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Multiple photomorphogenic repressors work in concert to regulate Arabidopsis seedling development

Jie Dong[1], William Terzaghi[2], Xing Wang Deng[1], and Haodong Chen[1,*]

[1]Peking-Yale Joint Center for Plant Molecular Genetics and Agro-biotechnology; State Key Laboratory of Protein and Plant Gene Research; Peking-Tsinghua Center for Life Sciences; College of Life Sciences; Peking University; Beijing, China; [2]Department of Biology; Wilkes University; Wilkes-Barre, PA USA

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Light is both a source of energy and a critically important environmental signal for plant development. Through decades of research, 2 groups of photomorphogenic repressors have been identified. The first group is *CONSTITUTIVE PHOTOMORPHOGENIC/DE-ETIOLATED/FUSCA* (*COP/DET/FUS*), which were first identified by genetic screening and then by purification of protein complexes. Another group is the Phytochrome-Interacting Factors (PIFs), which were identified by yeast 2-hybrid screens using phyB as bait. How so many factors work together to repress photomorphogenesis has long been an interesting question. Previously, we demonstrated that CULLIN4 (CUL4) works as a core factor connecting the COP1-SPA complexes, the COP9 signalosome (CSN), and the COP10-DDB1-DET1 (CDD) complex. Recently, we showed that DET1 represses photomorphogenesis through positively regulating the abundance of PIF proteins in the dark. Dr. Huq and his colleagues reported that PIFs may enhance the function of COP1-SPA complexes to promote the degradation of HY5, and thus they synergistically repress photomorphogenesis in the dark. Though much work still needs to be done, these recent breakthroughs shed light on the regulatory relationships among these multiple photomorphogenic repressors.

Multiple Photomorphogenic Repressors in Plants

Seedlings grown in the light display photomorphogenic development with short hypocotyls and open and expanded cotyledons. In contrast, seedlings grown in the dark exhibit skotomorphogenic development with long hypocotyls, closed and unexpanded cotyledons, and apical hooks.[1] *COP/DET/FUS* is a group of pleiotropic genes identified by genetic screens as photomorphogenic repressors since mutants displayed photomorphogenic phenotypes in the dark.[2] This group of proteins functions as part of 3 biochemical entities: the COP1-SPA complexes, the COP9 signalosome (CSN), and the CDD complex.

COP1 was the first of these proteins to be characterized molecularly,[3] and it was shown to interact with SUPPRESSOR OF PHYTOCHROME A (SPA) proteins to function as Ring-finger type E3 ligases.[4,5] In the dark, the COP1-SPA complexes ubiquitinate and degrade ELONGATED HYPOCOTYL 5 (HY5), HY5 HOMOLOG (HYH), LONG HYPOCOTYL IN FAR RED (HFR1) and others via the 26S proteasome to repress photomorphogenesis.[6-8] Upon light exposure, the function of COP1 is largely inhibited, at least partially through nuclear export, leading to stabilization of the targeted proteins.[9,10]

The CSN was initially identified as a repressor of light-dependent development in plants.[11] It was later found to be a nuclear-enriched complex consisting of 8 distinct subunits.[12] Interestingly, CSN is conserved between plants and mammals, and shows remarkable similarity to the lid subcomplex of the 19S regulatory particle.[13] The CSN can interact with CULLIN (CUL)-based E3 ligases to deconjugate Nedd8/Rub from CULLINs and regulate their activities.[14,15]

The CDD complex is composed of COP10, DET1, and DDB1.[16] DET1 is another central repressor of photomorphogenesis identified in 1989.[17] It can interact with DAMAGED DNA BINDING PROTEIN1 (DDB1) and bind histone H2B, indicating a role for chromatin remodeling in the regulation of photomorphogensis.[18,19] DET1 has also been shown to maintain normal peroxisomal activities in plants to repress photomorphogenesis.[20] Recently, DET1 has been found to interact directly with CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYL (LHY) to regulate the circadian clock in vivo.[21] COP10 encodes a small ubiquitin E2 variant protein, and both COP10 itself and the CDD complex are known to enhance the activity of several E2s.[16,22]

In addition to COP/DET/FUS, PIFs function as another group of photomorphogenic repressors in the dark, identified by yeast 2-hybrid screen using phyB as bait.[23,24] Although they have different functions in seed germination, hypocotyl elongation, chlorophyll biosynthesis, shade avoidance etc., PIFs (mainly PIF1, PIF3, PIF4 and PIF5) function redundantly to repress photomorphogenesis in the dark.[25-30] It is now clear that PIFs accumulate in darkness but are rapidly phosphorylated and degraded upon light exposure.[31-33]

*Correspondence to: Haodong Chen; Email: chenhaodong@pku.edu.cn
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ORCID ID: 0000-0001-7228-6341 (H.C.)

CUL4 Connects the COP Complexes

All the *cop/det/fus* mutants show similar photomorphogenic phenotypes in the dark, but how these COP complexes work together was unclear for a long period. COP/DET/FUS proteins are required for the proper nuclear localization of COP1 in the dark.[9,12] Gel filtration analysis showed that lack of the CSN reduced the stability of the COP10 complex, and affected its integrity.[22] Then our further studies showed that CUL4 might interact with the CDD complex to form an E3 ligase RBX1-CUL4-CDD, and the CSN complex could regulate its activity through derubylation of CUL4.[34] Another study showed that both COP1 and SPA proteins have DWD motifs, and could interact with CUL4-DDB1 to form large E3 ligases.[35] Interestingly, upon UV exposure, the COP1-SPA complexes may disassociate from CUL4-DDB1 and interact with the UV-B receptor UVR8 to form a UVR8-COP1-SPA complex and stabilize HY5 proteins in vivo.[36] These results indicate that the activity of COP complexes can be connected by the function of CUL4.

Cooperation of COP/DET/FUS and PIFs

COP/DET/FUS and PIFs are 2 groups of photomorphogenic repressors identified using different strategies. During the past decade, the underlying mechanisms regulating interactions between these 2 groups of factors were largely unknown, except that the abundance of PIF3 proteins was reduced in *cop1* mutants.[31]

We recently found that DET1 could physically interact with 4 PIF (PIF1, PIF3, PIF4 and PIF5) proteins and positively regulate their levels. The PIF3 levels in the mutants of DET1, COP10 and CUL4 were all lower than in wild type plants, and the protein levels correlated well with their photomorphogenic phenotypes, which indicated that this positive regulation might be accomplished by the CUL4-CDD complex. We then demonstrated that DET1 could stabilize the 4 PIF (PIF1, PIF3, PIF4 and PIF5) proteins in the dark, while DET1 only positively regulated the PIF3 transcription level. Genetic analysis showed that *pif* single mutations enhanced while PIF overexpression partially suppressed the phenotypes of *det1-1*. Most of the genes regulated by both light and DET1, were also regulated by PIFs. Taken together, we proposed that DET1 repressed photomorphogenesis partially by stabilizing PIFs in the dark.[37]

At the same time, Dr. Huq and his colleagues reported another functional relationship between COP1-SPA complexes and PIFs. They found that PIFs could promote COP1-SPA-mediated HY5 degradation in the dark. The *pif1* single mutant could promote photomorphogenesis synergistically with *cop1* and *spa123* mutants. The HY5 protein levels in *pif* single and higher order mutants indicated that PIFs redundantly promoted HY5 protein degradation. Further genetic analysis showed that *hy5* mutants could partially suppress the photomorphogenic phenotypes of both *cop1-6 pif1* and *pifq* mutants in the dark. Biochemical analyses showed that PIF1 could form complexes with COP1, HY5, and SPA1 and that PIF1 could enhance the substrate recruitment and autoubiquitylation and transubiquitylation activities of COP1. Together, PIFs can enhance the ligase activity of COP1-SPA and synergistically repress photomorphogenesis in the dark.[38]

Known and Unknown

Based on the current knowledge, we propose a model of how COP/DET/FUS and PIFs work together to repress photomorphogenesis in the dark (**Fig. 1**). However, there are still many questions waiting to be further investigated. For example, what's the regulatory mechanism that controls the COP1-SPA complexes to act as E3 ligases themselves or as adaptors of CUL4-based E3 ligases? How does DET1 stabilize PIFs in the dark? Do PIFs also promote the E3 ligase activity of COP1-SPA toward other targets of COP1? Why do the functions of COP1- and DET1-containing E3 ligases rely on each other during photomorphogenesis? These photomorphogenic repressors play important roles not only in light signal transduction, but also in many other processes regulating plant development. Further

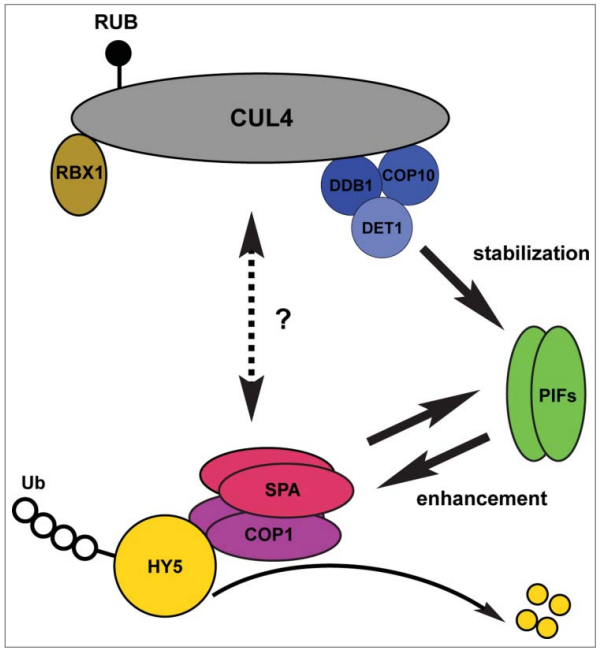

Figure 1. Multiple factors work together to repress photomorphogenesis in the dark. The CDD complex interacts with CUL4 to form the RBX1-CUL4-CDD E3 ligase in the dark. The RBX1-CUL4-CDD complex positively regulates PIF abundance primarily by stabilizing PIF proteins. On the other hand, PIFs promote the degradation of HY5 by enhancing the interactions between COP1-SPA complexes and HY5 and enhancing the ubiquitylation activities of COP1. Thus, these 2 newly- identified regulatory processes explain why the functions of COP1-SPA complexes rely on the CUL4-CDD complex to a certain extent. COP1 also positively regulates PIF3 abundance, although the mechanism is unclear. CSN regulates the activity of CUL4-based E3 ligases by deconjugating Nedd8/Rub from CULLINs (CSN is not shown in the model). The complete mechanism(s) by which these multiple photomorphogenesis factors work in concert need further investigation.

investigation of these photomorphogenic repressors will advance our views of regulatory mechanisms underlying plant development and their responses to environmental cues.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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