redundant with *CCA1*)	Transcription factor	Arabadi et al., 2002, Mizoguchi et al., 2002, Farre et al., 2005
CKB3	At3g60250	Not reported	β subunit of CK2	Sugano et al., 1998, 1999
CKB4	At2g44680	Not reported	β subunit of CK2	Perales et al., 2006
PRR9	At2g46790	Long period (redundant with *PRR7*)	Transcription factor	Farre et al., 2005, Salome and McClung, 2005, Nakamichi et al., 2010
PRR7	At5g02810	Long period (redundant with *PRR9* and *PRR5*)	Transcription factor	Farre et al., 2005, Salome and McClung, 2005, Nakamichi et al., 2005, Nakamichi et al., 2010
PRR5	At5g24470	Short period (redundant with *PRR7*)	Transcription factor, Interact with TOC1	Nakamichi et al., 2005, Nakamichi et al., 2010, Wang et al., 2010
PRR3	At5g60100	Short period	Interact with TOC1	Para et al., 2007
TOC1	At5g61380	Short period	Transcription factor	Strayer et al., 2000, Pruneda-Paz et al., 2010
CHE	At5g08330	Not apparent	Transcription factor	Pruneda-Paz et al., 2010
PRMT5	At4g31120	Long period	Methyl transferase	Sanchez et al., 2010, Hong et al., 2010
ZTL	At5g57360	Long period (redundant with *FKF1* and *LKP2*)	Blue-light receptor, F-box	Somers et al., 2000, Kiba et al., 2007, Kim et al., 2007
FKF1	At1g68050	Not apparent (redundant with *ZTL* and *LKP2*)	Blue-light receptor, F-box	Imaizumi et al., 2003, Sawa et al., 2007, Baudry et al., 2010
LKP2	At2g18915	Not apparent (redundant with *ZTL* and *FKF1*)	Blue-light receptor, F-box	Imaizumi et al., 2003 Baudry et al., 2010
GI	At1g22770	Reduced robustness	Interact with ZTL family	Fowler et al., 1999, Park et al., 1999, Kim et al., 2007, Sawa et al., 2007
LUX (*PCL1*)	At3g46640	Arrhythmic in constant light (LL)	Transcription factor	Hazen et al., 2005, Onai and Ishiura, 2005, Helfer et al., 2011
ELF4	At2g40080	Arrhythmic in LL	Transcription factor	Doyle et al., 2002, Dixon et al., 2011
ELF3	At2g25930	Arrhythmic in LL	Transcription factor	Hicks et al., 1996, Dixon et al., 2011
TEJ	At2g31870	Long period	Poly (ADP-ribose) Glycohydrolase	Panda et al., 2002
LWD1	At1g12910	Not apparent (redundant with *LWD2*)	Associae with *PRR9* promoter	Wu et al., 2008 Wang et al., 2011
LWD2	At3g26640	Not apparent (redundant with *LWD1*)	Unknown	Wu et al., 2008
FIO1	At2g21070	Long period	Unknown	Kim et al., 2008
TIC	At3g22380	Short period	Unknown	Hall et al., 2003
JMJD5 (*JMJ30*)	At3g20810	Short period	Probable histone demethylase	Jones et al., 2010, Lu et al., 2011
MYB3R2	At4g00540	Not reported	Transcription factor	Hanano et al., 2008
bHLH69	At4g30980	Not reported	Transcription factor	Hanano et al., 2008

Fig. 1 List of genes involved in the Arabidopsis circadian clock. Genes colored in red act in the morning ('early shift'), green-colored genes are active from early daytime until midnight, and those in light blue and orange act during the night.

time with enough information to understand how and when clock-associated proteins act in the circadian clock. It is now apparent that these genes interact to form a 'genetic circuit' which underlies the 24 h endogenous cycle. In this review, recent studies on the temporal and functional characterization of clock-associated proteins are summarized, followed by a discussion of how these proteins are integrated into the genetic circuit in the clock.

Clock-associated genes in Arabidopsis

Identification of clock genes began with traditional genetic approaches in the 1990s. Several key genes, including *TIMING OF CAB EXPRESSION 1* (*TOC1*), *ZEITLUPE* (*ZTL*), *TEJ*, *TIME FOR COFFEE* (*TIC*), *LUXARRHYTHMO* [*LUX* or *PHYTOCLOCK 1* (*PCL1*)], *FIONA1* (*FIO1*) and *PROTEIN ARGININE METHYL TRANSFERASE 5* (*PRMT5*), were isolated via large-scale screening experiments using gene promoters controlled under the circadian clock (e.g. *chlorophyll a/b-binding protein*, *Cab*) (Somers et al. 2000, Strayer et al. 2000, Panda et al. 2002, Hall et al. 2003, Hazen et al. 2005b, Onai and Ishiura 2005, Kim et al. 2008, Hong et al. 2010, Sanchez et al. 2010). Furthermore, the screening of mutants impaired in biological processes regulated by the circadian clock, such as photoperiodic flowering and hypocotyl elongation, led to the isolation of *EARLY FLOWERING 3* (*ELF3*), *ELF4*, *GIGANTEA* (*GI*) and *LATE ELONGATED HYPOCOTYL* (*LHY*) (Schaffer et al. 1998, Fowler et al. 1999, Park et al. 1999, Hicks et al. 2001, Doyle et al. 2002).

Several approaches have been used to identify other key clock-associated genes. *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *CCA1 HIKING EXPEDITION* (*CHE*) were identified by isolating proteins that bind to rhythmic gene promoters (Wang et al. 1997, Wang and Tobin 1998, Pruneda-Paz et al. 2009). Reverse genetics approaches have identified four *TOC1* homologs, *PSEUDO-RESPONSE REGULATOR 9* (*PRR9*), *PRR7*, *PRR5* and *PRR3*, as components of the clock (Matsushika et al. 2000, Eriksson et al. 2003, Michael et al. 2003, Yamamoto et al. 2003, Para et al. 2007). Functional redundancies among genes showing high sequence similarities are often observed in Arabidopsis, which may make further discovery of novel clock-associated genes by a classical genetic approach technically challenging. For example, independent large-scale mutagenesis screenings identified allelic mutants, suggesting that these screenings were saturated (Onai et al. 2004, Hazen et al. 2005a). Biochemical and reverse genetic approaches, however, have identified new genes. Chemical genetics is emerging as a method which can also transcend the problem of functional redundancy by targeting specific receptor–ligand or other small molecule interactions.

MYB transcription factors take the 'early shift'

CCA1 and LHY (CCA1/LHY) are the closest paralogs of a single MYB transcription factor expressed with a morning acrophase, indicating that these genes take the 'early shift' in clock regulation, corresponding to early morning duty in a shift-work labor system (**Fig. 2A**) (Wang et al. 1997, Schaffer et al. 1998, Wang and Tobin 1998, Perales and Mas 2007). Overexpression of *CCA1* or *LHY* abolishes the rhythms of clock output genes under constant light conditions (Schaffer et al. 1998, Wang and Tobin 1998). Single mutations in the *cca1* or *lhy* loci have a short period phenotype, and *cca1 lhy* double mutants have a shorter period than either single mutation alone, indicating that CCA1/LHY are redundant but that both are required for proper clock function (Green and Tobin 1999, Alabadi et al. 2002, Mizoguchi et al. 2002). CCA1/LHY bind to a CCA1-binding site (AACAATCT or AAAAATCT) and to an evening element (AAAATATCT) (Wang et al. 1997, Alabadi et al. 2001). They repress the transcription of *TOC1*, *ELF4* and *LUX* (Alabadi et al. 2001, Hazen et al. 2005b, Perales and Mas 2007, Li et al. 2011), but activate the transcription of *PRR9* and *PRR7* through these binding sites (Farre et al. 2005) (**Fig. 2A**).

CCA1 function is subject to post-translational modification. CCA1 is phosphorylated by the protein kinase CASEIN KINASE 2 (CK2), which is required for the formation of a DNA–protein complex containing CCA1 (Sugano et al. 1998, Sugano et al. 1999). Overexpressors of the *CKB3* regulatory subunit of CK2 (Sugano et al. 1998), or *CKB4* (Perales et al. 2006), which exhibits higher CK2 activity, display a short-period phenotype. On the other hand, overexpression of a mutant CCA1 which cannot be phosphorylated by CK2 does not result in a hypermorphic phenotype (Daniel et al. 2004), suggesting that CCA1 phosphorylation by CK2 is necessary for its function in the Arabidopsis clock. However, in rice it is unlikely that CK2 is involved in the clock mechanism. This difference was partly attributed to the lack of a serine residue, which is a CK2 target site, in OsLHY (a CCA1 ortholog), implying the divergence of post-translational regulation of CCA1 and LHY in higher plants during evolution (Ogiso et al. 2010).

Among the seven close homologs of CCA1/LHY in Arabidopsis, EARLY-PHYTOCHROME-RESPONSIVE 1 or REVEILLE 7 (RVE7) (Kuno et al. 2003), CIRCADIAN 1 or RVE2 (Zhang et al. 2007), and RVE1 (Rawat et al. 2009) have been implicated in the output function of the clock. Recently, however, it was shown that one member of the homolog set, RVE8 or LHY-CCA1-LIKE 5, is involved in the clock by directly activating the expression of both *TOC1* and *PRR5* (Farinas and Mas 2011, Rawat et al. 2011).

PRR9, PRR7 and PRR5 take the 'day and swing shifts'

PRR9, PRR7 and PRR5 (PRRs) are sequentially expressed from early daytime until around midnight, corresponding to the 'day shift' and 'swing shift' in this metaphorical shift-work system (Farre and Kay 2007, Ito et al. 2007b, Kiba et al. 2007, Fujiwara et al. 2008, Nakamichi et al. 2010) (**Fig. 2B**). They possess a pseudo-receiver (PR) domain at their N-termini, and a CONSTANS, CONSTANS-LIKE1 and TOC1 (CCT) motif at their C-termini (Makino et al. 2000, Matsushika et al. 2000, Strayer et al. 2000).

Genetic studies have shown that *PRR9*, *PRR7* and *PRR5* function redundantly and/or synergistically within the clock mechanism (Farre et al. 2005, Nakamichi et al. 2005, Salome and McClung 2005). Given that expression of *CCA1* and *LHY* is decreased in *PRR7* or *PRR5* overexpression lines (Sato et al. 2002, Farre and Kay 2007), and increased in *prr9 prr7*, *prr7 prr5* and *prr9 prr7 prr5* mutants (Farre et al. 2005, Nakamichi et al. 2005, Salome et al. 2010), these *PRR* genes are likely to be negative regulators of *CCA1* and *LHY*. A recent study demonstrated that these PRR proteins associate with *CCA1* and *LHY* promoters in vivo, and repress these genes from early daytime until midnight (Nakamichi et al. 2010) (**Fig. 2B**). Each PRR protein works at a specific time; PRR9 functions during early daytime, PRR7 is active from early daytime until midnight, and PRR5 works from noon until midnight. PRRs seem to act as active transcriptional repressors, because the repression motif, which confers transcriptional repressor activity on the yeast GAL4 DNA-binding domain, is present in the PRRs (Nakamichi et al. 2010). However, it is not yet known how the PRRs are recruited to the *CCA1* and *LHY* promoter regions. Given that the CCT motif of CONSTANS is involved in interactions with the target DNA (Wenkel et al. 2006, Tiwari et al. 2010), it is possible that the CCT motif of PRRs is also involved

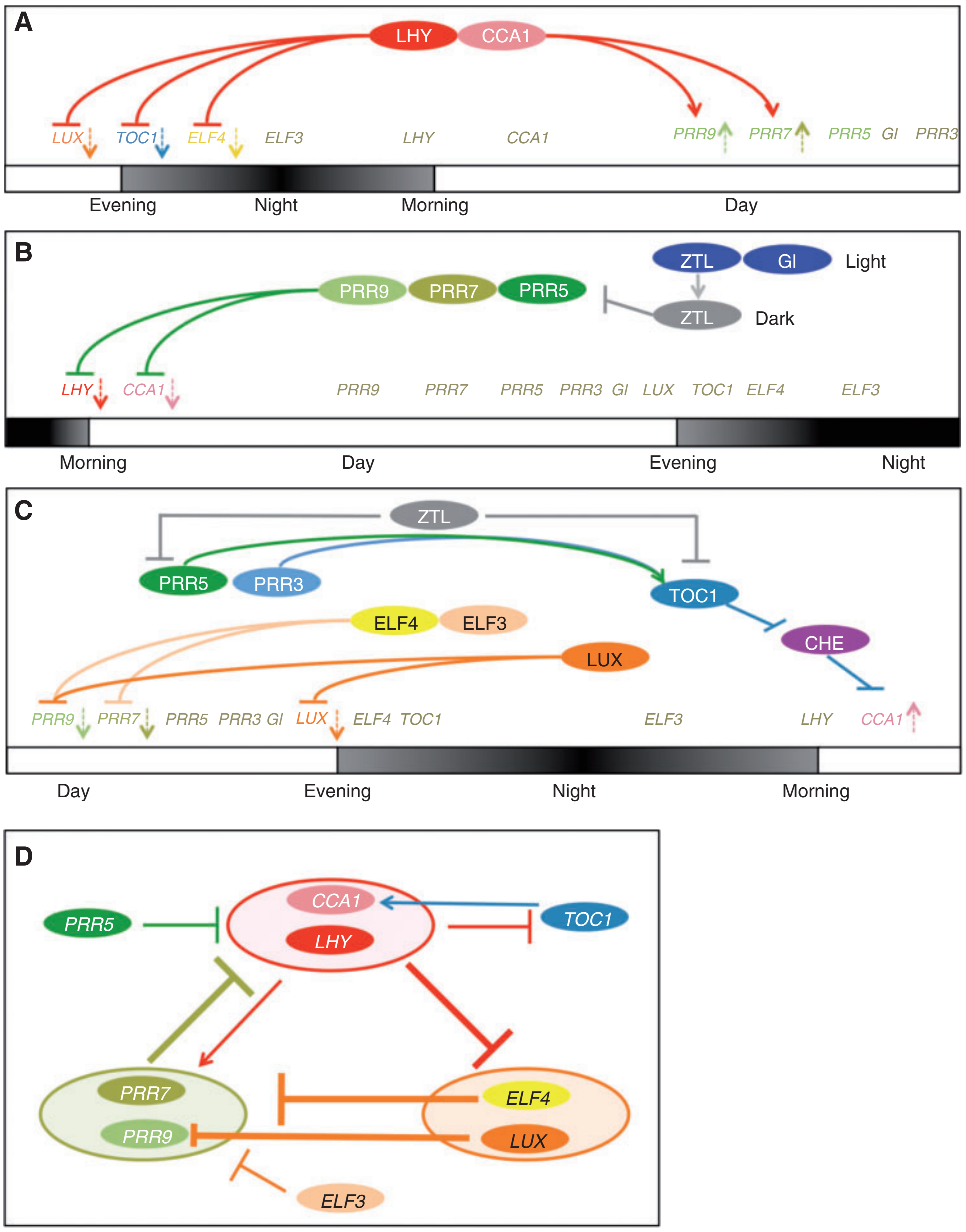

Fig. 2 Timetable for actions of clock-associated proteins. Genes colored in red act in the morning ('early shift'), green-colored genes are active from early daytime until midnight, and light blue and orange genes act during the night. (A) In the morning, LHY and CCA1 proteins repress the evening-phase genes *LUX*, *TOC1* and *ELF4*, and activate the day-phase genes *PRR9* and *PRR7*. (B) From early daytime until midnight, PRR9, PRR7 and PRR5 repress the morning-phase genes *CCA1* and *LHY*. Blue light enhances interaction of ZTL and GI. In the dark, the ZTL–GI complex is decoupled, allowing ZTL to promote the degradation of PRR5. (C) In the evening, LUX represses *PRR9* and *LUX* expression, and ELF4 and ELF3 repress *PRR9* and *PRR7*. PRR5 enhances nuclear localization of TOC1, and PRR3 stabilizes TOC1 in the evening. On the other hand, ZTL promotes the degradation of PRR5 and TOC1 at night (in darkness). TOC1 acts to activate *CCA1* expression by antagonizing CHE on the *CCA1* promoter. (D) Genetic circuit in the clock. CCA1 and LHY activate *PRR9* and *PRR7*, and repress *TOC1*, *ELF4* and *LUX*. ELF4, LUX and ELF3 repress *PRR9* and *PRR7*. PRR9, PRR7 and PRR5 repress *CCA1* and *LHY*, whereas TOC1 activates *CCA1*. These interactions illustrate the 'genetic circuit', which underlies the 24 h endogenous cycle.

in interactions with target DNA regions. In addition, PRR proteins participate in post-translational regulation. PRR5 interacts with TOC1 through its PR domains, and enhances phosphorylation and nuclear localization of TOC1 (Wang et al. 2010) (**Fig. 2C**).

'The graveyard shift': *TOC1*, *ELF3*, *ELF4* and *LUX*

The *toc1-1* mutant was the very first Arabidopsis clock mutant found, identified because of its short-period phenotype

(Millar et al. 1995, Strayer et al. 2000). *TOC1* proteins are expressed during the night, the time of the 'graveyard shift' (Makino et al. 2000, Matsushika et al. 2000, Strayer et al. 2000). Reduction of *TOC1* expression by RNA interference (RNAi) shortens the period length, whereas increased *TOC1* expression under the control of its own promoter results in a lengthened period (Mas et al. 2003a), suggesting that both the timing and level of TOC1 expression are crucial for maintaining a proper period length. *TOC1* is thus subjected to multiple layers of regulation. In addition to the transcriptional and post-transcriptional regulation of *TOC1* described in earlier sections (**Fig. 2A, C**), TOC1 is targeted for degradation by ZTL family proteins (**Fig. 2C**) (Mas et al. 2003b, Kim et al. 2007, Baudry et al. 2010). On the other hand, TOC1 is stabilized by PRR3 via protein–protein interactions (**Fig. 2C**) (Para et al. 2007).

The exact molecular function of TOC1 within the circadian clock remained elusive for a long time, but recent studies indicate that TOC1 is a transcriptional regulator. TOC1 activates *CCA1* expression by antagonizing the action of CHE, a repressor of *CCA1* (Pruneda-Paz et al. 2009) (**Fig. 2C**). CHE is a TCP (for TEOSINTE BRANCHED1, CYCLOIDEA and PCF) transcription factor. Other TCPs also have the ability to bind to TOC1 (Giraud et al. 2010), implying that TOC1 activates *CCA1* expression through its binding to TCPs.

ELF3, *ELF4* and *LUX* are essential for sustaining the circadian rhythm under constant light conditions, since mutations in each gene result in arrhythmia (Hicks et al. 1996, Doyle et al. 2002, Hazen et al. 2005b, Onai and Ishiura 2005). *ELF3*, *ELF4* and *LUX* are expressed from evening until midnight. All three genes are required for full *CCA1* and *LHY* expression, as evidenced by decreased expression of *CCA1* and *LHY* in *elf3*, *elf4* and *lux* mutants (Doyle et al. 2002, Hazen et al. 2005b, Kikis et al. 2005, Onai and Ishiura 2005). *ELF3*, *ELF4* and *LUX* encode structurally distinct proteins: ELF3 encodes a putative transcriptional regulator, ELF4 encodes a protein with unknown function, and LUX encodes a GARP-type MYB transcription factor. Recent studies indicate that ELF3 and ELF4 are transcriptional repressors of *PRR9* and *PRR7*, and that LUX is a night-time repressor of *PRR9* (Dixon et al. 2011, Helfer et al. 2011) (**Fig. 2C**). LUX also directly represses its own expression by binding to the *LUX* promoter, forming a negative feedback loop (Helfer et al. 2011).

The ZTL–GI complex as a light sensor

ZTL is the best characterized factor involved in post-translational regulation of clock-associated proteins. ZTL protein contains an F-box domain, which is a component of the Skp/Cullin/F-box E3 ubiquitin ligases acting in the proteasome-dependent protein degradation pathway. In addition, this protein possesses an N-terminal LOV (light, oxygen, voltage-dependent) domain that perceives blue light, suggesting that ZTL functions as a blue light-regulated F-box protein. Indeed, ZTL targets TOC1 and PRR5 for degradation in the absence of blue light (Mas et al. 2003b, Kiba et al. 2007). There are two ZTL homologs in Arabidopsis: FLAVIN BINDING, KELCH REPEAT, F-BOX1 (FKF1) (Nelson et al. 2000, Imaizumi et al. 2003, Sawa et al. 2007) and LOV KELCH PROTEIN2 (LKP2) (Schultz et al. 2001). A recent study indicated that all members of the ZTL protein family are engaged in PRR5 and TOC1 degradation (Baudry et al. 2010).

Recently, Kim et al. (2007) demonstrated that ZTL oscillation (low in the morning; high in the evening) is established and sustained by blue light-enhanced interaction with GI, which is a factor responsible for the robustness of the rhythm, but with no known functional domain (Kim et al. 2007). The blue light-activated ZTL forms a complex with GI, resulting in stabilization of ZTL. In darkness, the complex is attenuated, thereby destabilizing ZTL. Because the timing of GI expression is determined by the clock, complex formation is also under clock control (David et al. 2006). The external light conditions and internal circadian clock together confer fine-tuned rhythms to ZTL, leading to robust TOC1 and PRR5 oscillations (Kim et al. 2007) (**Fig. 2B, C**).

Other, but indispensable, factors

There are some clock-associated proteins which are known to be involved in rhythmic control, but whose specific molecular function remains unknown. For example, *TEJ* (*TEJ* means 'bright' in Sanskrit) encodes a poly(ADP-ribose) glycohydrolase, which is involved in poly(ADP-ribosyl)ation of proteins (Panda et al. 2002). A *tej* mutation lengthens the circadian period. TEJ targets have not been identified.

TIC was named for the evidence that the gene is required for maintaining metabolic rhythm from mid- to late night when human activity often gets a boost from caffeine coffee (Hall et al. 2003). *TIC* encodes a nuclear-localized protein with probable ATP/GTP-binding site A motifs (P-loop) (Ding et al. 2007). Interestingly, the abundance and cellular localization of TIC are not under clock control, implying that some unknown factor or factors restricts the activity of TIC during the night (Ding et al. 2007).

A mutation in *FIO1* (fiona means 'flowering' in Korean) results in longer circadian periods (Kim et al. 2008). *FIO1* encodes a nuclear-localized protein with a DUF890 domain, which is found in the methyltransferase superfamily, though the precise function of FIO1 remains to be elucidated.

LIGHT-REGULATED WD1 (*LWD1*) and its closest homolog, *LWD2*, encode WD repeat-containing proteins (Wu et al. 2008). The *lwd1 lwd2* double mutant has a short-period phenotype. Recently, it was shown that LWD1 associates with the promoter region of *PRR9* to activate *PRR9* expression (Y. Wang et al. 2011).

Two transcription factors, MYB3R2 and bHLH69A, were identified through systematic screening in which transcription factors were tested for their potential to alter circadian rhythms

(Hanano et al. 2008). Although circadian periods were lengthened both in MYB3R2 and bHLH69A overexpression lines, the direct target of MYB3R2 and bHLH69 has not been identified.

A jumonji-C (JmjC) domain-containing protein (JMJD5 = JMJ30) gene, encoding a possible histone demethylase, was identified as a clock component (Jones et al. 2010, Lu et al. 2011). Mutations in *JMJD5* result in shortened circadian periods, and the mutation enhances the effect of a *toc1* mutation, suggesting that *JMJD5* interacts synergistically with *TOC1* (Jones et al. 2010).

Recently, *PRMT5*, which is involved in site-specific alternative splicing, was implicated in the clock mechanism (Hong et al. 2010, Sanchez et al. 2010). PRMT5 acts by dimethylating Sm proteins which participate in pre-mRNA splicing. The loss-of-function *prmt5* mutation results in a long-period phenotype. Splicing of the third intron of *PRR9* is impaired in *prmt5*, and the *prr9 prr7* double mutation is epistatic to *prmt5*, suggesting that PRMT5 controls the period length in part by regulating *PRR9* splicing.

Genetic circuit in the clock

Based on our current knowledge of the regulation and molecular functions of clock-associated genes, a 'genetic circuit' model has been proposed (Helfer et al. 2011). Three classes of repressors constitute the genetic circuit: (i) morning-phase proteins CCA1 and LHY repress *ELF4* and *LUX*; (ii) evening-phase proteins ELF4 and LUX repress *PRR9* and *PRR7*; and (iii) midday-phase proteins PRR9 and PRR7 repress *CCA1* and *LHY*. Although there is no experimental evidence to show that the dynamics of the proposed circuit is responsible for clock function, a synthetic genetic circuit, known as 'the repressilator', which is a cyclic negative feedback loop composed of three transcriptional repressor genes, can produce an oscillating pattern (Elowitz and Leibler 2000). The sustainable oscillation in 'the repressilator' is dependent on similar decay rates of protein and mRNA, and large amounts of protein at its peak level. Oscillation patterns can also be generated in networks containing an activator and a repressor, or an odd number of repressors over three, if stochastic characters are negligible in the networks. Such theoretical approaches help to understand the molecular basis and dynamics of the clock.

Perspectives

Recent progress in genome research indicates that clock-associated genes identified in Arabidopsis are mostly conserved among angiosperms (Song et al. 2010). Furthermore, evidence is accumulating that these genes are orthologs of corresponding genes in Arabidopsis, suggesting that the proposed molecular clock mechanism is conserved among angiosperms [*ELF3*, *GI* and *LHY* in duckweed (Miwa et al. 2006, Serikawa et al. 2008), *LHY* and *TOC1* in poplar (Ibanez et al. 2010)], including agriculturally important plants [*ELF4* and *GI* in pea (Hecht et al. 2007, Liew et al. 2009), and *GI* and *LHY* in rice (Ogiso et al. 2010, Izawa et al. 2011)]. A key trait in crops under clock control is photoperiodic flowering, a critical aspect of crop production that has been selected during domestication. For example, photoperiod-insensitive wheat varieties predominate in relatively warm regions where wheat needs to flower and mature before the onset of high summer temperatures. A genetic locus responsible for advancing the heading date of such varieties is present at the upstream region of the *PRR7* homolog (*Ppd-D1a*), which causes misexpression of this gene (Beales et al. 2007). On the other hand, mutations in a *PRR7* homolog (*Ppd-H1*) were found as the genetic loci which delay the heading date of spring-sown barley (Turner et al. 2005). The *ppd-H1* varieties have an extended vegetative growth phase and ultimately higher yields in Western Europe and North America. Quantitative trait loci for flowering time overlap with *PRR7* homologs in rice and *Brassica rapa* (Murakami et al. 2005, Lou et al. 2011). Furthermore, *GI* and *ELF4* orthologs were identified as the genetic loci that alter photoperiodic flowering in pea (Hecht et al. 2007, Liew et al. 2009). Therefore, studies of the Arabidopsis circadian clock should enable us to understand how plants generate responses to photoperiod, and thus to induce flowering, which will ultimately be a significant boon for agriculture.

Thanks to recent studies, the molecular functions and functional timing (duration) of many Arabidopsis clock-associated proteins have been determined, which has enabled us to propose a 'genetic circuit' model. Although it is widely accepted that the genetic circuit plays an important role in the clock system, whether or not the genetic circuit alone drives these rhythms is an open question. Since the transcriptional process is generally stochastic and depends on temperature and metabolic conditions (Raser and O'Shea 2005), and the circadian period is constant over a wide temperature range (known as temperature compensation), a genetic circuit alone does not meet the theoretical requirements for clock function. Recently, a post-translational (or non-transcriptional) oscillation was detected in cyanobacteria and the unicellular green alga *Ostreococcus tauri* (Nakajima et al. 2005, Tomita et al. 2005, O'Neill et al. 2011), which also possess transcription-based feedback loops (Kitayama et al. 2008, Corellou et al. 2009). The post-translational oscillators are coupled with transcription-based feedback loops in cyanobacteria and the green algae under physiological conditions (Kitayama et al. 2008, O'Neill et al. 2011). Interestingly, the cyanobacterial post-translational oscillator is sufficient to drive circadian rhythm by itself under extremely poor metabolic conditions and throughout a wide temperature range, illustrating the resilient nature of circadian periodicity. Compensations in period length for temperature fluctuations and metabolic changes are embedded in the post-translational oscillator (Nakajima et al. 2005, Tomita et al. 2005, Ito et al. 2007a). Whether the post-translational oscillator functions in higher plants is an open and interesting question that needs to be addressed before we can understand how the circadian clock evolved, and exactly how it functions in controlling the plant circadian clock. It is likely that answers to these questions will provide insights into many of the critical features of plant behavior.

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