

The Photomorphogenic Protein, DE-ETIOLATED 1, Is a Critical Transcriptional Corepressor in the Central Loop of the *Arabidopsis* Circadian Clock

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In this issue of *Molecular Cell*, Lau et al. (2011) demonstrate that DET1, a component of the COP10-DET1-DDB1 (CDD) complex, is a transcriptional corepressor recruited to the promoters of core clock genes via interaction with two MYB transcription factors, CCA1 and LHY.

The circadian clock is an endogenous molecular oscillator with a period of approximately 24 hr that enables the anticipation of daily and seasonal changes in the environment. The plant circadian clock consists of multiple interlocked transcriptional feedback loops (McClung and Gutiérrez, 2010). In *Arabidopsis*, the central loop consists of an evening-expressed pseudoresponse regulator, TIMING OF CAB EXPRESSION 1 (*TOC1*), which positively regulates the transcription of two morning-expressed MYB domain transcription factor genes, *CIRCADIAN CLOCK ASSOCIATED 1* (*CCA1*) and *LATE ELONGATED HYPOCOTYL* (*LHY*), whose products in turn repress *TOC1* transcription (Alabadí et al., 2001). *CCA1* and *LHY* bind to the *TOC1* promoter and to the promoters of many other clock and clock-regulated genes. However, the mechanism by which *CCA1* and *LHY* repress transcription remains only incompletely explained. Moreover, *CCA1* and *LHY* also serve as positive regulators, for example, of the morning-expressed *PRR7* and *PRR9* (Farré et al., 2005). In this issue of *Molecular Cell*, Lau et al. (2011) show that *CCA1* and *LHY* recruit the COP10-DET1-DDB1 (CDD) complex to the *TOC1* and *GIGANTEA* (*G1*) promoters (Figure 1) and that DET1 (DE-ETIOLATED 1) possesses transcriptional corepressor activity that is necessary for *CCA1*- and *LHY*-mediated inhibition of *TOC1* and *G1* transcription. These results shed light on the roles of DET1 both in the circadian clock and in the widespread transcriptional reprogramming associated with the illumination of dark-grown seedlings.

The CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA (COP/DET/FUS) proteins were identified as repressors of seedling photomorphogenesis, but are broadly conserved among eukaryotes, including humans. COP/DET/FUS proteins are components of the COP1 complex, the COP9 signalosome, and the CDD complex (Jiao et al., 2007; Wei et al., 2008). COP1 is an ubiquitin E3 ligase, and the COP9 signalosome is a general regulator of all cullin-based ubiquitin ligases. COP10 is an ubiquitin E2 variant that lacks E2 activity, and DDB1 is the adaptor of CULLIN4-based E3 ligases. However, DET1 lacks recognized protein domains, and so its activity has remained enigmatic.

DET1 was first identified by its loss-of-function phenotype of de-etiolation, from where it takes its name, in which plants grown in the dark turn green due to chloroplast biogenesis and chlorophyll accumulation (Chory et al., 1989). Several light-induced genes, including *CHLOROPHYLL A/B-BINDING PROTEIN 2* (*CAB2*), are overexpressed in *det1*, suggesting that DET1 represses those genes in the dark. Indeed, Lau et al. (2011) first show that DET1, but not COP10 or DDB1, possesses transcriptional repressor activity in yeast and plants. Because DET1 lacks a known DNA-binding domain, Lau et al. looked for protein interactions that might recruit DET1 to DNA, discovered that DET1 interacts with *CCA1* and *LHY* in vitro and in yeast, and confirmed these interactions in planta. This interaction of DET1 (and COP10) with *CCA1* and *LHY* suggested that DET1 might contribute to the tran-

scriptional repression of *TOC1* and other *CCA1/LHY* targets. Chromatin immunoprecipitation (ChIP)-PCR confirmed that DET1 binds to the *TOC1* and *G1* promoters with higher binding at dawn than at dusk, consistent with the temporal pattern of *CCA1* and *LHY* accumulation. The dependence on *CCA1* and *LHY* for recruitment of DET1 to the *TOC1* promoter was tested in the *cca1 lhy* double mutant, in which DET1 binding was reduced to a background level.

The proposed mechanism that *CCA1* and *LHY* recruit DET1 as a transcriptional corepressor of *TOC1* and *G1* predicts that loss of DET1 function should have the same effect on the expression of *TOC1* and *G1* as the loss of both *CCA1* and *LHY* function. Lau et al. (2011) tested this prediction with a strong but incomplete DET1 loss-of-function mutant (*det1-1*), as complete loss of DET1 function is lethal. In *det1-1*, *TOC1* and *G1* transcripts cycle with shortened circadian period, similar to the effect observed in the *cca1* and *lhy* mutants and consistent with earlier studies (Millar et al., 1995; Mizoguchi et al., 2002). Overexpression of *CCA1* driven from a strong constitutive promoter yields arrhythmicity, with *TOC1* expression reduced to a nonoscillating basal level (Alabadí et al., 2001), and Lau et al. show a similar effect on *G1* transcript accumulation. Introduction of the *det1-1* mutation into *CCA1*-overexpressing plants partially rescues this phenotype in that *TOC1* and *G1* transcripts accumulate to about one-half the wild-type levels, indicating that DET1 is at least partially responsible for the effects of

CCA1 overexpression (Lau et al., 2011). However, neither *TOC1* nor *GI* transcripts show robust circadian oscillations in *CCA1-OX;det1-1* plants. It is possible that *CCA1* (and *LHY*) can interact with transcriptional corepressors other than *DET1*, or the residual *DET1* present in the partial loss-of-function *det1-1* mutant is sufficient to disrupt the circadian expression pattern. Alternatively, excess *CCA1* could have additional adverse effects on clock function through other regulatory interactions.

CCA1 and *LHY* are repressors of many genes, including other clock genes such as *LUX*, *ARRHYTHMIA* (*LUX*, also called *PHYTOCLOCK1*) and *EARLY FLOWERING 3* (*ELF3*) (Helfer et al., 2011). This raises the question of whether transcriptional repression by *CCA1* and *LHY* always requires *DET1* and the CDD. *CCA1* and *LHY* are positive regulators of a number of morning-expressed clock genes, including *PRR7* and *PRR9* (Farré et al., 2005) (Figure 1). *LUX* and *ELF3* are negative regulators of *PRR9*, so the induction of *PRR9* by *CCA1/LHY* could be indirect via the repression of *LUX* and *ELF3*. However, *LUX* does not bind to the *PRR7* promoter (Helfer et al., 2011), so an additional mechanism is required. Because *DET1* is present in the morning, it may be recruited to the *PRR7* and *PRR9* promoters by *CCA1/LHY*. If so, how is its repressive function blocked? If not, what is the mechanistic basis of its exclusion?

The phenotypic effects of loss of *DET1* are more severe than those of combined loss of *CCA1* and *LHY* (Chory et al.,

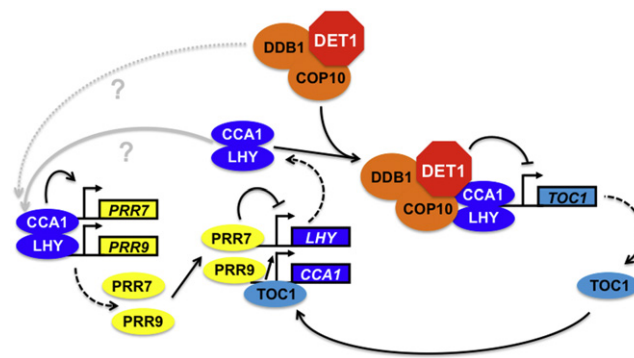


Figure 1. A Model for the Proposed Role of *DET1* in the *Arabidopsis* Circadian Clock

CCA1 and *LHY* (blue) genes are transcribed and proteins accumulate during the early morning. *CCA1* and *LHY* proteins bind to the *TOC1* (cyan) promoter, recruiting the CDD complex consisting of *DET1* (red), *DDB1*, and *COP10* (orange), where *DET1* acts as a transcriptional corepressor. *CCA1* and *LHY* are also positive regulators of the morning-expressed *PRR7* and *PRR9* (yellow) genes, although the mechanism is not understood (indicated with gray lines and question marks) and could be direct (shown) or indirect. Because the regulation is positive, it seems unlikely that the CDD complex is recruited to the *PRR7* and *PRR9* promoters (dashed gray line). *CCA1* and *LHY* proteins turn over during the day, releasing the CDD complex and relieving repression so that *TOC1* is expressed in the evening. *TOC1* protein is a positive regulator of *CCA1* and *LHY*, although the mechanistic details are not fully understood. *PRR7* and *PRR9* proteins bind to the *CCA1* and *LHY* promoters and repress transcription. *PRR7* and *PRR9* proteins turn over after dark, relieving repression so that *CCA1* and *LHY* are expressed in the early morning. Genes are shown as rectangles and proteins as ovals, except for *DET1*, which is shown as a red octagon. Many components (e.g., *GI*, *LUX*, and *ELF3*) have been omitted to simplify the model.

1989). This raises the possibility that *DET1* may act as a corepressor with other DNA-binding transcription factors. *DET1* expression is neither light nor clock regulated, so potential regulatory targets need not be limited to those expressed in the evening, in phase with *TOC1* and *GI*. The CDD complex has been proposed to be involved in chromatin remodeling, as tomato *DET1* interacts with the nonacetylated tail of H2B and *DDB1* interacts with histone acetyltransferases (Jiao et al., 2007). This is consistent with a potentially broad transcriptional corepressor role for *DET1* and the CDD complex. A genome-wide analysis of *DET1* recruitment to promoters by ChIP-Seq could be informative in future. Related to this point, it is

also interesting to speculate whether *DET1* is even restricted to the CDD complex or whether other *DET1* interactors bridge *DET1* to other transcription factors, for example.

Lau et al. shed new light on the central loop of the *Arabidopsis* circadian clock. Because *CCA1* is a key node in the clock and clock output networks (McClung and Gutiérrez, 2010), their study has broad implications for the temporal organization of plant molecular, physiological, and behavioral responses to the environment.

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