Cryptochromes Go Toe to Toe with TOEs Too

To breed or not to breed, that is the question. The switch from vegetative to reproductive growth is one of the most important steps in a plant’s life cycle. Flower too early or too late and there is a risk that the environment will not support the development of healthy offspring. To avoid this, the timing of flowering is tightly controlled by environmental cues. In Arabidopsis (Arabidopsis thaliana), flowering transition is promoted by long-day photoperiods.

Long photoperiods are detected by the blue light photoreceptor cryptochrome 2 (cry2). For blue light to promote flowering, it must coincide with internal cues. The expression of the floral integrator CONSTANS (CO) peaks around 16 h after dawn. When photoperiods are short, the peak CO expression occurs in the dark and CO is quickly degraded by the E3 ligase CONSTITUTIVELY PHOTOMORPHOGENIC1 (COP1). However, if the expression of CO coincides with light (as occurs in long days), COP1 is inhibited by cry2, allowing CO protein to accumulate. CO induces the expression of FLOWERING TIME (FT) and thereby promotes the transition to reproductive growth (Fig. 1; Song et al., 2015). cry2 also promotes flowering more directly through interaction with the transcription factor CRY2 INTERACTING bHLH1 (CIB1). CIB1, CO, and cry2 form a complex that accumulates at the FT promoter and enhances FT expression (Fig. 1; Song et al., 2015; Liu et al., 2018).

In this issue of Plant Physiology, Du et al. (2020) add yet another string to cry2’s bow. In a screen for cry1-interacting proteins, the group identified TARGET OF EAT1 (TOE1), an APETALA2-like (AP2-like) family transcription factor. TOE1 has previously been shown to control flowering by binding to CO and blocking its activity (Zhang et al., 2015). Because cry2 is the predominant cryptochrome in the regulation of flowering, the group tested whether cry2 also bound to TOE1. Indeed, they found that cry2 binds to TOE1, TOE2, and other members of the AP2-like family. Curiously, the group found that the interaction between cry2 and AP2-like transcription factors occurred in the dark in yeast but was blue light dependent in plants. It is unclear why the blue light requirement differs between these two systems.

To investigate whether the cry2:TOE interaction plays a role in flowering regulation, the group created a cry1 cry2 toe1 toe2 quadruple knockout mutant. These plants flowered slightly earlier than the cry1 cry2 mutant, suggesting that cryptochromes promote flowering at least in part through the inhibition of TOEs. The group also showed that the overexpression of either TOE1 or TOE2 represses flowering much more strongly in the cry1 cry2 mutant. They went on to demonstrate that cry2 blocks the interaction between TOEs and CO in a blue light-dependent manner. They propose that reduced TOE:CO interaction promotes CO activity and allows for flowering induction. The group also established that the interaction between cry2 and TOE1 blocked TOE1 from binding to a specific site of the FT promoter. They suggest that cry2-mediated suppression of TOEs promotes flowering in two ways, both by increasing the pool of functional CO and by releasing the direct repression of FT expression by TOEs (Fig. 1).

In addition to improving our understanding of cry2-mediated flowering, this study brings up some important questions. It is curious that TOE1 binding to the FT promoter was increased in the cry1 cry2 background at only one of four TOE1-binding sites. If cry2 simply inhibits TOE1 DNA binding, should we not expect all binding sites to be enriched in the absence of cry2? Selectivity in this response hints that cry2-mediated inhibition of TOE1 DNA binding is more nuanced than simply through sequestration. Another aspect that could be further explored is the effect of TOEs on the cry2:CIB1:CO complex. CO is a B-box family transcription factor. Recently, it was shown that other members of the B-box family act as rate-limiting cofactor2:CIB1:CO multimer, TOE:CO interaction could potentially modulate the transcriptional activity of the complex. Finally, it is currently unclear when cry2 mediates the suppression of TOEs.

toe1 toe2 mutants flower early in both short days and long days (Zhang et al., 2015), whereas cry2 affects

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Figure 1. The molecular mechanisms proposed by Du et al. (2020). In the dark, cry2 is inactive and COP1 promotes CO degradation. TOEs bind to the remaining CO to block its transcriptional activity. TOEs also directly repress FT expression. In long days, cry2 suppresses COP1 activity, leading to a stabilization of CO. cry2 also inhibits TOEs to reduce their interaction with CO and repression of FT expression. cry2 additionally forms a complex with CIB1 and CO at the FT promoter to directly promote FT expression. Figure adapted from Du et al. (2020).
flowering only in long days (Song et al., 2015). This implies that cry2 suppression of TOEs mainly plays a role toward the end of the day. Future research should provide some valuable insights into these questions and improve our understanding of how plants make that important decision to switch to reproductive growth.

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