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PIF3 and HY5 regulate light responses both competitively and collaboratively

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Objectives

We have tried to figure out that complex role of PIF3 is partly arisen by the complex functional relationships with other components.

Materials and Methods

1. Material

Plant *Arabidopsis,* Anti-myc antibody

2. Methods

Hypocotyl length : Arabidopsis seeds were sown in the 1/2 MS, 8% phytoagar, imbibed in 4℃, dark condition for 3days. White light was treated for 6 hours and plants were transferred to continuous R, FR light, and D respectively for additional 4days. Hypocotyl length of plants was measured by Scion Image software.

Anthocyanin accumulation : 50 seeds of Arabidopsis were sown in the 1/2 MS include 1% sucrose, imbibed in 4℃, dark condition for 3days. White light was treated for 6 hours and plants were transferred to continuous FR light for additional 4days. Seedlings were harvested and anthocyanin was extracted by MeOH+1% HCl.

ChIP assay : Chromatin Immunoprecipitation assay was performed using myc-tagged PIF3, HY5, and GFP transgenic plants. Chromatin bound PIF3, HY5, and GFP proteins were pulled down by anti-myc antibody and protein bound DNA was eluted, quantified by real-time PCR.

Results and Discussion

Phytochromes are red/far-red light receptors that regulate various light responses by initiating transcriptional cascades leading into the expressional changes of 10 30% of whole genes. Several transcription factors that are thought to participate in this process have been identified, but the exact roles of them are largely elusive. PIF3 is a phytochrome-interacting bHLH transcription factor that regulates a subset of light responses either positively or negatively. Previous report showed that it acts as a negative component in PHYB-, but not PHYA-mediated inhibition of hypocotyl elongation, while it acts as a positive component in PHYA-mediated anthocyanin biosynthesis. We report that complex role of PIF3 is partly arisen by the complex functional relationships with other components. In the absence of functional HY5, PIF3 acts as a negative component both in PHYA- and PHYB- mediated inhibition of hypocotyl elongation. For anthocyanin biosynthesis, PIF3 positively regulates transcription of anthocyanin biosynthetic genes by directly binding to the promoters of them *in vivo*. The positive role of PIF3 in anthocyanin biosynthesis, however, requires a functional HY5 as shown by epistasis of hy5 mutation to PIF3 overexpression. Taken together, our data show that the phenotypic manifestation of PIF3 function in light signaling is partly dependant on the function of HY5.

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