

PICKLE is a repressor in seedling de-etiolation pathway

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Light plays a vital role in seedling de-etiolation during which it remarkably inhibits hypocotyl growth and promotes cotyledon opening and the synthesis of chlorophyll and anthocyanin. After light perception, photoreceptors act to repress two main branches of the light signaling, PIFs and COP1-HY5. We recently identified PKL/EPP1, a chromatin remodeling factor, as a new component in regulating light-mediated hypocotyl growth. In this study, we found that EPP1 acts additively with SPA1 to repress seedling de-etiolation. Moreover, the expression of *EPP1* is downregulated specifically in the hypocotyl region of the *cop1* mutant compared with that of the wild type. We further found that EPP1 drastically inhibits both the protein and transcript levels of HY5, but not vice versa, indicating that HY5 acts downstream of EPP1. We thus propose a model in which EPP1 defines a new repressor and mediates a distinct signaling pathway of photomorphogenesis.

Light provides plants important environmental information for variety of growth and developmental processes, including seedling de-etiolation (also termed photomorphogenesis) in the early stage of their life cycle. Plants have thus evolved an array of photoreceptors, including phytochromes and cryptochromes, to monitor the light signals, which are then transduced to regulate physiological responses through the signaling transduction pathway.¹ An increasing body of evidence has established a primary network composed of both positive and negative components in regulating seedling de-etiolation in the model plant *Arabidopsis thaliana*.¹⁻³ COP1 (for constitutive photomorphogenic 1), a RING-type E3 ubiquitin ligase, functions as a central repressor controlling photomorphogenesis downstream of all photoreceptors.⁴ A group of bHLH transcription factors, designated as PIFs, physically interact with phytochromes and repress photomorphogenesis under red and far-red light conditions.⁵ Recently, we identified the chromatin remodeling factor EPP1 (enhanced photomorphogenic 1) PKL (pickle) as a new negative regulator in the light signaling pathway.⁶ PKL/EPP1 is an ATP-dependent chromatin remodeling enzyme that plays vital role in plant development.⁷ Genetic studies had also discovered a large number of photomorphogenesis-promoting factors. For example, HY5 (elongated hypocotyl 5) is one of the most important positive transcription factors that are targeted by COP1 and thereafter degraded through the 26S proteasome pathway.^{8,9} The negative and positive factors work together to fine-tune the downstream gene expression and modulate physiological responses that allows plants to adapt to the light environments properly.

SPA1 and PKL/EPP1 Additively Regulate Seedling De-etiolation

Previous genetic experiments identified SPA1 (suppressor of phytochrome A 1) as a negative component in the phyA signaling pathway.¹⁰ It has been well documented that SPA1 physically interacts with COP1 to form E3 ligase complex and represses photomorphogenesis through destabilizing positive transcription factors, such as HY5, in *Arabidopsis*.^{11,12} Our previous study revealed that EPP1 acts in parallel with COP1 in repressing photomorphogenesis.⁶

To study the relationship between EPP1 and SPA1, we generated *epp1 spa1* double mutant by genetic crossing of *epp1-1* and *spa1* mutants. When grown under far-red light, the *epp1 spa1* double mutant seedlings showed much shorter hypocotyls and accumulated more anthocyanin than their single parent mutants (Fig. 1). The double mutant also displayed reduced hypocotyl growth in blue and red light conditions (Fig. 1C). These results indicate that EPP1 functions additively with SPA1, similar to with COP1, in modulating seedling de-etiolation process.

COP1 Activates PKL/EPP1 Expression

Consistent with the role of EPP1 in repressing light-inhibited hypocotyl elongation, the expression of *EPP1* transcript is downregulated by light specifically in the hypocotyl regions and this inhibition is mediated by both phytochromes and cryptochromes.⁶ To examine a possible regulation by COP1, we performed a reverse-transcription with quantitative PCR assay and found that the transcript level of *EPP1* was remarkably reduced in the *cop1-4*

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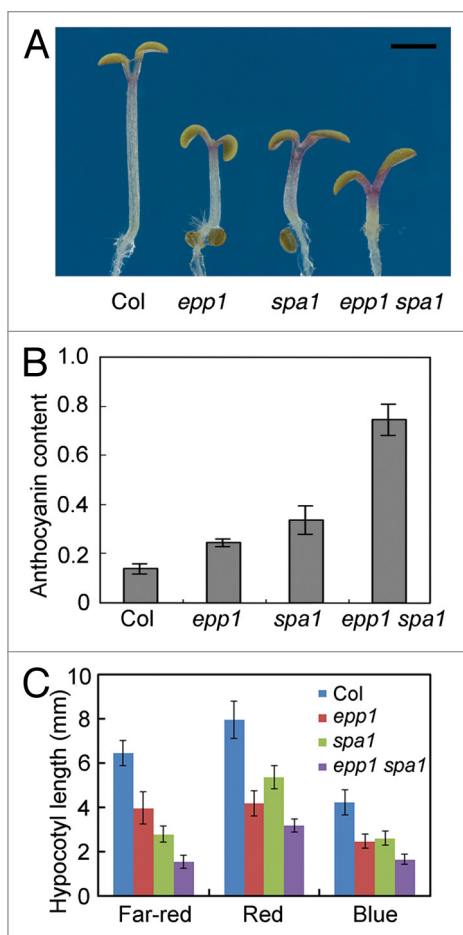


Figure 1. SPA1 and EPP1 additively regulate seedling de-etiolation. (A and B) Seedling phenotype (A) and anthocyanin content [(A530-0.25 × A657)/100 seedlings] (B) of wild-type and mutant seedlings in far-red light for 5 d. Bar in (A) denotes 2 mm. (C) Hypocotyl length of 5-d-old seedlings. Data in (B and C) represent the mean ± SD of triplicate assays.

mutant compared with Col wild type (Fig. 2A). Furthermore, by visualizing the expression of *EPP1* using a *GUS* reporter gene driven by the *EPP1* promoter sequence (*ProPKL:GUS*), we observed that *GUS* staining was greatly decreased in the hypocotyl of *cop1* related to that of the wild-type seedlings (Fig. 2B). However, no pronounced difference was observed in the root region between *cop1* and the wild type. These data suggest that COP1 promotes *EPP1* gene expression in the hypocotyl. On the contrary, COP1 expression was upregulated in the *epp1* mutant (Fig. 2C), suggesting a negative feedback role of EPP1.

PKL/EPP1 Negatively Regulates HY5 Activity

HY5 defines a master point of signaling converged from multiple photoreceptors and itself is regulated mainly at the post-translational level. In the dark, HY5 is targeted by COP1 and further degraded, whereas light triggers nuclear export of COP1, thus stabilizing HY5.⁸ On the other hand, HY5 possesses transcriptional repression activity toward downstream genes involved in cell elongation to promote photomorphogenesis. However, the recruitment of EPP1 through interaction is proposed to dampen the activity of HY5 from over entry into photomorphogenesis, providing an additional regulatory mechanism to fine-tune the light signaling pathway.⁶ Using an immunoblotting assay against HY5 antibody, we also detected a significant increase of HY5 protein in the *epp1* mutant alleles compared with the wild type (Fig. 3A). This HY5 repression by EPP1 is modulated, at least in part, at the transcriptional level as *HY5* expression was greatly increased in the *epp1* mutant (Fig. 3B). However, mutation in *HY5* does not affect the protein level of EPP1 (Fig. 3C). Thus, EPP1 plays a dual role on HY5, de-repressing the transcriptional activity of HY5 toward target loci and inhibiting the transcriptional level of *HY5* as well.

The light signaling pathway composes of different layers of components, ranging from early photoreceptors, central negative regulators and positive transcription factors, to downstream responsive genes (Fig. 4). Although COP1, PIFs (including PIF1, PIF3, PIF4 and PIF5) and EPP1 all act as repressors in regulating photomorphogenesis, their functions and the underlying regulatory mechanisms are distinct from each other due to their divergent biochemical features. COP1 is an E3 ubiquitin ligase targeting positive factor for degradation, while PIF proteins bind to and regulate the expression of downstream genes in phy-mediated pathway.^{5,9} Consistently, PIF4 directly activates the expression of a set of genes involved in hypocotyl cell elongation.¹³ The stability or activity of HY5 is negatively regulated by COP1 and EPP1, respectively.

Chromatin remodeling plays critical roles in specifying gene expression and maintaining transcriptional states in yeast, plants and animals.¹⁴ Strikingly, the identification of EPP1 uncovers the chromatin remodeling-mediated regulatory mechanism by which plants precisely respond to light environment in a temporal and/or spatial manner. Future efforts on identifying more factors and elucidating various histone modification mechanism involved

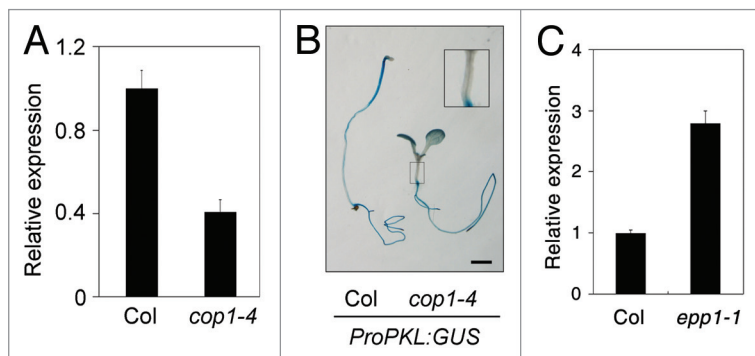
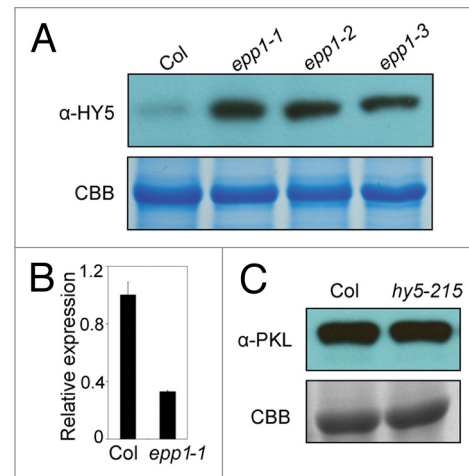


Figure 2. *EPP1* expression is downregulated by *COP1* mutation. (A) A quantitative RT-PCR assay of *EPP1* transcript levels in *cop1-4* and wild-type seedlings grown in darkness for 5 d. (B) *GUS* staining of *ProPKL:GUS* transgenic seedlings grown in darkness for 5 d. The enlarged image of *cop1* hypocotyl is shown up-right. Bar, 2 mm. (C) *COP1* transcript level in 5-d-old light-grown wild type and *epp1* mutant. In (B and C), relative expression was normalized to that of *UBQ1* and data represent mean ± SD of three biological replicates.

Figure 3. EPP1 inhibits the mRNA and protein levels of *HY5*. (A) Immunoblotting assay showing *HY5* protein level in the *epp1* mutants and Col wild type. (B) *HY5* expression level by qRT-PCR. Relative expression was normalized to *UBQ1* and data represent mean \pm SD of three biological replicates. (C) EPP1 protein level in *hy5* mutant and Col wild type. Seedlings were grown in the dark for 5 d in all assays. Staining with coomassie brilliant blue of the gel serves as a loading control in (A and C).



in epigenetic controlling of light signaling are highly desired. Previous studies suggest that light integrates hormonal signaling pathways to modulate diverse growth and development and that EPP1 is involved in regulating multiple processes.^{9,15} It will be interesting to investigate whether and how EPP1 integrates multiple signaling to modulate a particular developmental program.

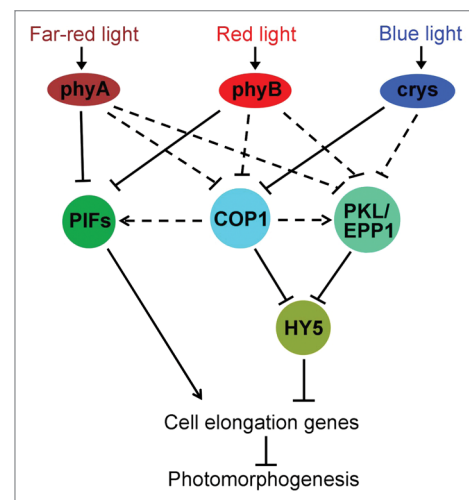
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Figure 4. A simplified model of photomorphogenic pathway in *Arabidopsis*. Different spectrum of light are absorbed by the corresponding photoreceptors (phytochromes and cryptochromes) and the light signals are then transduced to suppress three main types of negative factors, including COP1, PIFs and PKL/EPP1. COP1 represses *HY5* through mediating its protein turnover, while EPP1 inhibits the transcriptional activity of *HY5*. PIFs (including PIF1, PIF3, PIF4 and PIF5) and *HY5* are transcription factors that promote or suppress the transcription of cell elongation genes, respectively. Solid lines indicate a direct regulation, while dotted lines denote an indirect effect. Modified from Lau and Deng.⁹



References

- Chen M, Chory J, Fankhauser C. Light signal transduction in higher plants. *Annu Rev Genet* 2004; 38:87-117; PMID:15568973; <http://dx.doi.org/10.1146/annurev.genet.38.072902.092259>
- Jiao Y, Lau OS, Deng XW. Light-regulated transcriptional networks in higher plants. *Nat Rev Genet* 2007; 8:217-30; PMID:17304247; <http://dx.doi.org/10.1038/nrg2049>
- Lau OS, Deng XW. Plant hormone signaling lightens up: integrators of light and hormones. *Curr Opin Plant Biol* 2010; 13:571-7; PMID:20739215; <http://dx.doi.org/10.1016/j.pbi.2010.07.001>
- Yi C, Deng XW. COP1 - from plant photomorphogenesis to mammalian tumorigenesis. *Trends Cell Biol* 2005; 15:618-25; PMID:16198569; <http://dx.doi.org/10.1016/j.tcb.2005.09.007>
- Leivar P, Quail PH. PIFs: pivotal components in a cellular signaling hub. *Trends Plant Sci* 2011; 16:19-28; PMID:20833098; <http://dx.doi.org/10.1016/j.tplants.2010.08.003>
- Jing Y, Zhang D, Wang X, Tang W, Wang W, Huai J, et al. *Arabidopsis* chromatin remodeling factor PICKLE interacts with transcription factor *HY5* to regulate hypocotyl cell elongation. *Plant Cell* 2013; 25:242-56; PMID:23314848; <http://dx.doi.org/10.1105/tpc.112.105742>
- Ogas J, Kaufmann S, Henderson J, Somerville C. PICKLE is a CHD3 chromatin-remodeling factor that regulates the transition from embryonic to vegetative development in *Arabidopsis*. *Proc Natl Acad Sci USA* 1999; 96:13839-44; PMID:10570159; <http://dx.doi.org/10.1073/pnas.96.24.13839>
- Osterlund MT, Hardtke CS, Wei N, Deng XW. Targeted destabilization of *HY5* during light-regulated development of *Arabidopsis*. *Nature* 2000; 405:462-6; PMID:10839542; <http://dx.doi.org/10.1038/35013076>
- Lau OS, Deng XW. The photomorphogenic repressors COP1 and DET1: 20 years later. *Trends Plant Sci* 2012; 17:584-93; PMID:22705257; <http://dx.doi.org/10.1016/j.tplants.2012.05.004>
- Hoecker U, Tepperman JM, Quail PH. SPA1, a WD-repeat protein specific to phytochrome A signal transduction. *Science* 1999; 284:496-9; PMID:10205059; <http://dx.doi.org/10.1126/science.284.5413.496>
- Hoecker U, Quail PH. The phytochrome A-specific signaling intermediate SPA1 interacts directly with COP1, a constitutive repressor of light signaling in *Arabidopsis*. *J Biol Chem* 2001; 276:38173-8; PMID:11461903
- Saijo Y, Sullivan JA, Wang H, Yang J, Shen Y, Rubio V, et al. The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of *HY5* activity. *Genes Dev* 2003; 17:2642-7; PMID:14597662; <http://dx.doi.org/10.1101/gad.1122903>
- Oh E, Zhu JY, Wang ZY. Interaction between BZR1 and PIF4 integrates brassinosteroid and environmental responses. *Nat Cell Biol* 2012; 14:802-9; PMID:22820378; <http://dx.doi.org/10.1038/ncb2545>
- Ho L, Crabtree GR. Chromatin remodelling during development. *Nature* 2010; 463:474-84; PMID:20110991; <http://dx.doi.org/10.1038/nature08911>
- Zhang H, Bishop B, Ringenberg W, Muir WM, Ogas J. The CHD3 remodeler PICKLE associates with genes enriched for trimethylation of histone H3 lysine 27. *Plant Physiol* 2012; 159:418-32; PMID:22452853; <http://dx.doi.org/10.1104/pp.112.194878>