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The dark side of clock-controlled flowering

Vicente Rubio[1]* and Xing Wang Deng[2]

Addresses: [1]Department of Plant Molecular Genetics, Centro Nacional de Biotecnología-CSIC, Darwin 3, Campus UAM, Cantoblanco, Madrid, 28049, Spain; [2]Department of Molecular, Cellular, and Developmental Biology, Yale University, 165 Prospect Street, OML 352A, New Haven, CT 06520, USA

* Corresponding author: Vicente Rubio (vrubio@cnb.csic.es)

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Abstract

Perception of seasonal changes in day length allows plants to properly maintain daily biological rhythms and determine the most favorable time for flowering. Important knowledge has been gained recently on the molecular basis of this process, which depends not only on light perception at certain times of day but also on its dark phase.

Introduction and context

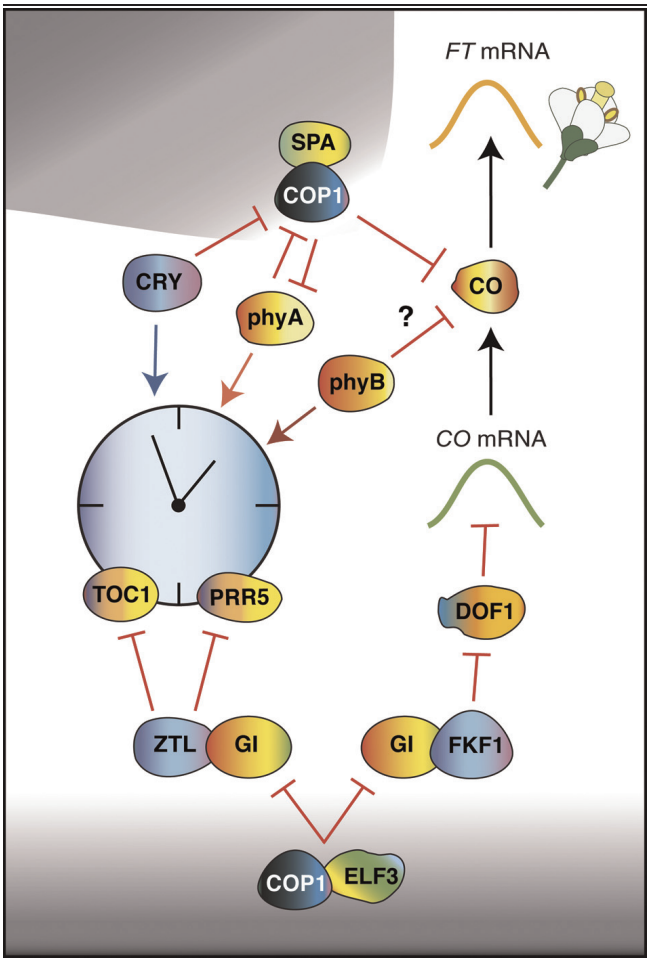
As in many other organisms, plants rely on circadian clocks, endogenous self-sustained molecular oscillators, to keep track of time and to generate biological rhythms that occur on a daily basis [1]. Additionally, clock function allows flowering plants to estimate the most appropriate time of year to flower, thus favoring their reproductive success. For this, the plant clock needs to be reset (or synchronized) to the external day/night cycles. This process implies the perception of fluctuations in day length (photoperiod) and temperature, and subsequent transmission of such environmental information through specific 'input pathways' to the clock. Three major classes of photoreceptors are known to mediate light-input signaling to the clock in the model plant *Arabidopsis*: blue light (BL) receptors of the ZEITLUPE (ZTL) and cryptochrome (CRY) families and the red/far-red light-sensing phytochromes (Phys) [2]. Studies over the past two years have shed light on the molecular mechanisms that regulate their function in controlling clock resetting by light (CRL) and photoperiodic flowering. These mechanisms largely involve CONSTITUTIVE PHOTOMORPHOGENESIS 1 (COP1), a master repressor of light-mediated development in darkness.

Major recent advances

Members of the ZTL/FKF1 (FLAVIN-BINDING, KELCH REPEAT, F-BOX 1)/LKP2 (LOV, KELCH PROTEIN 2) family of photoreceptors display two signature motifs: a BL-sensing LOV domain and an F-box motif involved in ubiquitination and degradation of specific protein substrates [3]. Indeed, both domains are essential for ZTL and FKF1 control of CRL and photoperiodic flowering, respectively. Thus, upon BL perception, ZTL and FKF1 form stable complexes with GIGANTEA (GI), a clock-regulatory protein [4]. BL-enhanced ZTL-GI interaction enables ZTL stabilization during the late evening, thus favoring degradation of the ZTL targets, such as TIMING OF CAB1 (TOC1) and PSEUDORESPONSE REGULATOR 5 (PRR5) [4,5] (Figure 1). TOC1 and PRR5 are clock oscillator components whose rhythmic accumulation is necessary to drive robust oscillations of clock-generated biological rhythms. Therefore, day-length information can be transmitted to the clock through ZTL-GI action on TOC1 and PRR5 to influence timing and amplitude of circadian rhythms. FKF1-GI complexes also accumulate during the evening to effectively promote degradation of CYCLING DOF FACTOR 1 (CDF1) [6]. CDF1 negatively controls flowering by repressing expression of the floral inducer gene *CONSTANS* (*CO*) [7]. Therefore, day-length-dependent formation of FKF1-GI-CDF1 complexes allows precise timing of *CO* expression, thus helping to determine the most advantageous time for flowering.

Recently, we reported that COP1, an E3 ubiquitin ligase that promotes degradation of positive regulators of

Figure 1. Photoreceptor-mediated signaling pathways converge to allow clock resetting by light and photoperiodic flowering



The molecular mechanisms by which these pathways exert their function significantly involve control of the accumulation of limiting regulatory proteins that modulate circadian oscillation and/or the promotion of flowering (for example, TOC1, PRR5, DOF1, or CO). COP1 plays a central role in reducing the effectiveness of these light-input pathways. For this, COP1, likely with the help of substrate adaptors (for example, ELF3 and SPA proteins), triggers dark-driven degradation of regulators of clock function and/or flowering, such as GI, CO, and PhyA. CDF1, CYCLING DOF FACTOR 1; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENESIS 1; CRY, cryptochrome; ELF3, EARLY-FLOWERING 3; FKF1, FLAVIN-BINDING, KELCH REPEAT, F-BOX 1; FT, FLOWERING LOCUS T; GI, GIGANTEA; Phy, phytochrome; PRR5, PSEUDORESPONSE REGULATOR 5; SPA, SUPPRESSOR OF PHYA-105; TOC1, TIMING OF CAB1; ZTL, ZEITLUPE.

photomorphogenesis (light-mediated development) in darkness, also limits the accumulation of GI [8] (Figure 1). This process is enabled by EARLY-FLOWERING 3 (ELF3), a repressor of CRL and flowering which probably acts as a substrate adaptor bringing together COP1 and GI. By reducing GI protein levels at night, COP1 and ELF3 may reduce the abundance of the ZTL- and FKF1-GI complexes, desensitizing the clock to light signals after dusk and repressing photoperiodic flowering. An interesting additional finding was that ELF3 is degraded upon interaction with COP1, suggesting that a negative-feedback mechanism limits the extent of ELF3 activity to the night phase. This inhibitory mechanism may help to restrict clock responsiveness to light to certain times of day, a process known as circadian gating of light.

Two recent studies have shown that CO protein is also a target of COP1 E3 ubiquitin-ligase activity [9,10] (Figure 1). Therefore, COP1 control of CO function may be accomplished by two separate mechanisms: transcriptional regulation of the *CO* gene, by controlling FKF1-GI activity, and post-translational control of CO accumulation, by promoting its proteasomal degradation in darkness [8-11]. Both regulatory mechanisms may be repressed by light-activated CRYs, which is in agreement with previous results showing that CRYs physically interact with COP1 and impair its function under BL conditions [12,13]. Besides controlling CO and, potentially, GI accumulation, a direct mechanism by which photoactive CRYs regulate flowering gene expression was recently unveiled [14]. It has been found that CRY2 physically interacts with transcription factor (TF) CRY-INTERACTING BASIC-HELIX-LOOP-HELIX (CIB1) to promote expression of *FLOWERING LOCUS T* (*FT*), a gene that encodes a flowering-inducing mobile protein: the florigen [15-18]. Whether COP1 controls CRY2-mediated activation of CIB1 remains to be demonstrated.

Concerning Phys, there is evidence that PhyA and PhyB differentially control CO stability, with PhyA stabilizing CO in the evening and PhyB promoting its degradation at dawn [9,11]. These two receptors are also known to transmit light information to the clock [19]. However, contrary to the case of ZTL, FKF1, and CRYs, the molecular mechanisms by which Phys affect CRL and flowering are poorly understood. One possibility is that Phys act in concert with CRYs to modulate COP1 function toward GI or CO proteins. This may be supported by the fact that PhyA, which is known to interact with CRY1, transmits both low-fluence red light and BL to the clock [19,20]. Functional relationships between CRYs and Phys are also supported by physical interaction of CRY2 with PhyB [21]. Another possibility is that, as in the case of CRY2, Phys directly affect the function of TFs controlling circadian and flowering gene expression. In agreement with this, PhyA and PhyB bind PHY-INTERACTING FACTOR 3 (PIF3), a TF that negatively regulates *CO* and *FT* gene expression [22].

Similarly, it has been shown that FAR-RED ELONGATED HYPOCOTYL 3 (FHY3), a TF that mediates Phys signaling, also participates in the gating of red light signals for clock resetting [23]. FHY3 association with underphosphorylated PhyA was recently found to protect PhyA from targeted degradation by COP1, thus adding a new layer of regulation to COP1-mediated control of photoreceptors [24].

Future directions

How COP1 can recognize diverse unrelated protein targets, such as PhyA, CO, or GI, represents an important question arising from these studies. The fact that ELF3 likely functions as a substrate adaptor for COP1 to recognize GI may answer this question [8]. Thus, COP1 may interact with additional specific adaptors to discriminate between targets depending on precise developmental stages or light-controlled biological processes. In this regard, it was shown that COP1 is part of a multi-protein complex that contains, among others, members of the SUPPRESSOR OF PHYA-105 (SPA) protein family [25] (Figure 1). SPA proteins share sequence similarity with COP1 and modulate its E3 ubiquitin-ligase activity toward light-response regulators such as ELONGATED HYPOCOTYL 5 (HY5), LONG AFTER FAR-RED 1 (LAF1), and PhyA [25-29]. This function is likely extended to other substrates, such as CO [27]. It would be interesting to analyze whether different combinations of COP1, SPA members, and additional proteins, like ELF3, determine target specificity of COP1.

Despite important insights, our knowledge about the regulatory mechanisms governing CRL and photoperiodic flowering is still very limited. Thus, studies in this field very often reveal new genetic and molecular interactions as well as new regulatory activities (for example, phosphorylation, sumoylation, and histone acetylation) within these processes. These findings should allow us to further explore how light/dark signaling pathways converge to control light resetting of the clock and flowering time.

Abbreviations

BL, blue light; CDF1, CYCLING DOF FACTOR 1; CIB1, CRYPTOCHROME-INTERACTING BASIC-HELIX-LOOP-HELIX 1; CO, CONSTANS; COP1, CONSTITUTIVE PHOTOMORPHOGENESIS 1; CRL, clock-resetting by light; CRY, cryptochrome; ELF3, EARLY-FLOWERING 3; FHY3, FAR-RED ELONGATED HYPOCOTYL 3; FKF1, FLAVIN-BINDING, KELCH REPEAT, F-BOX 1; FT, FLOWERING LOCUS T; GI, GIGANTEA; HY5, ELONGATED HYPOCOTYL 5; LAF1, LONG AFTER FAR-RED 1; LKP2, LOV, KELCH PROTEIN 2; Phy, phytochrome; PIF3, PHY-INTERACTING FACTOR 3; PRR5, PSEUDORESPONSE REGULATOR 5; SPA, SUPPRESSOR OF PHYA-105; TF, transcription factor; TOC1, TIMING OF CAB1; ZTL, ZEITLUPE.

Competing interests

The authors declare that they have no competing interests.

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