

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 3' 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 cofactors for a master regulator of photomorphogenesis, *ELONGATED HYPOCOTYL5* (Bursch et al., 2020). If *CO* acts as the rate-limiting component of the *cry2*:*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*

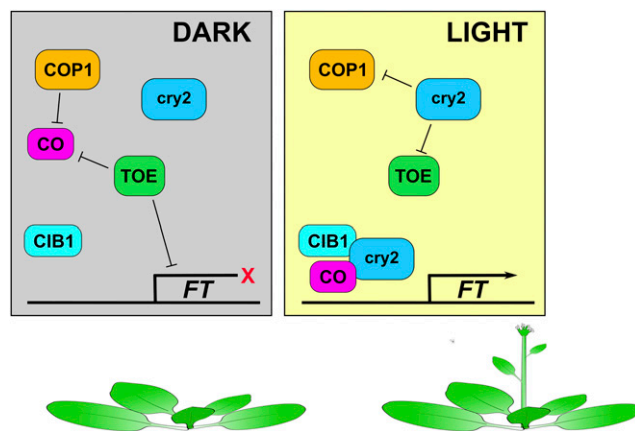


Figure 1. The molecular mechanisms proposed by Du et al. (2020). In the dark, *cry2* is inactive and *COP1* promotes *CO* degradation. *TOEs* bind to the remaining *CO* to block its transcriptional activity. *TOEs* also directly repress *FT* expression. In long days, *cry2* suppresses *COP1* activity, 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).

¹Author for contact: scott.hayes@wur.nl.

²Senior author.

www.plantphysiol.org/cgi/doi/10.1104/pp.20.00983

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

Scott Hayes^{1,2}

ORCID ID: 0000-0001-8943-6238

**Laboratory of Plant Physiology,
Wageningen University, Wageningen 6708 PB,
The Netherlands**

LITERATURE CITED

- Bursch K, Toledo-Ortiz G, Pireyre M, Lohr M, Braatz C, Johansson H** (2020) Identification of BBX proteins as rate-limiting cofactors of HY5. *Nat Plants* **6**: 921–928
- Du S-S, Li L, Li L, Wei X, Xu F, Xu P, Wang W, Xu P, Cao X, Miao L, et al** (2020) Photoexcited cryptochrome2 interacts directly with TOE1 and TOE2 in flowering regulation. *Plant Phys* **184**: 487–505
- Liu Y, Li X, Ma D, Chen Z, Wang JW, Liu H** (2018) CIB1 and CO interact to mediate CRY2-dependent regulation of flowering. *EMBO Rep* **19**: 1–10
- Song YH, Shim JS, Kinmonth-Schultz HA, Imaizumi T** (2015) Photoperiodic flowering: Time measurement mechanisms in leaves. *Annu Rev Plant Biol* **66**: 441–464
- Zhang B, Wang L, Zeng L, Zhang C, Ma H** (2015) Arabidopsis TOE proteins convey a photoperiodic signal to antagonize CONSTANS and regulate flowering time. *Genes Dev* **29**: 975–987