

that partition into such rafts display an increased resistance to detergent solubility and a decreased two-dimensional diffusion compared to nonraft components. Accordingly, cholesterol/sphingomyelin conferred detergent resistance on the bound minimotors and caused a 10-fold decrease in their diffusion coefficient. These lipids had no effect on liposome binding but enhanced transport by the minimotors. The results suggest that vesicle-bound Unc104 motors partition into  $\text{Ptlns}(4,5)\text{P}_2$  rafts and that this decreases the lateral mobility of the motors and facilitates vesicle transport.

How does the association of Unc104 with membrane rafts activate transport? Clustering of Unc104 motors within the rafts could increase their local concentration and drive their oligomerization, possibly producing processive dimers. Although native Unc104, KIF1A, and the monomeric minimotors lack the coiled-coil extension found in dimeric DdUnc104, they do contain a short heptad repeat adjacent to the motor domain that could drive dimerization when Unc104 motors partition into rafts. In support of this hypothesis, mutagenesis of residues in the a and d positions of this heptad repeat that were designed to inhibit coiled-coil formation had no effect on liposome binding by Unc104 minimotors but inhibited the activation of vesicle transport induced by raft formation. This suggests that raft formation normally activates vesicle transport by promoting the formation of Unc104 dimers on the surface of vesicles that are capable of walking processively along MTs by a mechanism similar to that of kinesin-1.

The speed and persistence of vesicle transport observed by Klopfenstein et al. more closely resembles the movement of Unc104::GFP motors in axons of living worms than does the saltatory movement displayed by KIF1A. Assuming that Unc104::GFP in axons faithfully mimics native Unc104, this suggests that the movement of vesicles by raft-induced Unc104 dimers is the dominant form of transport occurring in vivo and that the two-headed walking mechanism employed by kinesin-1 may be more widespread than previously thought. However, it is possible that processive movement by biased one-dimensional diffusion of Unc104 may occur under

certain circumstances in cells. The observation that the organization of membrane lipids into rafts activates vesicle transport is a fascinating and unexpected discovery that focuses attention on how changes in the properties of a bound cargo can profoundly influence cargo transport, although the significance of raft-induced vesicle transport isn't fully understood at this stage; for example, it is not known whether the Unc104-bound rafts are stable structures or whether they form and dissipate transiently as part of a regulatory mechanism that switches on and off vesicle transport. Finally, one wonders how these findings on Unc104 relate to other motors, such as monomeric chromokinesins, that could form active dimers on the surface of their chromosomal cargo and to other forms of intracellular transport, such as vesicle trafficking during animal cell division, which is currently a topic of growing interest (Finger and White, 2002).

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## The Role of CCA1 and LHY in the Plant Circadian Clock

**Our understanding of plant circadian rhythms has been advanced by two papers investigating the roles of the transcription factors CCA1 and LHY in the circadian oscillator.**

Circadian (from the Latin, *circa dies*) clocks are complex timekeeping systems that generate endogenous rhythms with periods of about a day. They are found in a wide range of organisms including mammals, insects, fungi, cyanobacteria, and plants, and they control a variety

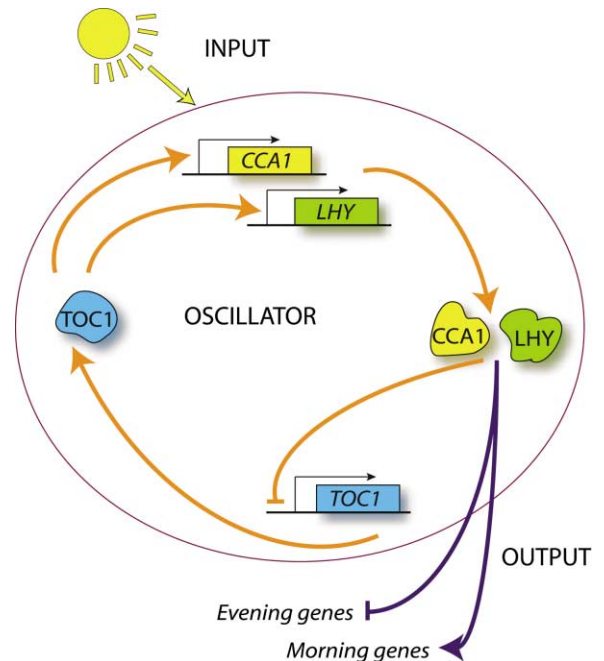
of activities from sleep/wake cycles in animals to leaf movements and flowering time in plants. Studies have revealed that the core of the circadian systems is an oscillator based on a transcriptional-translational negative feedback loop. While the basic architecture of oscillator systems seems to be conserved, the molecules comprising the oscillator vary. Although plant circadian rhythms were the first to be described, it is only recently that we have obtained experimental insight into what comprises the components of the plant oscillator (reviewed by Barak et al., 2000).

Three genes have recently been proposed to encode molecular components of the circadian oscillator in the model plant *Arabidopsis thaliana*. Two of these genes, CCA1 (CIRCADIAN CLOCK ASSOCIATED 1; Wang and Tobin, 1998) and LHY (LATE ELONGATED HYPOCOTYL;

Schaffer et al., 1998), encode closely related single MYB domain transcription factors. The *TOC1* gene (*TIMING OF CAB 1*; Strayer et al., 2000 and also referred to as *APRR1*; Matsushika et al., 2002) encodes a protein with a receiver domain similar to that of response regulators from two-component signaling systems and a motif implicated in nuclear localization. There are several lines of evidence that these three genes are oscillator components and comprise the core negative feedback loop. All three genes show rhythmic expression under constant conditions of light and temperature. Constitutive expression of either *LHY* or *CCA1* causes arrhythmicity in constant conditions and represses the expression of both the endogenous *LHY* and *CCA1* genes and of *TOC1* (Wang and Tobin, 1998; Fowler et al., 1999; Matsushika et al., 2002). In addition, loss-of-function mutants of *CCA1* (*cca1-1*) and *TOC1* (*toc1-2*) have shorter periods of clock-controlled rhythms than their wild-type counterparts (Green and Tobin, 1999; Strayer et al., 2000). However, the single loss-of-function mutations do not stop the clock, which implies that if they are really oscillator components, there must be one or more additional components that can function at least partially in the absence of either *CCA1* or *TOC1*. The close relationship between the *CCA1* and *LHY* proteins suggests that they are likely to have redundant functions and, thus, that *LHY* could maintain rhythmicity in the absence of *CCA1*. Similarly, there are other proteins in *Arabidopsis* that are closely related to *TOC1* (Matsushika et al., 2002).

Two recent papers have now addressed the question of what happens in the absence of both *CCA1* and *LHY*. In a paper published in this issue of *Developmental Cell*, Coupland and his coworkers used an elegantly designed suppressor screen of *LHY* gain-of-function plants to generate three loss-of-function *LHY* mutants (Mizoguchi et al., 2002). The Kay lab used double-stranded RNA interference (RNAi) technology to disrupt *LHY* function in three lines of plants (Alabadi et al., 2002), in a study presented in the April 30<sup>th</sup> issue of *Current Biology*. In both cases, plants that have lost *LHY* alone (*lhy*) showed a period of gene expression that was 2 to 3 hours shorter than wild-type, consistent with results for the *cca1-1* mutants (Green and Tobin, 1999). By making *lhy cca1-1* plants that lack both *CCA1* and *LHY*, the two groups have now been able to report the effects of losing both proteins. For the first 24–48 hr in continuous light, the *lhy cca1-1* plants showed further shortening of the period of rhythmicity of several clock-controlled genes (by ~6 hr compared to wild-type plants). However, by 2 days in constant light, these rhythms and those of leaf movements damped out completely. This result contrasts with the single mutants, which continued to show robust rhythms with shortened periods for many days. Thus, circadian rhythms cannot be sustained without both *CCA1* and *LHY*, and the results are consistent with them being components of the central oscillator.

Flowering is also under the control of the central oscillator, and *Arabidopsis* flowers earlier when grown under long days and short nights. Coupland's group found that under short days, both *lhy* and *cca1-1* single mutants flowered earlier than wild-type plants, while the *lhy cca1-1* plants flowered significantly earlier than either of the two single mutants. Together with the gene expression data, it is clear that *LHY* and *CCA1* have at least some



A Model of the Putative Roles of *LHY*, *CCA1*, and *TOC1* in the *Arabidopsis* Circadian Oscillator

Light induces the expression of *CCA1* and *LHY*. *CCA1* and *LHY* repress *TOC1* transcription. At the same time, *CCA1* and *LHY* may directly activate clock-regulated genes that are expressed early in the day while possibly repressing genes that are only expressed in the evening. As the levels of *CCA1* and *LHY* fall during the course of the day, *TOC1* repression is alleviated and *TOC1* levels rise. *TOC1* then directly or indirectly induces the expression of *CCA1* and *LHY*.

redundant functions and is further evidence that they are functioning as part of the oscillator itself.

But how can the plant show any oscillations in the absence of both *LHY* and *CCA1*, if *LHY* and *CCA1* are really oscillator components? What other components might result in some rhythmicity when both *LHY* and *CCA1* are absent? A second oscillator may exist whose presence is uncovered in the absence of *LHY* and *CCA1*. Another possibility is that other single MYB domain proteins (Barak et al., 2000) that show some homology with *LHY* and *CCA1* might partially substitute for them, but not be able to sustain the rhythms. If *CCA1* and *LHY* have much higher binding affinities, such a protein would not normally bind, but in the absence of both *CCA1* and *LHY*, it might serve as a weak substitute and, thus, not be able to sustain normal rhythms. Further experiments should provide answers to these questions.

Finally, another recently published paper from the Kay laboratory (Alabadi et al., 2001) has suggested a way in which *CCA1* and *LHY* might interact with *TOC1* to form a negative feedback loop that acts as an oscillator. Light, which can act through a phytochrome photoreceptor, induces transcription of *CCA1* and *LHY* (see Figure). These transcription factors then bind the *TOC1* promoter, repressing its transcription. Consistent with this idea, when levels of *LHY* or *CCA1* are constitutively high, *TOC1* mRNA levels are low (Alabadi et al., 2001). Conversely, in the *lhy cca1-1* plants, *TOC1* mRNA levels remain high (Mizoguchi et al., 2002). As *LHY* and *CCA1*

levels fall toward evening, *TOC1* expression rises. *TOC1* then directly or indirectly induces expression of *CCA1* and *LHY*.

In summary, the two papers discussed here bring us closer to fully understanding the molecular components of the plant circadian system. Are *CCA1*, *LHY*, and *TOC1/APRR1* the whole story of the plant central oscillator? Begging forgiveness in advance, we conclude by saying that time will tell.

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## Cyclin G: A Regulator of the p53-Mdm2 Network

A recent study published in the April issue of *Molecular Cell* has shown that cyclin G, a p53 target, is a regulatory component of the active PP2A holoenzyme, which activates Mdm2 through dephosphorylation. These findings suggest that cyclin G is a key regulator of the p53-Mdm2 network.

p53 is a stress sensor and is activated in response to a variety of stress stimuli. Activated p53 then regulates a diverse array of target genes, which mediate p53 activity in the control of cell cycle arrest, apoptosis, and DNA repair (Ko and Prives, 1996). Many cellular genes have been identified, including p21, 14-3-3 $\sigma$ , and MCG10, which induce cell cycle arrest; Bax, Puma, and Killer/Dr5, which induce apoptosis; and p53R2 and GADD45, which participate in DNA repair. However, after stress subsides and cellular damage such as damaged DNA is repaired, the activated p53 becomes harmful to normal cell growth and needs to be eliminated. It has been clearly demonstrated that the main negative regulator of p53 is Mdm2, which is itself a p53 target gene (Michael and Oren, 2002).

In response to various stress conditions, stress kinases such as ATM are activated, which then phosphorylate and activate p53 (see Figure). Recent studies have shown that some of these stress kinases also phosphorylate Mdm2, and phosphorylated Mdm2 has a reduced ability to interact with, and inhibit, p53 (Michael and Oren, 2002). Therefore, removal of an inhibitory phosphate from Mdm2 is necessary for its activation. In addition, recent evidence indicates that prosurvival kinases,

such as AKT, can phosphorylate Mdm2 and increase its ability to target p53 (Michael and Oren, 2002). Therefore, the activity of Mdm2 appears to be regulated by phosphorylation in a manner similar to that of cyclin-dependent kinases (CDKs).

Cyclin G was one of the earliest p53 target genes to be identified, but its function in the p53 pathway has been elusive (Okamoto and Beach, 1994). A number of studies have shown that cyclin G has growth-promoting functions and is highly expressed in regenerating hepatocytes and motoneurons and in rapidly growing cancer cells (Morita et al., 1996; Skotzko et al., 1995). In addition, cyclin G can also increase the sensitivity of cancer cells to TNF $\alpha$ -induced apoptosis (Okamoto and Prives, 1999). As a cyclin, cyclin G can associate with cdk5 and GAK, a cyclin G-associated kinase (Kanaoka et al., 1997), but the significance of these associations is unclear. Cyclin G was also known to interact with the B' subclass of PP2A phosphatase (Okamoto et al., 1996). PP2A is a serine/threonine phosphatase, consisting of a catalytic (C), a structural (A), and a regulatory (B) subunit. Until the recent Okamoto et al. study, the significance of the cyclin G-PP2A interaction was also not clear. Their new data have provided convincing evidence that cyclin G interacts with the enzymatically active PP2A phosphatase (Okamoto et al., 2002). In addition, cyclin G directly interacts with Mdm2 and can stimulate the ability of PP2A to dephosphorylate Mdm2, and thus serves as a recruitment factor. Specifically, they provide strong evidence that the PP2A holoenzyme is capable of dephosphorylating two sites in Mdm2 (S166 and T216) both in vitro and in vivo.

These data raise an important question: does cyclin G have an impact on the activity of Mdm2 toward p53? Using mouse embryo fibroblasts (MEF) that are deficient in cyclin G, Prives and colleagues provide the most interesting and compelling evidence that in the absence