

## Addendum

# Flowering regulation by tissue specific functions of photoreceptors

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**Abbreviations:** PHY, phytochrome; CRY, cryptochrome; GFP, green fluorescent protein; FT, flowering locus T

**Key words:** photoreceptor, light, flowering, phytochrome, cryptochrome, inter-tissue signal

Flowering is one of the most important steps in a plant life cycle. Plants utilize light as an informational source to determine the timing of flowering. In *Arabidopsis*, phytochrome A (phyA), phyB and cryptochrome2 (cry2) are major photoreceptors that regulate flowering. These photoreceptors perceive light stimuli by leaves for the regulation of flowering. A leaf is an organ consisting of different tissues such as epidermis, mesophyll and vascular bundles. In the present study, we examined in which tissue the light signals are perceived and how those signals are integrated within a leaf to regulate flowering. For this purpose, we established transgenic *Arabidopsis* lines that expressed a phyB-green fluorescent protein (GFP) fusion protein or a cry2-GFP fusion protein in organ/tissue-specific manners. Consequently, phyB was shown to perceive light stimuli in mesophyll. By contrast, cry2 functioned only in vascular bundles. We further confirmed that both phyB-GFP and cry2-GFP regulated flowering by altering the expression of a key flowering gene, *FT*, in vascular bundles. In summary, perception sites for different spectra of light are spatially separated within a leaf and the signals are integrated through the inter-tissue communication.

The timing of flowering is strictly regulated by environmental conditions such as light. Two aspects of light, spectral nature and photoperiod, dramatically affect flowering. In *Arabidopsis*, phyB and phyA/cry2 are the major photoreceptors mediating these responses. Although photoreceptors are expressed in almost all organs,<sup>1</sup> partial irradiation and grafting analyses have demonstrated that plants perceive light signals only in leaves.<sup>2-4</sup> However, roles for different tissues in a leaf remained unknown due to a lack of a proper method.

To answer the question, we established *Arabidopsis* transgenic lines that expressed phyB-GFP or cry2-GFP on the respective mutant backgrounds. The resultant transgenic lines were examined for their flowering phenotype. Consequently, we found that phyB-GFP in mesophyll but not in other tissues regulated flowering.<sup>5</sup> By contrast, cry2-GFP functioned only in vascular bundles.<sup>6</sup>

A strong genetic interaction between phyB and cry2 in the regulation of flowering is known.<sup>7,8</sup> Cry2 regulates the flowering by suppressing the inhibitory effect of phyB on flowering. Hence, cry2 function is observed only in the presence of phyB. Conversely, the effect of phyB is exaggerated in the cry2 mutant, because phyB is not counteracted by cry2 in its absence. Here, we tested how phyB and cry2 in different tissues regulated flowering in the absence of the other photoreceptor. For this purpose, we took a physiological approach. Phenotype of the phyB-GFP lines was examined under monochromatic red light, in which phyB but not cry2 is activated. As expected, phyB-GFP in mesophyll but not in vascular bundles strongly affected the flowering in this condition (Fig. 1A). We also tested the cry2-GFP function when phyB was not activated. Namely, plants were placed under blue light supplemented with strong far-red light. As expected, cry2-GFP failed to affect the flowering even under this condition regardless of where it was expressed (Fig. 1B).

Photoreceptors regulate flowering by altering the expression of a key flowering regulator, *FT*.<sup>9,10</sup> Interestingly, the *FT* gene is expressed specifically in vascular bundles.<sup>11</sup> Indeed, mesophyll phyB-GFP controlled the expression of *FT* in vascular bundles. Hence, there must be a mechanism by which the light signal is transduced from mesophyll to vascular bundles to regulate the *FT* expression in vascular bundles. It should be noted here that *FT* is not the sole factor involved in the light regulation of flowering. Factors such as CO, SPA, COP1 and PFT1 are known to link the photoreceptors and *FT*.<sup>12-14</sup> These factors most likely function in leaves. However, their function sites at the tissue level remain totally unknown except for CO. The biological clock is another class of machinery that is tightly related to the light signal transduction pathway.<sup>15</sup> Unfortunately, function sites of the clock components for the regulation of flowering remain unclear. The future work should reveal those sites. Such analyses should finally provide a complete picture illustrating a network of the inter-tissue signaling for the regulation of flowering.

The present work urges us to identify the molecule that mediates the inter-tissue signaling between mesophyll and vascular bundles. Potential candidates include phytohormones, microRNA<sup>16</sup>

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and

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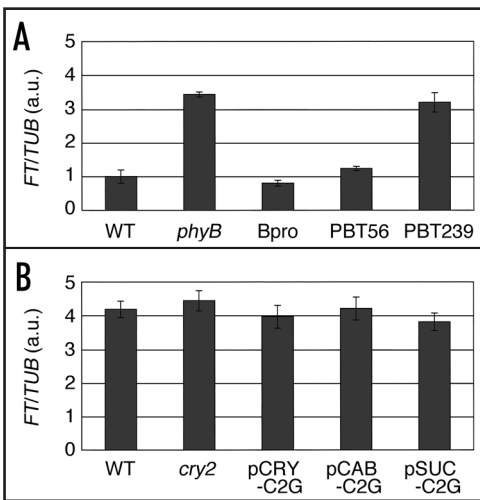


Figure 1. *FT* expression under *phyB* or *cry2* inactive conditions. Total RNA was extracted from the seedlings grown under long-day condition for 10 days and subjected to qRT-PCR for *FT* expression analysis. Data were normalized to the level of *FT* mRNA in (A) of the wild type, which was set to 1 arbitrary unit (a.u.). Mean  $\pm$  SE ( $n = 4$ ). WT, wild type. (A) Long-day red light, (16L 8D;  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). WT, wild type; *phyB*, *phyB* mutant; Bpro, *PHYB* promoter-*PHYB*-GFP; PBT56, *phyB*-GFP in mesophyll; PBT239, *phyB*-GFP in vascular bundles.<sup>5</sup> (B) Long-day blue and far-red light (16L 8D; blue light,  $3 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; far-red light,  $10 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). WT, wild type; *cry2*, *cry2* mutant; pCRY-C2G, *CRY2* promoter-*CRY2*-GFP; pCAB-C2G, *CAB3* promoter-*CRY2*-GFP; pSUC-C2G, *SUC2* promoter-*CRY2*-GFP.<sup>6</sup>

and peptides.<sup>17</sup> Among phytohormones, gibberellin promotes flowering.<sup>18</sup> However, gibberellin is probably not the answer because gibberellin does not alter the *FT* expression directly. Except gibberellin, no exogenously added phytohormone dramatically affects flowering in *Arabidopsis*. It is known that microRNA such as *miR172*, *miR159* and *miR156* are involved in the regulation of flowering time.<sup>19</sup> However, those microRNAs neither regulate the *FT* expression nor are regulated by light. Since most of microRNAs has not been intensively studied yet, it remains possible that one of them may mediate the above inter-tissue signal. Another potential candidate is a peptide. Although not much is known about peptide hormones in plants yet, peptides such as PSK,<sup>20</sup> xylogen<sup>21</sup> and CLE<sup>22</sup> have been shown to regulate cell growth and differentiation. Although none of peptides is known to regulate flowering in plants at present, a future work may reveal a novel peptide that mediates the inter-tissue signals for flowering.

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