Posttranslational Photomodulation of Circadian Amplitude

D.E. Somers,* S. Fujiwara,* W.-Y. Kim,*† AND S.-S. Suh*

*Department of Plant Cellular and Molecular Biology/Plant Biotechnology Center, Ohio State University Columbus, Ohio 43210, USA; †Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju 660-701, South Korea

The transcription-translation feedback loops that form our current view of how the core mechanism of the clock operates is being challenged, as more and more posttranslational events are seen as essential to a full understanding of oscillator function. But in addition to phosphorylation, other processes may be involved. Here, a novel mechanism of posttranslational photomodulation of circadian amplitude is described that uniquely ties together light perception, protein stabilization, and proteolysis. In the process, the waveform of a core clock component is sharpened or “sculpted,” resulting in appropriately high amplitude and proper phasing to obtain normal clock function.

INTRODUCTION

The earliest models that emerged in understanding eukaryotic clock function converged on a molecular view of the oscillator that involves a transcription-translation autoregulatory negative feedback loop (Young and Kay 2001; Bell-Pedersen et al. 2005). In this model, core transcription factors positively activate transcription of clock genes which are transcribed and translated into proteins in the cytoplasm. Certain of these cytoplasmic proteins undergo posttranslational modifications, generally phosphorylation, that facilitate their nuclear import. These factors then act to inhibit the positive activation of their own genes by interfering with the transcriptional activity of the dedicated core transcription factors. The cycle can begin again once the autoregulatory inhibitors are degraded and the transcriptional activators are free to bind.

This very basic scheme has been greatly elaborated on in both general and unique ways in each circadian system studied. It is now clear that more than one autoregulatory loop is present and that positive- and negative-acting branches are interlocked, providing greater stability to the system (Dunlap and Loros 2004; Hardin 2005, 2006; Dunlap 2006). Some of the core transcription factors are constitutively expressed, whereas others are under circadian control, with robust cycling of both mRNA and protein.

The focus here is the intersection between the light environment and the molecular components of the plant circadian system. Resetting of the clock occurs with each sunrise and, in some cases, sunset (Stoleru et al. 2007). How this occurs in each organism reflects the sensitivity of the circadian system to light. Here, two well-studied circadian systems with relatively direct light input pathways (Drosophila and Neurospora) are compared to new findings in the Arabidopsis system.

PHOTOENTRAINMENT IN DROSOFLA

Classic assays of Drosophila rhythms used eclosion and then locomotor activity to follow clock function. Under constant light (LL), rhythmic activity of flies quickly diminishes, in striking contrast to the sustained robust cycles observed in extended darkness. Similarly, per-luciferase-based luminescence rhythms are robust in constant darkness (DD), and this assay was instrumental in the discovery of the first element in the entrainment pathway when the cryb mutant was identified (Emery et al. 1998; Stanewsky et al. 1998). This mutation allows continued locomotor rhythmicity under LL, and mutant flies are recalitrant to a phase-shifting light pulse. This finding identified cryptochrome, a protein already identified as a blue light photoreceptor in plants, as the first step in the light-signaling pathway to the clock in flies. As a loss-of-function mutation, cryb indicates that light acts to disrupt clock, although pulses are effective, and essential, for photic entrainment (Dolezelova et al. 2007).

Subsequently, two-hybrid studies identified TIME-LESS (TIM) as a CRY interaction partner. TIM had already been shown to be a core element of the fly clock, interacting with PERIOD (PER) to suppress transcription driven by the CLOCK/CYCLE heterodimer (Gekakis et al. 1995; Darlington et al. 1998). A light-dependent interaction between CRY and TIM in yeast, abrogated by the CRYB mutation, helped establish CRY as a bona fide circadian photoreceptor, with TIM as a direct interaction partner (Ceriani et al. 1999). In the fly, blue light absorption by CRY facilitates TIM interaction, leading to TIM phosphorylation and degradation via the proteasome (Naidoo et al. 1999; Hardin 2005). The F-box protein JETLAG binds TIM, possibly after release from CRY, and is the link to the proteasome-dependent degradation of TIM. Light activation of CRY appears to facilitate TIM release and phosphorylation, making it receptive to JETLAG interaction (Fang et al. 2007). In this way, light enters the fly clock system via CRY-dependent changes in TIM abundance. It is through the rapid light-dependent reduction of TIM that phase resetting and entrainment are effected, although other light-input pathways exist in other cells and tissues of the fly (Dolezelova et al. 2007).

PHOTOENTRAINMENT IN NEUROSPORA

In Neurospora, FREQUENCY (FRQ) and the WHITE COLLAR COMPLEX (WCC) are central elements of the
circadian system (Liu 2003; Dunlap and Loros 2004; He and Liu 2005; Dunlap 2006). The WHITE COLLAR-1 (WC-1) and WC-2 heterodimer (WCC) act to positively regulate transcription from the *frequency* promoter, as well as other light-regulated genes. WC-1 is the photoreceptive partner of the pair, containing an amino-terminal LOV domain that binds flavin adenine dinucleotide (FAD) that confers the blue-light-absorptive properties to the molecule (He et al. 2002). Upon blue light absorption, the WCC binds to the light-responsive elements (LRE) of the *frq* promoter, promoting transcription (Froehlich et al. 2002). At the same time, FRQ protein acts negatively to disrupt the complex, inhibiting its own transcription. But FRQ also promotes *wc-1* and *wc-2* transcription, positively acting to enhance *frq* activation (He and Liu 2005; Brunner and Schaffmeier 2006). Hence, in this system, photocontrol of resetting and entrainment begins with transcriptional activation, via light-enhanced binding of the WCC to the *frq* promoter.

The WCC is required for *frq* transcription in the dark as well, but light absorption causes a transient increase in WCC-binding capacity, nicely accounting for an acute induction of FRQ and other light-regulated genes (He and Liu 2005). Following initial light absorption, WCC becomes progressively phosphorylated, which results in diminished affinity for LREs and a reduction in transcription from light-inducible promoters (He and Liu 2005; He et al. 2005). Hence, this direct light-enhanced DNA binding by a photoreceptive transcriptional complex (WCC) differs fundamentally from the light-enhanced degradation of protein, initiated by the CRY/TIM interaction in *Drosophila*.

**PHOTOENTRAINMENT IN ARABIDOPSIS**

The circadian system in plants involves numerous gene families, including Myb transcription factors (LHY and CCA1), pseudoresponse regulators that likely act as transcriptional cofactors (PRR1/TOC1, PRR3, PRR5, PRR7, PRR9), and two classes of classic red and blue photoreceptors (phytochromes and cryptochromes, respectively) (Mizuno and Nakamichi 2005; McClung 2006; Hotta et al. 2007). Mutations in both classes of photoreceptors lengthen free-running period, similar to the effects of light absorption: TOC1 (PRR1) and PRR5 (Mas et al. 2003a; Kiba et al. 2007; S. Fujiwara et al., unpubl.). Both are clock-controlled components of the plant circadian system, with a peak phase of expression near subjective dusk similar to the peak of *ZTL* abundance. Mutations in either TOC1 or PRR5 shorten period, and overexpression lengthens period (Somers et al. 1998b; Strayer et al. 2000; Makino et al. 2002; Sato et al. 2002; Mas et al. 2003b; Michael et al. 2003). This is similar to the long period of *ztl* mutants, consistent with the observed increase in *frq* mRNA of LKP2 overexpression causes arrhythmicity, similar to that of *ztl* mutants (Schultz et al. 2001; Somers et al. 2004 and unpubl.). *FKF1* transcription is clock-controlled, but at least one target is CDF1, which regulates the activity of CONSTANS, a key component of flowering time control (Imaizumi et al. 2005). *βkf1* mutants are late flowering but have no circadian defect (Nelson et al. 2000; Imaizumi et al. 2003).

In contrast, *ZTL* mRNA is constitutively expressed under all conditions. However, *ZTL* protein is rhythmic with a peak near ZT13, indicating some form of posttranscriptional circadian regulation (Somers et al. 2000; Kim et al. 2003). ZTL targets two members of the PRR family of pseudoresponse regulators for proteasome-dependent degradation; TOC1 (PRR1) and PRR5 (Mas et al. 2003a; Kiba et al. 2007). The phenotype of *ztl* mutants are fluence-rate-dependent, with a more severe long period at lower light intensities, relative to wild type (Somers et al. 2000, 2004). This suggests that ZTL has a stronger role at low fluence rates, and other photoreceptors feature more prominently in high light.

Further insights into how the ZTL LOV domain functions in the context of the full-length protein have come with the identification of GIGANTEA (GI) as a ZTL interaction partner. GI was first reported more than 45 years ago as a late-flowering mutant (Redei 1962). Subsequently, *gi* mutants were found to have circadian period defects, as well as aberrantly high starch accumulation and long hypocotyls (Table 1) (Eimert et al. 1995; Park et al. 1999; Huq et al. 2000; Tseng et al. 2004; Mizoguchi et al. 2005; Gould et al. 2006; Kim et al. 2007; Martin-Tryon et al. 2007; Oliverio et al. 2007). The large (1173 amino acids) GI protein has no recognizable domains that could suggest a mechanism for its action...
darkness or in red light in both the double mutant and wild levels were significantly higher than those observed in blue light. Extension into the subjective dark period, ZTL domain. Because, like the phototropins, the ZTL family is conserved in all LOV domains and forms a thiocysteine adduct with FMN in the phototropin LOV moiety upon blue light absorbance (Salomon et al. 2000; Crosson and Moffat 2001). Thus, this residue is the critical defining element that confers photosensitivity to the LOV domain. Because, like the phototropins, the ZTL family binds FMN (Imaizumi et al. 2003), these results demonstrate that ZTL is a blue light circadian photoreceptor.

Because the blue-light-dependent effects on ZTL could also be mediated through the cryptochromes, we tested the levels of ZTL in the cry1 cry2 background. Under all light conditions, we found no differences between wild type and the cry double mutant (Fig. 1). During a 12-hour blue light extension into the subjective dark period, ZTL levels were significantly higher than those observed in darkness or in red light in both the double mutant and wild type. Similar tests were conducted with the photochrome mutants, and no effect on ZTL levels was observed (data not shown). These results indicate that all light-dependent effects on ZTL levels result entirely from ZTL photoabsorption properties.

### Table 1. Effect of gi Alleles on Circadian Period and Reproductive and Morphological Development

<table>
<thead>
<tr>
<th>Allele</th>
<th>LL</th>
<th>RR</th>
<th>BB</th>
<th>DD</th>
<th>LD</th>
<th>SD</th>
<th>Hypocotyl length</th>
</tr>
</thead>
<tbody>
<tr>
<td>gi-1</td>
<td>short&lt;sup&gt;1&lt;/sup&gt;</td>
<td>short&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>~WT&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (LL, RR, BB, FR)&lt;sup&gt;1,7&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-2</td>
<td>short/WT (leaf movement)</td>
<td>short&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (LL, RR, BB, FR)&lt;sup&gt;1,7&lt;/sup&gt; or long (RR) and WT (DD, FR)&lt;sup&gt;1,7&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-3</td>
<td>short (leaf movement)</td>
<td></td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (LL, RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-4</td>
<td>short (leaf movement)</td>
<td></td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (LL, RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-5</td>
<td>short (leaf movement)</td>
<td></td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (LL, RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-6</td>
<td>short (leaf movement)</td>
<td></td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>~WT&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (RR, SD)&lt;sup&gt;2,5&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-11</td>
<td>short (leaf movement)</td>
<td></td>
<td></td>
<td></td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>late&lt;sup&gt;6&lt;/sup&gt;</td>
<td>long (RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-201</td>
<td>short (LL, R+B)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>short&lt;sup&gt;4&lt;/sup&gt;</td>
<td>short&lt;sup&gt;4&lt;/sup&gt;</td>
<td>short&lt;sup&gt;4&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;4&lt;/sup&gt;</td>
<td>long (LL, RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-596</td>
<td>long (R+B)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>long&lt;sup&gt;3&lt;/sup&gt;</td>
<td>short&lt;sup&gt;4&lt;/sup&gt;</td>
<td>short&lt;sup&gt;4&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;3&lt;/sup&gt;</td>
<td>WT&lt;sup&gt;3&lt;/sup&gt;</td>
<td>long (LL, RR, BB)&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>gi-611</td>
<td>short (R+B)&lt;sup&gt;4&lt;/sup&gt;</td>
<td>short&lt;sup&gt;1&lt;/sup&gt;</td>
<td></td>
<td></td>
<td>WT&lt;sup&gt;1&lt;/sup&gt;</td>
<td>~WT&lt;sup&gt;1,5&lt;/sup&gt;</td>
<td>short (RR, SD)&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
</tbody>
</table>


CIRCADIAN CLOCK ELEMENTS WITHOUT PHOSPHORYLATION: A NOVEL MODE OF REGULATION

These recent data demonstrate that clock-controlled GI transcription, and consequent GI protein rhythms, confers a posttranslational rhythm on ZTL protein by virtue of their mutual cooperative stabilization (Fig. 2A,B) (Kim et al. 2007). Following on this, how does the blue-light-enhanced binding of GI and ZTL affect circadian cycling?

Figure 1. Protein extracts from wild type (Ler) and cry1 cry2 mutants entrained in 12-hour light/12-hour dark cycles (WD) for 10 days and transferred to dark (D) (left panel), blue (B), or red (R) light (right panel) at ZT12 were immunoblotted and probed for ZTL abundance at the times indicated. Representative blots are shown below; adenosine kinase (ADK) was used as a loading control. ZTL levels expressed relative to wild type at ZT13 (D). Means of three trials §S.E.M.
In contrast to the long-period ztl mutants (Kevei et al. 2006), gi mutants generally show a short free-running period, although some alleles cause long period for some outputs, and light quality is also a factor (Table 1) (Kim et al. 2007; Martin-Tryon et al. 2007). Therefore, GI likely affects other aspects of clock function in addition to its effects on ZTL. TOC1 protein in a gi null still cycles in light/dark but with much reduced amplitude (Kim et al. 2007). During the early part of the day (photoperiod), when TOC1 is normally low, levels are strongly increased compared to wild type. Later, during the dark phase (skotoperiod), TOC1 levels are somewhat lower in a GI-deficient background compared to wild type. This overall blunting of the TOC1 waveform points to the importance of ZTL cycling in maintaining the appropriate amplitude and sharpness of the TOC1 rhythm (Fig. 2C). In a gi null, ZTL levels are constitutively low throughout the light/dark cycle, different from a line completely lacking ZTL. If this protein is functional, this could partially explain why TOC1 levels are not pegged to maximal levels at all times, as seen in ztl null mutants (Mas et al. 2003a).

This aspect of the gi phenotype may also provide insight into the puzzle of why, in the wild type, ZTL and TOC1 proteins cycle in-phase. As the substrate of ZTL, TOC1 levels should be low at times of peak ZTL, and high when ZTL is minimal. Their synchronous phasing suggests that other factors may be involved. One attractive model to explain their similar peak levels depends on the light-mediated GI/ZTL complex dissociating in the dark. Although this will lead to subsequent ZTL degradation, until then, the newly freed ZTL will be available to bind and degrade TOC1. This scheme shows GI with the dual role of simultaneously stabilizing ZTL and protecting TOC1 from degradation through the binding of GI to ZTL during the photoperiod, only to release ZTL for action during the skotoperiod. Thus, the GI/ZTL interaction heightens the peak of ZTL through the stabilizing effect of GI and also heightens TOC1 peak expression through the sequestration of ZTL. Therefore, in gi mutants, peak TOC1 levels are lower during the skotoperiod than in wild type because although ZTL levels are low throughout the cycle, free ZTL may effectively be higher than normal soon after entrance into the skotoperiod because no GI is present to complex with ZTL. That there is any TOC1 cycling in gi lines at all likely arises from the tracking of TOC1 mRNA rhythms.

In comparison to the fly and Neurospora light-input mechanisms, ZTL bears closer similarity to cryptochrome’s role in Drosophila. In both systems, blue light facilitates a protein–protein interaction that leads to the degradation of a key component of the oscillator. In Drosophila, cryptochrome binds directly to that component (TIM) in a light-dependent way, resulting in

Figure 2. Posttranslational control of ZTL protein rhythms through blue-light-enhanced stabilization by GI. (A) Robust diurnal rhythms of GI message contrasts with constitutive expression of ZTL mRNA. (B) Increasing GI protein in the light (high amplitude) associates with ZTL, stabilizing both and allowing increasing ZTL and GI protein levels, following on the clock-controlled rise of GI mRNA. In darkness, decreasing GI message and reduced stabilization of the GI/ZTL interaction hasten clearance of both proteins from the system. (C) One consequence of ZTL oscillations is to sharpen the TOC1 protein profile (dotted line). In the absence of GI (–GI), ZTL levels are constitutively low. TOC1 protein oscillates with low amplitude, tracking its own message rhythms. In the wild type (+GI), ZTL protein rhythms, resulting from GI cycling (thin line), allow high-amplitude TOC1 protein cycles, resulting in a normal circadian period and phasing of clock-controlled outputs.
increased TIM phosphorylation, which leads to better recognition of TIM by the F-box protein JETLAG. In Arabidopsis, light perception comes through the F-box protein itself (ZTL) but acts to facilitate binding of a stabilizing factor (GI) that leads to the appropriate phasing of peak ZTL. The ZTL/TOC1or ZTL/PRR5 interaction may require the phosphorylation of these two substrates, but there is no evidence that if this occurs, it is light-dependent. Similarly, there is no evidence that the GI/ZTL interaction is affected by phosphorylation of either protein.

Another key difference is that ZTL is most likely not the sole or even primary light-input pathway to entrainment and phase resetting in plants. Although there is good evidence that other ocular photoreceptors in flies can have a role in entraining locomotor activity, cryptochrome is clearly primary in its effect in controlling TIM levels acutely and in response to light (Dolezelova et al. 2007). In plants, as noted earlier, the phy and cry are essential in phase resetting, and other proteins, such as ELF3 and ELF4 are clearly involved in shaping the light responsiveness of the clock (Covington et al. 2001; Doyle et al. 2002; McWatters et al. 2007).

It is important to note that the effect of blue light absorption by ZTL is to stabilize the protein, not to initiate a light-dependent transduction chain. In this way, ZTL is not a photoreceptor in the “classic” sense of initiating a signaling cascade through a change in phosphorylation status of downstream components or through the activation of second messengers (e.g., rhodopsin). These signaling pathways act to amplify the original light input. The light-enhanced interaction of ZTL with GI is largely an act of self-preservation, and the partnering with GI is essential simply to maintain sufficiently high levels of ZTL, at the appropriate circadian phase, to diminish TOC1 and PRR5 levels.

PHOSPHORYLATION AND CLOCK FUNCTION

Phosphorylation in the Drosophila Clock

Protein maturation or stabilization controlled by a clock-regulated factor such as GI is a novel mechanism of conferring posttranslational regulation. More common is protein phosphorylation/dephosphorylation of clock components (Lee et al. 2001; Merrow et al. 2006; Gallego and Virshup 2007). In Drosophila, timely phosphorylation of TIM and PER regulates the timing of nuclear import as well as the susceptibility to degradation (Hardin 2005; Bae and Edery 2006). Casein kinase Iε (CKIε) associates with and phosphorylates PER in either the nucleus or cytoplasm when unbound to TIM, potentiating it for proteasome-dependent degradation. Mutations in CKIε were recovered as period mutants (doubletime), the first demonstration of the importance of phosphorylation in the Drosophila clock (Kloss et al. 1998; Price et al. 1998). This same kinase is found in the nucleus, acting on both PER and CLK, priming both for degradation (Bae and Edery 2006; Kim and Edery 2006). A likely collaborator of CKIε is casein kinase 2 (CK2), contributing to the progressive phosphorylation of PER in the cytoplasm and helping to regulate nuclear import (Nawathean and Rosbash 2004). Additionally, a third kinase, glycogen synthase kinase-3 (GSK-3), is essential in the phosphorylation and subsequent degradation of TIM by JETLAG, as well as the nuclear import of the TIM/PER complex (Naidoo et al. 1999; Martinek et al. 2001; Koh et al. 2006). Finally, in addition to these three kinases, protein phosphatase 2A (PP2A) and protein phosphatase 1 (PP1) act to remove phosphates from PER/CLK and TIM, respectively.

Phosphorylation in the Neurospora Clock

In Neurospora, phosphorylation has critical roles in a number of clock components. Progressive phosphorylation of FRQ by CKII, and likely other kinases (CAMK-1, CK1α), controls FRQ degradation through interaction with the F-box protein FWD-1 and is highly dependent on the phosphorylation status of certain key residues (He et al. 2003; Yang et al. 2003; Brunner and Schafmeier 2006). Two phosphatases, PP1 and PP2A dephosphorylate FRQ (Yang et al. 2004). PPI positively regulates FRQ abundance, consistent with an antagonism toward the CKII activity that promotes FRQ degradation. Still unclear is how the opposing activities of these two protein classes work together to establish a particular state of FRQ phosphorylation during the circadian cycle and how critical this state is to FRQ function apart from dictating its degradation potential.

As noted earlier, WCC binding to the frq promoter is enhanced by blue light, but there is no evidence that this occurs through light-dependent phosphorylation. Indeed, hyperphosphorylation of WCC peaks 15–30 minutes after light exposure, but this is much later than the binding of WCC to LREs, which is detectable within 5 minutes (He and Liu 2005). Instead, light appears to potentiate WCC for subsequent phosphorylation which, paradoxically, reduces the affinity of WCC for LRE binding. Additionally, FRQ is required for this phosphorylation, which recruits or interacts with both CK1α and CKII at WCC (Schafmeier et al. 2005; He et al. 2006). Extensive FRQ-dependent phosphorylation (in complex with CK1α and CKII) of WCC results in inactivation and removal of WCC from the frq promoter. Increasing FRQ phosphorylation results in its degradation, and dephosphorylation of WCC, via PP2A, can reactivate the complex and frq transcription may begin again (He and Liu 2005; Schafmeier et al. 2005, 2006; He et al. 2006).

Phosphorylation in the Arabidopsis Clock

Although less well elaborated, phosphorylation does have a role in the plant circadian system. A series of papers concerned with CK2 and the myb transcription factor CCA1 have established a clear role for this kinase. Recombinant CK2 can phosphorylate CCA1 in vitro, and the DNA-binding activity of CCA1 from plant extracts requires CK2 phosphorylation, as demonstrated by CK2-specific inhibitors (Sugano et al. 1998). Overexpressors of a CK2 regulatory subunit (CKB3) have severely shortened periods (4 hours) with few other pleiotropic effects, further suggesting that CCA1 phosphorylation by CK2 is necessary for clock function (Sugano et al. 1999). Six key
CK2 phosphorylation sites in CCA1 were mutated (mCCA1) and overexpression of mCCA1 had no significant effect on period, in contrast to the arrhythmia seen in wild-type CCA1-OX plants. Interestingly, CCA1 homodimerization was abolished by the mutations, which together with the previous in vitro DNA-binding results suggests that a phosphorylated CCA1 homodimer may be the functional product of this gene (Daniel et al. 2004). CCA1 and the related LHY both bind the TOC1 promoter to negatively regulate translation (Alabadi et al. 2001), so CK2 activation of CCA1 may contribute to TOC1 repression.

WNK1, one of a nine-member Arabidopsis family of WNK serine/threonine kinases, related to mitogen-activated protein kinases (MAPK) was found to interact with PRR3 and PRR5 in a yeast two-hybrid assay (Murakami-Kojima et al. 2002; Nakamichi et al. 2002). WNK1 is able to phosphorylate PRR3 and PRR5 in vitro and is expressed rhythmically with the same phase as that of PRR3 and PRR5. This is strong circumstantial evidence that suggests a role for WNK1 in the plant circadian clock and possibly others of the family whose transcription is also rhythmic (Nakamichi et al. 2002). If true, this would mark a departure from other kinase families described earlier but put plants into close company with the mammalian circadian system, where a MAPK cascade has been implicated in resetting (Butcher et al. 2002; Weber et al. 2006).

**CONCLUSION**

It is becoming evident that the concept of the transcription-translation feedback loop needs further revision as the importance of posttranslational events such as phosphorylation becomes more apparent in eukaryotic clocks. Conservation of kinase and phosphatase classes across groups as diverse as flies and fungi—which find no common ground in transcriptional regulators—indicates just how fundamental this mode of regulation is. We are still uncovering many of the basic elements of the plant circadian clock, and phosphorylation will certainly have a key role in determining subcellular location and protein half-lives. However, entirely different regulatory mechanisms may emerge in the plant clock, as the ZTL/GI relationship will certainly have a key role in understanding many of the basic elements of the plant circadian clock. Whether similar surprises will be uncovered many of the basic elements of the plant circa-

**REFERENCES**


Dolezelova E., Dolezel D., and Hall J.C. 2007. Rhythm defects from other kinase families described earlier but put plants into close company with the mammalian circadian system, where a MAPK cascade has been implicated in resetting (Butcher et al. 2002; Weber et al. 2006).
Posttranslational Photomodulation

Timeless by PER protein interaction: Defective interaction between timeless protein and long-period mutant PER.


He Q., Cha J., He Q., Lee H.C., Yang Y., and Liu Y. 2006. CKJ1 and CKJ2 mediate the FREQUENCY-dependent phosphorylation of the WHITE COLLAR complex to close the Neurospora circadian negative feedback loop. Genes Dev. 20: 2552.


Muranakami-Kojima M., Nakamichi N., Yamashino T., and Mizuno T. 2002. The APRR3 component of the clock-associated APRR1/TOC1 quintet is phosphorylated by a novel protein kinase belonging to the WNK family, the gene for which is also transcribed rhythmically in Arabidopsis thaliana. Plant Cell Physiol. 43: 675.


Posttranslational Photomodulation of Circadian Amplitude


Access the most recent version at doi:10.1101/sqb.2007.72.035

References

This article cites 87 articles, 59 of which can be accessed free at:
http://symposium.cshlp.org/content/72/193.full.html#ref-list-1

Creative Commons License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or click here.